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Specialisation in veterinary medicine

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The acknowledgement of the specialisation in a veterinary discipline represents the confirmation of the importance of that specific discipline in professional study and practice.

The paper presents the historic of Veterinary Professional Colleges in USA and Europe, the organizational model of European Colleges, study disciplines in veterinary medicine in France that offers Veterinary Specialisation Studies Diplomas (DESV), the validity of the diplomas offered not as academis studies diplomas but as certificates of high professional competence.

Presently, the quality of veterinary specialisation in Europe is verified and certified following the criteria established by the European Board of Veterinary Specialisation (EBVS), which acnowledged 21 European Veterinary Specialisation Colleges.

Key Words: Veterinary Professional Colleges in USA, European Colleges, Veterinary Specialisations

The recognition and development of the concept of specialization in a precise veterinary disciplinary field represents an important step for the professional veterinary knowledge and for the professional practice.

The first **professional specialized veterinary Colleges** were founded in United States just after last world war; they still represent references for the foundation of comparable European Colleges. Effectively, since years 90, generally under impulse of national associations, **European Colleges of veterinary specialists** were progressively founded and presently cover a large part of veterinary professional activities recognized as specialty fields of veterinary practice, which means able to provide, for a veterinary practitioner particularly well formed in a disciplinary field, an exclusive or major professional specialized activity, directly or by way of referred cases.

European Colleges, after a short period of spontaneous aggregation of their "Charter members" and then of "***de facto*** members" restricted access to the certification to the way of the "**board examination**" organized by an examination committee nominated by the Colleges authorities ; the level aimed is generally a professional **excellence** one.

"Diplomas" obtained by successful veterinary candidates **are not academic ones** but only certifications of high professional knowledge in a specific field; nevertheless even if the diplomas are not of academic value, they are presently recognized by all professional European instances or world-wide.

In some fields and particularly paraclinical ones, "Colleges diplomates" are highly appreciated for recruitment and frequently obtain high standard positions with interesting levels of responsibility and fees. This is for example the situation for veterinary pathologists for which the label "**board certified**" is the best key for a quick and interesting recruitment the board certification being American (American College) or European (European College).

In clinical disciplines, the certification permits organization of an exclusive professional specialized practice and justifies better fees, correlative to high proved competences.

The quality of the specialized formation is nowadays under control of EBVS (European Board of Veterinary Specialization www.ebvs.be). This European Association presently identifies 21 Colleges of veterinary specialties. It requires and controls that the candidate to the board examination really followed a residency formation program (3 years at least) under direction of an active diplomate of the corresponding College.

Table I : list of European Veterinary Colleges identified by EBVS

Acronym	Name of Collège	year of foundation
ECVS	European College of Veterinary Surgery	1991
ECVD	European College of Veterinary Dermatology	1992
ECVO	European College of Veterinary Ophthalmologists	1992
ECAMS	European College of Avian Medicine and Surgery	1993
ECVN	European College of Veterinary Neurology	1993
ECVA	European College of Veterinary Anaesthesiology	1994
ECVDI	European College of Veterinary Diagnostic Imaging	1994
ECVIM-CA	European College of Veterinary Internal Medicine - Companion Animals	1994
ECVP	European College of Veterinary Pathology	1995
ECVPT	European College of Veterinary Pharmacolgy and Toxicology	1997
ECAR	European College of Animal Reproduction	1998
EVDC	European Veterinary Dentistry College	1998
ECVCN	European College of Veterinary Comparative Nutrition	1999
ECVPH	European College of Veterinary Public Health	2000
ECEIM	European College of Equine Internal Medicine	2002
ECLAM	European College of Laboratory Animal Medicine	2000
ECVBM-CA	European College of Veterinary Behavioural Medicine - Companion Animals	2002
ECVCP	European College of Veterinary Clinical Pathology	2002
EVPC	European Veterinary Parasitology College	2003
ECBHM	European College of Bovine Health Management	2003
ECPHM	European College of Porcine Health Management	2004

In some European countries, veterinary specialization is organized at a national level with formalization of a specialized residency curriculum ; this is the situation for France country in which **Diplômes d'Etudes Spécialisées Vétérinaires (DESV)** are developed for **13 disciplinary fields** and conduct to delivery of a specialty diploma and recognition of the professional national title of "specialist"

Table II

Veterinary disciplines for which specific specialist residency programs are officially organized in France:

Anatomie pathologique vétérinaire
Chirurgie des animaux de compagnie
Dermatologie vétérinaire
Elevage et pathologie des équidés
Gestion de la santé et de la qualité en productions avicoles et cunicoles
Gestion de la santé et de la qualité en production laitière
Gestion de la santé et de la qualité en production porcine
Hygiène et technologies alimentaires
Médecine interne des animaux de compagnie
Ophtalmologie vétérinaire
Sciences de l'animal de laboratoire
Santé publique vétérinaire
Santé et productions animales en régions chaudes

In France, only titular of an official DESV diploma are authorized to use the qualification of "specialist" in…

The program attached to a DESV is strictly codified – requirements are :

- **three "full time" years of studies** in the corresponding Veterinary Faculty clinical service or laboratory of one of the 4 French veterinary Schools,
- attendance to a teaching program comprising at least, **annually, 300 hours of "presential" teachings** (teachings where students are really physically present) and practicals, program regularly actualized by the pedagogic committee of the formation,
- yearly exams with required minimal mark of **50 % for theoretical subjects and 60% for practical or clinical ones,**
- organization, on three years program, of **9 months of extramural practical sessions in professional agreed structures,**
- performance of a **personal research program** (preferably applied than fundamental) with personal presentation in front of a jury named by the organizing committee with presence of professional non academic members…

The DESV formation is organized by an Organizing Committee (COF) generally comprising six teachers (the status of which in France being at the same time teacher and searcher) and six qualified professional members representing various fields of the specialty practice.

For Veterinary pathology, this type of formation was open in 1987 first diplomas being delivered in 1990; all titular of the diploma are presently 84, ten of them coming of non European countries (Mexico, Burkina-Faso, Mauritania, Senegal, Ethiopia, Brasil, Japan…).

From another hand, since opening of ECVP board examination, the curriculum developed for the French DESV has proved to be a good preliminary formation only being to be complemented by language adaptation as English has been retained by ECVP as official language for the examination; ECVP boarded veterinary pathologists coming from the French preparation actually represent more than one half of all ECVP diplomates by way of the board examination.

Specialized teaching belongs in France to **veterinary professional third cycle** teaching system which is apart the other way of third cycle defined by LMD Bologna system.

Time and energy consuming for teachers, it meanwhile represents an emerging part of their teaching practice: as the second cycle teaching is obligatory generalist and sometimes has routine

character, DESV teaching requires from teacher to maintain high level of competence in one or several domains of his discipline.

Development of a specialized program along three years cycles also frequently requires federative collaboration of several veterinary faculties in order to have benefit of special competences and complementary knowledge. It also needs participation of foreign teachers required for their special competence in a precise field of the discipline. Such organization needs strong motivation and availability from professors.

When a collective system is not established, the preparation passes through an individual residency system and has to be surveyed by a certified senior member of the corresponding College. This individual way also gives good results but does not provide the veterinary teaching establishments which such a regular flow of residents.

The veterinary specializing system now being definitely installed in USA and in Europe, this level of veterinary teaching will soon preoccupy newly entered European countries: they must prepare their own teaching members to this new system in order to assume residents formation possibilities. Escaping this valorization of veterinary teaching would be, at term, strongly negative for the evaluation of the teaching veterinary system and its recognition.

Furthermore, we have to be conscious that specialized veterinary teaching is in progress in other continents countries where veterinary teaching is strongly structured with an open eventuality of occupation of interesting professional positions by specialized practitioners coming from foreign countries as India or China for example, this being possible *in situ* as well as outside for some disciplines non strictly clinical as veterinary pathology, veterinary clinical pathology, veterinary imagery… possible to delocalize.

Warrant of an excellence level in veterinary practice, the specialized teaching system is yet an important goal for the veterinary profession which will represent in a close future a strategic global challenge for the most interesting veterinary activities. Having developed such system, European countries must comfort their advance and help newly entered ones to reach the excellence level in veterinary formation.

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Respiratory distress syndrome in full-term newborn calves

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Hundreds of mature calves showing tachypnoea in the hours following birth are observed each year in Belgium, mainly in the Belgian Blue (BB) double-muscled breed, but also in France in other breeds of cattle. A total of 53 RDS calves ranging from 6 hours to 6 days old (32 males and 21 females, 51 BB and 2 Aquitaine Blond, 49.5 kg on average) was physically examined on the field and compared to 42 healthy BB 48-hour-old calves (17 males and 25 females, 48.0 kg on average). After an asymptomatic period variably lasting between ½ to 40 hours, these double-muscled calves suddenly showed spectacular tachypnoea and tachycardia (mean of 105 breaths and 151 beats.min^{-1}), quite often after the first colostrum intake. Pulmonary necropsy findings on all these RDS calves were a combination of atelectasis, emphysema, interstitial oedema, congestion and red hepatization. The extended atelectasis lesions systematically found in dead RDS calves (i.e. depressed ones) led to hypothesize a primary or secondary surfactant deficiency resulting in major pulmonary functional disturbances. Results of the microbiological analyses performed on 48 dead RDS calves revealed a septicaemia in 15 (31%) of them, 13 due to Escherichia coli, 1 due to Salmonella typhimurium and the last due to a Streptococcus, group B of Lancefield. Nineteen out of 24 intestinal content samples contained non-haemolytic E. coli while 4 of them were positive for Clostridium perfringens (> 10^8 CFU/ml intestinal content). Concerning viruses, one calf was positive for IBR..

RDS of full-term newborn calves is a very fine multifactorial disease that could certainly be used as an experimental model for mature babies suffering from RDS.

Key Words: Hyaline membrane disease, surfactant, etiopathogenesis, endotoxaemia, iodine and selenium deficiency

INTRODUCTION

The respiratory distress syndrome (RDS) is well-known in premature infants and other newborn mammals. Prematurity has also been associated with RDS in calves but, in the veterinary literature, few RDS descriptions concern calves born at term. An incidence study performed in 50 randomly selected BB farmings revealed that nearly two thirds of them had to face RDS in some of their newborn calves and that 36% of them had to deplore one or more losses each year due to this clinical entity[1]. First, a field study was designed to get more insight into this RDS by means of clinical, laboratory and necropsy observations on these newborn calves. After that, several pathogenic hypotheses were tested with the aim of effectively treating and especially preventing the disease.

ETIOPATHOGENESIS

The extended atelectasis lesions systematically found in dead RDS calves (i.e. depressed ones) led us to hypothesize a primary or secondary surfactant deficiency resulting in major pulmonary functional disturbances. Indeed, the physiological roles of surfactant are of major importance in pulmonary mechanics and include a decrease in surface tension at the air-liquid interface during lung deflation, preventing alveolar collapse and stabilising the small alveoli. It also minimizes the work required for respiration, offers a barrier to fluid transudation and is involved in the innate non-specific defence mechanisms of the lungs. RDS due to a primary quantitative surfactant deficiency has been well described in premature Holstein Friesian calves[2] but the problem here described is clearly not associated with prematurity.

However, primary qualitative surfactant deterioration could not be excluded. It is the reason why we analysed for composition and surface activity the pulmonary surfactant isolated from bronchoalveolar lavage fluids recovered from 14 BB newborn calves that died from RDS and from 7 healthy controls[3]. The surface tension properties of the samples of crude surfactant were assessed by means of a pulsating bubble surfactometer. Pulmonary surfactant consists mainly of a mixture of about 90% of lipids (mostly phospholipids) and 10% of proteins by weight. The presence of phosphatidylcholine and especially the disaturated form, dipalmitoylphosphatidylcholine, is believed to be of major importance in the surface tension lowering properties of surfactant. Four surfactant-associated proteins (SP) have also been identified. Two small hydrophobic surfactant proteins, SP-B and SP-C, are thought to be essential for the rapid adsorption of the phospholipids to the interface[4], while two large hydrophilic surfactant proteins, SP-A and SP-D, have been implicated in defence mechanisms of the lungs and in the secretion and recycling of surfactant[5,6]. In our study, major biochemical modifications associated with altered static and dynamic surface tension properties were demonstrated in pulmonary surfactant isolated from the RDS calves[3]. In particular, alterations in the ratio of total proteins to phospholipids, the phospholipid profile, SP-A levels and especially extremely low or undetectable SP-C levels were found in these samples. This last abnormality associated with protein alveolar flooding and secondary surfactant inhibition was thought to be responsible for the death of these animals. However, the cause of the very low SP-C content is still not identified (mutation/deletion in the gene encoding pro-SP-C or inhibition of one or several steps in the intracellular enzymic processing of pro-SP-C to the mature SP-C peptide ?) and, on the other hand, surfactant from undepressed as well as depressed RDS calves that survived was not investigated.

Beside this low SP-C content, we had to consider other possible causes leading to secondary surfactant deficiency with functional defect in the hours following birth, i.e. asphyxia, acidosis, hypercapnia, septicaemia, endotoxaemia and all kinds of shock. It has been demonstrated that the C-section performed without prior traction on the calf, as soon as the dam gets prepared, considerably reduces neonatal metabolic acidosis and anoxia associated with calving, and does not impair the calf respiratory adaptation during the first 48 hours of life[7,8]. These conclusions led us to exclude hypoxaemia, hypercapnia, acidosis, the non-resorption of fetal lung fluids and all kinds of shock as an aetiology of secondary surfactant deficiency in the RDS calves.

However, the hypothesis that the affected newborn calves suffer a septicaemia-endotoxaemia from digestive origin with various pulmonary repercussions had to be considered at the light of the necropsy findings. It was tested by measuring the endotoxin level in the blood of 14 RDS (10 depressed and 4 undepressed) and 9 healthy (4 BB and 5 Holstein Friesian) calves by the Limulus Amebocyte Lysate test. Results clearly demonstrated a higher endotoxaemia in the depressed RDS calves (0.14 Endotoxin Unit (EU)/ml) than in undepressed RDS (undetectable level) and control (0.01 EU/ml) calves. The fact that no endotoxin was detected in the undepressed RDS calves pleads for endotoxaemia being a consequence rather than the cause of the RDS. In any case, when endotoxaemia is present, the calf enters in a vicious circle, given that endotoxin suppresses

surfactant synthesis by type II alveolar epithelial cells[9] but also that the cellular inflammatory response to endotoxin includes the increase of the pulmonary arterial pressure and permeability of the pulmonary capillaries with the following disastrous consequences : oedema, crossing of proteins in the alveoli, hyaline membranes formation and alteration of the alveolar surfactant system.

Lastly, seeing the lung immaturity of these full-term RDS calves, it was still legitimate to consider subclinical trace elements deficiencies in their mothers, especially iodine (I). Indeed, the thyroid hormones T3 and T4 are known to play an important role in the maturation of the surfactant system. In fact, type II alveolar epithelial cells have receptors to these thyroid hormones[10] and the cellular response to stimulation by the fibroblast-pneumocyte factor, necessary for the production of surfactant of good quality, is considerably increased by T3[11]. The effects of this I deficiency are exacerbated by a lack of selenium (Se) since the deiodinase responsible for the transformation of T4 in T3 is a selenoenzyme and since T3 is ten times more active than T4[12].

More prosaically, a recent study performed in 29 beef herds distributed in the southern part of Belgium clearly revealed the importance of deficiencies in copper (Cu), zinc (Zn) and Se[13]. In a preliminary study, we have then selected 10 additional BB farms where many RDS cases occurred (morbidity and mortality rates due to RDS = 5-33% and 6-80%, respectively) and investigated their status in Cu, Zn, Se and I in comparison with those in 7 control BB farms with no RDS cases. Plasmatic Cu and Zn concentrations and glutathion peroxidase activity in the red blood cells were measured in 99 and 70 healthy cows from the RDS and control farms, respectively. I status was evaluated by determining I content of cow's milk. An I deficiency was diagnosed when the milk I content was < 80 □ g/L. All RDS farms were deficient in Cu, Zn and Se while 8 RDSfarms were proved to be deficient in I. In the 2 other RDS farms, lactating cows were not deficient in I but were supplemented by a mineral complex containing I that was not distributed during pregnancy. Consequently, I deficiency could not be excluded in pregnant cows in these 2 farms. Concerning control farms, 5 of them were deficient in Cu and Zn while only 2 were deficient in I and Se. Moreover, and this fact is stronger than a Lord Mayor, no other calf has presented RDS in the 10 selected farms since the adequate trace elements supplementation of the cows during pregnancy. We deduce that the trace elements deficiencies in general and the ones in I and Se in particular might take on a dominating responsibility in the RDS pathogenesis in newborn calves.

In summary, whatever the mechanisms leading to the initial surfactant inhibition, that probably are in close connection (low SP-C level, septicaemia-endotoxaemia, I and Se deficiencies), the resulting atelectasis decreases lung compliance, increases the work of breathing and leads to a rapid and shallow respiratory pattern. The extent of the pulmonary shunt is function of the importance of the atelectatic lung area and the resulting hypoxaemia brings the newborn calf into a critical vicious circle : pulmonary hypoxic vasoconstriction and pulmonary hypertension with alveolar flooding of oedema fluid and secondary surfactant inhibition, maintenance of the foetal circulation, bad oxygenation of the tissues and metabolic acidosis. As a consequence, type II pneumocytes are injured and are not able to synthesize surfactant in adequate amount anymore. Through the description of the physiopathological mechanisms leading to respiratory failure, we recognize the clinical and necropsy symptoms described for the RDS calves.

CLINICAL FINDINGS

A total of 53 RDS calves ranging from 6 hours to 6 days old (32 males and 21 females, 51 BB and 2 Aquitaine Blond, 49.5 kg on average) was physically examined on the field and compared to 42 healthy BB 48-hour-old calves (17 males and 25 females, 48.0 kg on average). All calves were born at term, as confirmed by their high birth weight but also by the date of mating, insemination or

transplantation of the dams that delivered exclusively by caesarean section (C-section) mostly performed after the spontaneous beginning of the parturition process, with the exception of the 2 Aquitaine Blond calves that were born by vaginal delivery.

After an asymptomatic period variably lasting between ½ to 40 hours, these double-muscled calves suddenly showed spectacular tachypnoea and tachycardia (mean of 105 breaths and 151 beats.min^{-1}), quite often after the first colostrum intake. These values are to be compared to the 63 breaths and 120 beats.min^{-1} obtained on average in control calves, that is to say also rather high values that could however be explained by the proportionally reduced weight of their heart (- 15%) and lungs (- 19%) in comparison with Holstein Friesian calves. One of the most important anamnestic elements was the precise age of the RDS calves at beginning of symptomatology. Indeed, this data combined with information on their mental status and appetite led us to distinguish two subgroups of calves: the depressed (n = 30) and undepressed (n = 23) RDS calves. In depressed RDS calves, the general clinical assessment was rapidly affected and their appetite was decreased or even nil. They balked at standing up, developed dyspnoea in addition to tachypnoea, and sometimes presented cyanotic mucous membranes, then possibly associated with turgescent jugular veins in dying calves. Now, mortality has only been recorded in this depressed subgroup of calves (n = 11/30). Respiratory rate (RR) tended to be slightly lower and heart rate (HR) higher in depressed (mean RR = 101 and HR = 158) than in undepressed RDS calves (mean RR = 110 and HR = 144). Hyperthermia (> 39.5°C) was sometimes recorded in undepressed as well in depressed RDS calves but was far from being the general rule. RDS calves showed no abnormal nasal discharge, cough or stridor. Attentive auscultation of their lungs only revealed increased breath sounds and rarely crackles and wheezes.

Symptoms appeared significantly earlier in depressed (after 2 hours of life on average) than in undepressed (after 20 hours of life on average) RDS calves, thus giving a non negligible prognostic value to the age at symptoms appearance. Unless the calf previously died, the symptomatology lasted for about 5-7 days before dramatically solving without sequelae. Treatment with antibiotics and anti-inflammatory drugs, either steroidal or nonsteroidal, associated with very good nursing practices, did not seem to influence to a great extent the course of the disease. On the contrary, treatment of RDS calves with laevothyroxine *per os* (Forthyron®, Eurovet) gives promising results.

CLINICAL PATHOLOGY

Physical examination was supported by arterial and venous blood sampling in order to measure blood gases and pH, glycaemia and lactacidaemia on the field, using portable analysers. Haematological analysis, total protein electrophoresis, ions (Na, K) and enzymes (AST, LDH, CPK) measurements were committed to the laboratory care.

In comparison with the control group (PaO_2 = 78.1 mm Hg, $PaCO_2$ = 46.1 mm Hg, pHa = 7.38 and lactacidaemia = 2.4 mmol/L, on average), depressed RDS calves were in arterial mixed (respiratory and metabolic) acidosis, severe hypoxaemia and hypercapnia (PaO_2 = 51.1 mm Hg, $PaCO_2$ = 56.3 mm Hg, pHa = 7.31 and lactacidaemia = 4.6 mmol/L, on average) that correlated well with the observed symptomatology, the most hypoxaemic calves also being those showing cyanotic mucous membranes. Undepressed RDS calves were normocapnic ($PaCO_2$ = 45.2 mm Hg) and their degree of metabolic acidosis (lactacidaemia = 3.8 mmol/L, on average) was not sufficient to significantly influence their blood pH value (pHa = 7.36), even if their mean arterial oxygen partial pressure tended to be lower (PaO_2 = 70.2 mm Hg) than in control calves.

Despite great variability, a significantly lower glycaemia value was measured in the depressed RDS calves (70 mg/dL) in comparison with the control group (93 mg/dL). Some of these depressed RDS calves were even hypoglycaemic (< 54 mg/dL).

Compared to the values of the control group, the ions, haematocrit and red blood cells levels were unremarkable in the 2 RDS groups. On the other hand, a leukocytosis was demonstrated

only in the depressed RDS group (14,200 WBC/ l). However, considering all RDS calves individually, nearly half the calves presented a leukocytosis and a leukocytes count compatible with an infection, even if the intervention of the stress induced by the disease or by the use of therapeutical glucocorticoids cannot totally be excluded.

On the average, RDS calves had a significantly lower total serum protein content (53.0 and 55.2 g/L for depressed and undepressed RDS calves, respectively) than healthy control calves (59.1 g/L). If the value of 10 g -globulins/L is taken as the threshold value for an adequate passive transfer of colostral immunoglobulins, 60% of the depressed and 40% of the undepressed RDS calves showed a relative failure at this point of view.

Considering that no noteworthy increase of the muscular enzymes was registered in all but one RDS calves, it was concluded that it was not a primary myopathic problem with associated dyspnoea.

NECROPSY FINDINGS

Every time a calf died, a complete necropsy was performed in the shortest delay as possible, associated with bacteriological, virological and histopathological analysis. Blood from the heart, lung aspirate, carpial synovial fluid, mesenteric lymph node aspirate and small intestine content were aseptically sampled for bacteriological culture. Viruses (RSV, IBR, PI_3, adenovirus and torovirus) were investigated by immunofluorescence in lung pieces as well as the BVD virus in the spleen. Several lung pieces were used for histopathology. The reports of 67 necropsies performed on BB calves dead after a clinical course of RDS were also retrospectively analysed.

Pulmonary necropsy findings on all these RDS calves were a combination of atelectasis, emphysema, interstitial oedema, congestion and red hepatization. Atelectasis involved always the apical and cardiac lung lobes and, in the most severe cases, more than half of the diaphragmatic lobes. Emphysema could be present in 2 different forms: either a multitude of small interstitial bubbles of $\pm$ 1 mm diameter localized on the entire lung, and/or greater bubbles 2-3 cm diameter localized preferentially on the back of the diaphragmatic lobes and under the pleura. Lesions found by optic microscopy on some of these collapsed lungs were various combinations of congestion, haemorrhagic intra-alveolar oedema, hyaline membranes and the infiltration of the interstitium and the airspaces mainly by mononucleated cells but also by polymorphonuclear neutrophils.

Interestingly, even though they did not present diarrhoea in their life-time, 42% of the necropsied calves also presented an acute enteritis, sometimes haemorrhagic, with associated mesenteric adenitis.

Results of the microbiological analyses performed on 48 dead bodies of RDS calves revealed a septicaemia in 15 (31%) of them, 13 due to *Escherichia coli*, 1 due to *Salmonella typhimurium* and the last due to a *Streptococcus* of the group B of Lancefield. Nineteen out of 24 intestinal content samples contained non-haemolytic *E. coli* while 4 of them were positive for *Clostridium perfringens* (> 10^8 CFU/ml intestinal content). When possible (only 2 opportunities), the *E. coli* strain found in the intestinal content was compared with the one isolated in the heart blood. In each of these 2 calves, the strains could not be distinguished from each other by typage or comparison of their antibiograms. All these results are also to be interpreted taking into account the high amounts of antibiotics previously administered as treatment, mainly by the intravenous route, to these valuable animals.

Concerning viruses, the lung of only one calf was positive for IBR by immunofluorescence.

CONCLUSION

In conclusion, the RDS of full-term newborn calves is a very fine multifactorial disease that could certainly be used as an experimental model for mature babies suffering from RDS. Indeed, in human medicine, RDS is the leading cause of neonatal death throughout the industrialized world, especially in countries that are known to be I and Se deficient[14]. Human beings will rarely have been so closely related to cattle.

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Emergences, zoonoses et franchissement de la barriere d’espece

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Différents exemples d’émergence de maladies virales seront analysés, illustrant différents mécanismes : l’apparition d’une nouvelle espèce virale, les contaminations iatrogènes ou alimentaires, les risques liés au transport, la modification des écosystèmes. Ces exemples illustrent l’imprévisibilité des émergences virales. Ils posent la question de l’évaluation des risques pour l’homme liés à certains virus animaux. Le laboratoire travaille sur des virus dont la transmission interspécifique est possible mais dont la réalité ou la fréquence sont méconnues, ainsi que les mécanismes sous-jacents. Les travaux conduits au laboratoire pour l’Hépatite E, l’encéphalomyocardite, le virus Borna et les coronavirus des carnivores seront présentés.

Key Words: Emergences, zoonoses, franchissement de la barriere d’espece

Lentiviral vectors for eliciting humoral responses against West Nile virus infection

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Studies based on applications of lentiviral vector in the field of vaccinology have focused on their abilities to elicit a cellular immune response and little is known on their capacities to stimulate a specific B cell response. To address this question, we evaluated the potential of a lentiviral vector-based vaccine to elicit a protective humoral immunity against West Nile Virus (WNV). WNV is maintained in a natural cycle between mosquitoes and birds but also infects humans, horses and other animals. This flavivirus is endemic in parts of Africa, Europe, Middle East and Asia and it caused recently the largest recognized epidemic of neuroinvasive human disease in North America. Currently, no specific therapy or vaccine is approved for human use. To investigate the ability of lentiviral vector to initiate a protective B cell response, we produced lentiviral vector coding for the secreted form of the envelope glycoprotein of WNV (TRIP-sEwnv) which possesses neutralizing epitopes. Remarkably, we demonstrated that a single injection with TRIP-sEwnv induced a strong antibody response and elicited a long-lasting, protective and sterilizing humoral immunity against a lethal challenge of WNV (Iglesias et al., 2006). Moreover, immunizations with TRIP-sEwnv conferred also a protection against a high viral challenge as early as one week after vaccination. We showed recently that the protective dose 3 weeks post-immunization is at least 10 times lower (unpublished data). This entirely fulfills the requirements for an emergency veterinary vaccination campaign in case of a West Nile outbreak.

Taken together, these results broaden the applicability of lentiviral vector as efficient non-replicating vaccines against pathogens for which a neutralizing humoral response is one active arm of the protective immunity.

Applications of lentiviral vector technology to veterinary vaccination purposes will be discussed.

Key Words: West Nile Virus, lentiviral vector technology, vaccination

Mitochondrial DNA analysis of Salamandra infraimmaculata larvae from habitats in northern Israel

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*The molecular DNA variation in Salamandra infraimmaculata larvae from populations represented in eight breeding sites in Israel was studied. Samples from larvae were analyzed by sequence analysis of the mitochondrial cytochrome b fragment and D-loop regions. The highest variation was discovered to be between salamanders from winter ponds and from perennial water sources. The cytochrome b fragment (determined from a 361 base pair [bp] clone) differed at one point, position 77, in which salamanders from one site were polymorphic for the nucleotides A and G. The control region (D-loop) sequence was determined from an 807 bp clone and differed at three nucleotide positions: 24, 459 and 520. With respect to positions 24 and 459, salamanders from two breeding sites had a single nucleotide C (group A), while those from three other breeding spots had T (group B), and at the nucleotide position, 520, group A had T and group B had C. At the sixth breeding site, all nucleotide positions were polymorphic, with C and T. The molecular mitochondrial DNA (mtDNA) of the partial cytochrome b gene in the larvae from Israel differed from that of European salamanders. The evolutionary distance between S. salamandra from Europe and Israel was greater than among different populations within Israel, while genetic differences between various habitats in Israel were relatively low. The control region (D-loop) sequence of the Israeli S. infraimmaculata differs by 6.5-7% to that of Salamandra corsica. The lowest level of genetic differentiation (0%) was found in the control region of populations from springs and streams (Humema Spring, Tel Dan Stream and Navuraya Spring**),** all of which are located in the northern part of the area studied. The cytochrome b gene of the S. infraimmaculata population in Israel varied from that of the salamanders in Europe and North Africa, S. lansai, S. algira and S. salamandra, with genetic differentiation levels of approximately 14%, 17% and 7%, respectively. The lowest amount of genetic differentiation (0%) with regard to the cytochrome b gene was found between salamanders in springs and streams in the northern part of the area studied.*

Key Words: Mitochondrial DNA, Salamandra

INTRODUCTION

The "true" salamander (Salamandridae) is distributed predominantly across Europe, North Africa, Anatolia and the Middle East, providing a group suitable for the investigation of the historical biogeography of these two regions (WEISROCK et al., 2001, 2006). Two main hypotheses have been proposed for the phylogenetic relationships among genera and species of "true" salamanders. The traditional monophyletic hypothesis (O¨ ZETI, 1967; WAKE & O¨ ZETI, 1969) suggests that the Anatolian populations have a single historical biogeographical origin followed by a north–south vicariant event across the Anatolian Plateau. Recent molecular phylogenetic studies suggest an alternative phylogenetic hypothesis, which places *Chioglossa lusitanica* as the sister taxon to *M. caucasica* and the genus *Salamandra,* as the sister taxon to *M. luschani* (TITUS & LARSON, 1995; VEITH et al*.,* 1998).

Salamanders of the genus *Salamandra* are present throughout Europe, North Africa and East Asia, surviving in various habitats and climates (reviewed by DEGANI, 1996). Based on the morphological data, the species *S. salamandra* is subdivided into 16 subspecies that can be found throughout Europe, the Middle East and North Africa (EISELT, 1958; FACHBACH, 1976; DEGANI, 1986; KLEWEN 1991).

The *Salamandra* subspecies, which can be differentiated by plasma protein electrophoresis data (GASSER 1978; DEGANI 1986; JOGER & STEINFARTZ 1994, 1995) and allozyme data (VEITH 1994), do not show dramatic variations from one another. MIZUNO & MACGREGOR (1974) and MIZUNO et al. (1976) used chromosomes and DNA sequences to study the evolution of salamanders belonging to the genus *Plethodonital* (MIZUNO et al., 1976). Based on the sequence analysis of the mitochondrial D-loop region and geological dates, STEINFARTZ et al. (2000) suggested that six major monophyletic groups exist in Europe (*S. salamandra, S. infraimmaculata*, *S. corsica, S. atra* and *S. lanzai*) and that the salamanders in Israel belong to *S. infraimmaculata*. In Africa *S. algira* (ESCORIZA et.al., 2006).

The *S. infraimmaculata* populations in northern Israel are located near breeding spots in isolated populations. There are physiological (DEGANI 1981a,b), morphological and biological differences among salamanders of the isolated populations, which are effected by habitat conditions (DEGANI & WARBURG 1978; 1996). There are various types of breeding places situated in three (DEGANI, 1986; 1996) different regions in Israel. The relatively drier area of Mount Carmel and the central Galilee has predictable (perennial springs and streams) and unpredictable (seasonal water) breeding places (WARBURG, 1992, 1997; DEGANI & KAPLAN, 1999). There are many types of breeding places in xeric areas, including streams, springs, rock pools, rain pools and large ponds, where water is available during different periods and according to different conditions. No variations in the dorsal pattern, serum protein (DEGANI, 1986) or ten enzyme systems with 14 loci (VEITH et al*.*, 1992) were found among the populations of these areas.

Salamanders from semi-arid habitats are significantly larger than those from moist habitats (DEGANI et al., 1999). The molecular DNA variation in salamanders from these two habitats has been studied using the randomly amplified polymorphic DNA polymerase chain reaction (RAPD PCR). The genetic variation differed between the two habitats, but not between the two populations from the semi-arid habitat. Band sharing between salamanders from the semi-arid habitats was 94%, whereas between these and salamanders from the moist habitat band sharing was only 85-86% (DEGANI et al., 1999).

In the present study, we examine the genetic variation, with respect to the mitochondrial region cytochrome *b* (CACCONE et al. 1997) and the control region (D-loop) of salamanders from eight populations derived from streams, springs and rain ponds in northern Israel.

MATERIALS AND METHODS

Samples

Population tissue samples were obtained from *S. infraimmaculata* individuals from eight locations in the Galilee in the northern part of Israel (Fig. 1). The temporary water bodies (the water available during winter between the months, March and July included Manof Pond - a winter pond in Gush Segev (elevation 340 m), Dovev Pond (740 m), Matityahu (682 m) and the Maalot pit (596m)- a two-meter deep hole on a hill. The permanent water bodies, which are those available all year round, comprised Al Balad - a year-round spring on Mount Carmel, Navuraya Spring (663 m) and Humema Spring (900m) - a layer spring in a cave located on Mount Meiron.The eighth sampling location was the Tel Dan Stream, with year-round water at 16ºC.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from the clipped or whole tail of larvae, with the QIAamp DNA Mini Kit that consisted of proteinase K lysis of the tissue and specific DNA binding to the QIAamp silica-gel membrane through which contaminants passed. The primer combination L-Pro-ML (5'-GGCACCCAAGG-CCAAAATTCT 3') and H-12S1-ML (5'-CAAGGCCAGGACCAAACCTTTA 3') was used for amplifying and sequencing the D-loop of the control region. These primers were based on control region sequences from *Mertensiella luschani atifi* (STEINFARTZ et al., 2000). The primer combination of L14841 (5'- AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA 3') and the modified primer, cyt *b* B2 (5'-CCCTCAGAATGATATYTGTCCTCA 3', H15149) were employed for the amplification and sequencing of cytochrome *b* (cDNA; KOCHER et al., 1989).

PCR amplification was performed in a 50 µl solution containing 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM of each dNTP, 0.5 µM of each primer, 10-500 ng gemonic DNA and 2.5 units of Taq DNA polymerase (Promega, USA). PCR was performed in a PTC-150 MiniCycler (MJ Research, USA) with the following parameters: 3 min denaturation at 94°C, followed by 32 cycles of 1 min at 94°C, annealing for 1 min at 52°C and elongation at 72°C for 1 min. An additional 10 min elongation period at 72°C followed the last cycle. After amplification, the PCR products were separated by electrophoresis on a 1.3% agarose gel and stained with ethidium bromide. The DNA bands were excised from the gel and the DNA was extracted by the Jet Quick Gel Extracting Spin Kit (Genomed, Germany) and suspended in distilled, deionized water. Purified DNA was sequenced directly with an ABI PRISM 3100 Genetic Analyzer (PE Biosystems, USA). Both cytochrome *b* and control region fragments were sequenced in both directions.

Tree Construction

Multiple sequences of the cytochrome *b* and the D-loop were aligned. Trees were constructed by the MegAlign program of the DNASTAR software package, which employs the neighbor-joining algorithm of SAITOU & NEI (1987).

RESULTS

DNA fragments, encoding portions of the cytochrome *b* gene and the D-loop control region, were amplified as described above. The nucleotide sequences of the DNA fragments were determined from a 361 bp clone of cytochrome *b* and an 807 bp clone of the control region (Fig. 2; Table 1). The cytochrome *b* fragment varied at one nucleotide site, 77, in which the Dovev, Maalot, Tel Dan and Humema population had A, while all other populations had G (Fig. 2). Three nucleotide sites in the control region fragment, 24, 59 and 520, varied among breeding locations. The Al Balad, Maalot and Manof populations had C, C and T, respectively, the Navuraya, Dovev, Humema and Tel Dan populations had T, T and C, respectively, while the Matityahu population was polymorphic with C/T in all positions (Fig. 2; Table 2).

Based on the tree representing the degree of sequence similarity (constructed by the MegAlign program [DNASTAR]), all of the *S. infraimmaculata* populations in Israel varied greatly from the salamanders in Europe. The control region (D-loop) sequence of the Israeli *S. infraimmaculata* differed from *S.corsica* by 6.5-7% (Fig. 3). The lowest level of genetic differentiation for the control region (0%) was found between individuals of the springs and streams (Humema Spring, Tel Dan Stream, and Navuraya Spring**),** which are located in the northern part of the area studied (Table 2).

A similar situation was revealed when comparing the cytochrome *b* gene variation of *S. infraimmaculata* populations. The genetic differentiation between the *S. infraimmaculata* cytochrome *b* gene of the European and North African salamanders (*S. lansai, S. algira and S. salamandra*) and that of the *S. infraimmaculata* population in Israel was approximately 14%, 17% and 7%, respectively (Fig. 4). Also, with regard to the cytochrome *b* gene, the lowest genetic difference (0%) was found among the populations of the springs and streams, Humema Spring, Tel Dan Stream and Navuraya Spring (Table 2)**.**

When both the cytochrome *b* and the control region sequences of the salamanders are taken into account, a variation between those of the springs and those of the ponds is detected (with the exception of Balad Spring).

DISCUSSION

The evolutionary genetic variation among *S. salamandra* larvae from different populations in Israel is smaller than the variation between *S. salamandra* from Europe and *S. salamandra* from Israel (DEGANI, 1996; STEINFARTZ et al., 2000; WEISROCK et al., 2006; ESCORIZA et al., 2006). Genetic variation in *S. salamandra* has been studied intensively (see review by STEINFARTZ et al. 2000), but earlier studies have focused on geographical and taxonomical aspects, examining differences between subspecies rather than between different populations of a single species, as in the present study. The parameters by which differences among *S. salamandra* populations are determined, include morphology (EISELT, 1958; THORN, 1968; DEGANI, 1986, 1994; VEITH, 1994; DEGANI & WARBURG, 1995), plasma proteins (DEGANI, 1986; JOGER & STEINFARTZ, 1994) and isozyme studies (VEITH et al., 1992; NICKLAS, 1992; ALCOBENDAS et al., 1994; NICKLAS et al., 1994), but these parameters have a relatively limited sensitivity in elucidating genetic variation among populations belonging to the same species or subspecies, in comparison to the RAPD PCR method (Degani *et al*., 1999).

According to RAPD-PCR, there are genetic differences among salamander populations from different habitats (DEGANI et al., 1999), although no variations have been revealed according to the dorsal pattern, serum protein (DEGANI, 1986) or ten enzyme systems with 14 loci (VEITH et al., 1992). In the present study, both mitochondrial genes showed a very low variation among populations, with high similarities among the larvae in springs and streams.

Plasma protein patterns and enzyme system analyses are limited to protein variation, so it is difficult to use them to determine genetic variations within populations or between populations of the same subspecies. However, it seems that at least some of the physiological (reviewed by DEGANI, 1994) and biological (reviewed by DEGANI, 1996) variability comes from adaptation to different habitats. The present study shows differences among *S. infraimmaculata* belonging to the same site. This result is in contrast to that of DEGANI (1986) and VEITH et al. (1992), who found that individual populations were completely monomorphic, but coincides with DEGANI et al. (1999). The difference results from the method employed, which in this case was based on genetic markers. The DNA variation, revealed in this study, indicates that there are differences between salamanders within a population. The geographical distances between the breeding places seem to have influenced genetic variation as well as ecological conditions in the habitats (DEGANI, 1996). Here genetic variation between eight populations was established according to

two sequences: that of the control region of the mitochondrial DNA; Fig. 3), and that of the gene, cytochrome *b* (Fig. 4). The variation between the cytochrome *b* sequences of the salamanders in Israel, as compared to sequences of those in Europe, was even greater.

There are many differences among salamanders from different areas, e.g., in body size (DEGANI, 1986), number of larvae born per parturition (DEGANI & WARBURG, 1995), survival in dry conditions (DEGANI, 1981a) and their reproductive cycle (SHARON et al., 1996; DEGANI et al., 1997). The eight habitats studied in this report are geographically close to each other, with minor differences in rainfall, photoperiod or other meteorological conditions. However, the semi-arid conditions in four of them, where the water source dries up during the summer, differ greatly from the moist conditions in the other four, where water is available year-round. Evidently, this is the difference, which gives rise to the biological, morphological, and physiological variations in these parameters, as mentioned above, as well as to the genetic variations, found for the first time in this study.

We have shown here molecular polymorphisms in mitochondrial DNA, which reflect a sharp ecological separation between temporary pools and permanent sources of water. These are presumably adaptive changes caused by natural selection. Future analysis at the nuclear DNA level of stress genes, such as heat shock proteins (HSP), among others, could reinforce the underlying molecular-genetic ecological difference, indicating that natural selection indeed operates at the genotypic level, as it does at the phenotypic level, adapting organisms to their local environmental stresses.

Table 1.

Nucleotide differences among populations.

	Nucleotide position		
Cytochrome *b* nucleotide differences	77		
Al Balad Spring	G		
Manof Pond	G		
Navuraya Spring	G		
Dovev Pond	A		
Matityahu Pond	G		
Tel Dan Stream	A		
Maalot Pit	A		
Humema Spring	A		
Control region nucleotide differences	24	459	520
Al Balad Spring	C	C	T
Manof Pond	C	C	T
Navuraya Spring	T	T	C
Dovev Pond	T	T	C
Matityahu Pond	C/T	C/T	C/T
Tel Dan Stream	T	T	C
Maalot Pit	C	C	T
Humema Spring	T	T	C

Table 2.

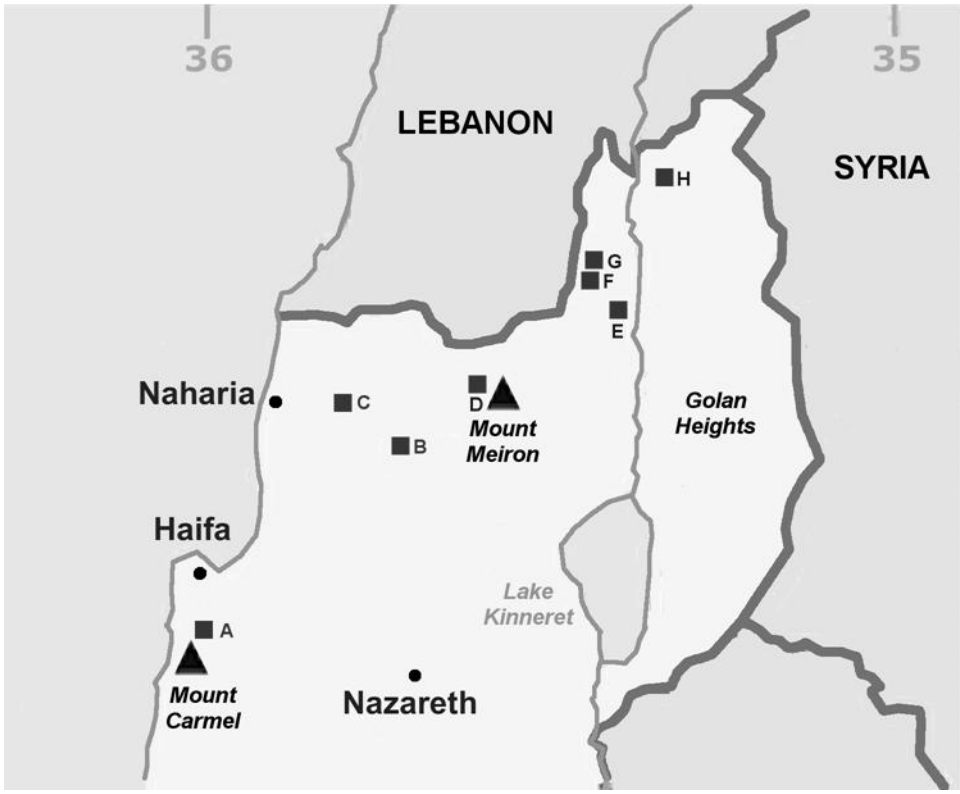
Percent identity of the control region and the cytochrome b fragments

	Balad	Dovev	Humema	Maalot	Manof	Matityahu	Navuraya
Tel Dan	99.6	100	100	99.6	99.6	99.6	100
Balad	*	99.6	99.6	100	100	99.6	99.6
Dovev	*	*	100	99.6	99.6	99.6	100
Humema	*	*	*	99.6	99.6	99.6	100
Maalot	*	*	*	*	100	99.6	99.6
Manof	*	*	*	*	*	99.6	99.6
Matityahu	*	*	*	*	*	*	99.6
Navuraya	*	*	*	*	*	*	*

Percent of identity of the control region

	Balad	Dovev	Humema	Maalot	Manof	Matityahu	Navuraya
Tel Dan	99.7	100	100	99.7	99.7	99.7	100
Balad	*	99.7	99.7	100	100	100	99.7
Dovev	*	*	100	99.7	99.7	99.7	100
Humema	*	*	*	99.7	99.7	99.7	100
Maalot	*	*	*	*	100	100	99.7
Manof	*	*	*	*	*	100	99.7
Matityahu	*	*	*	*	*	*	99.7
Navuraya	*	*	*	*	*	*	*

Percent of identity of cytochrome b



Name of Site	Latitude	Longitude	*Altitude* (m above sea level)	Region
Al Balad Spring (A)	157022	236119	446	Mount Carmel
Manof Pond (B)	172086	250470	340	Gush Segev
Maalot Pit (C)	176000	267450	596	Upper Galilee
Humema Spring (D)	187299	276819	900	Upper Galilee
Navuraya Spring (E)	197970	267307	663	Upper Galilee
Dovev Pond (F)	189086	271195	740	Upper Galilee
Matityahu Pond (G)	192784	274580	682	Upper Galilee
Tel-Dan Stream (H)	211100	294800	190	Hula Valley

Figure 1. Various ponds and springs in Northern Israel colonized with salamanders examined in the study.

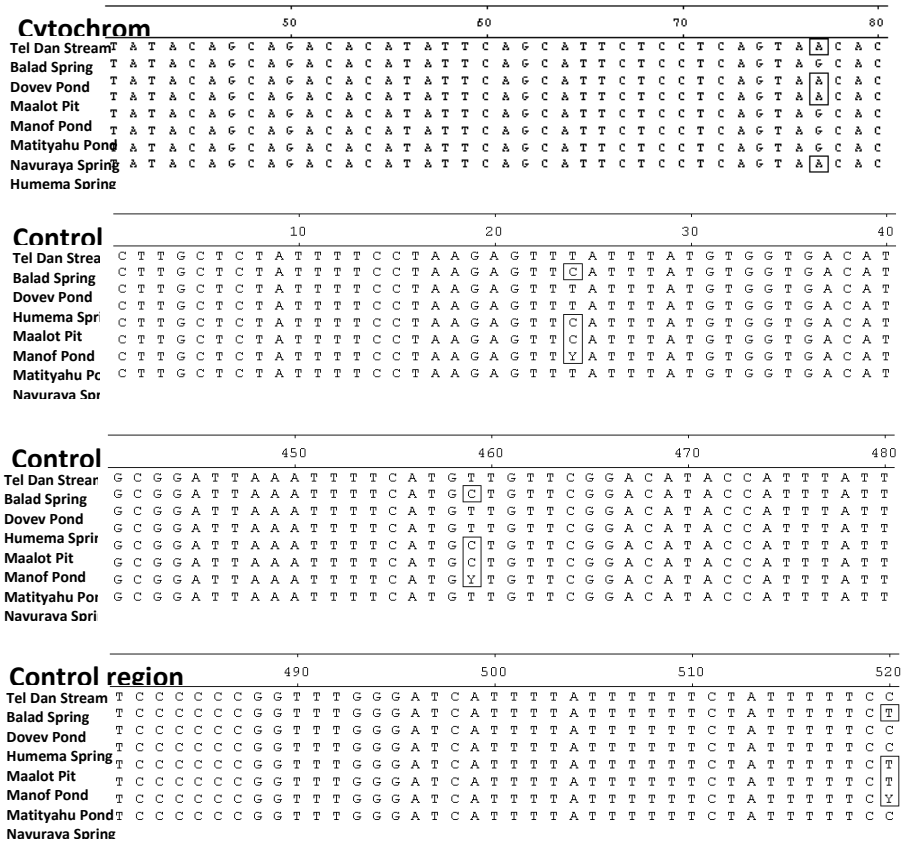


Figure 2. Alignment of the nucleotide sequences of the cytochrome *b* and control region fragment, of *S. infraimmaculata* larvae from eight breeding sites in northern Israel.

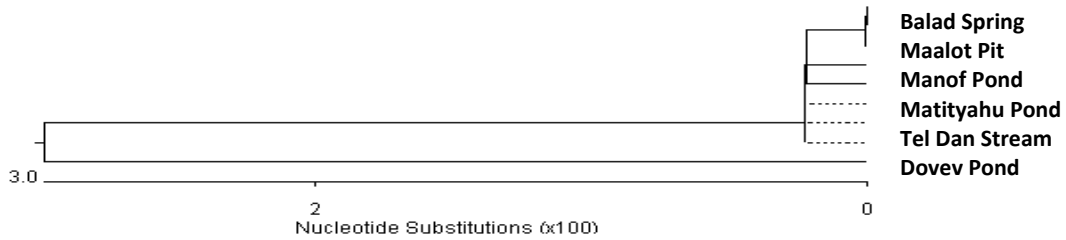


Figure.3 Unrooted phylogenetic tree of the control region (D – loop) fragment based on nucleotide sequences of the genus Salamandra. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. The phylogenetic tree was constructed using the MegAlign program (DNASTAR) by the CLUSTALW method. The branch length represents the evolutionary distance.

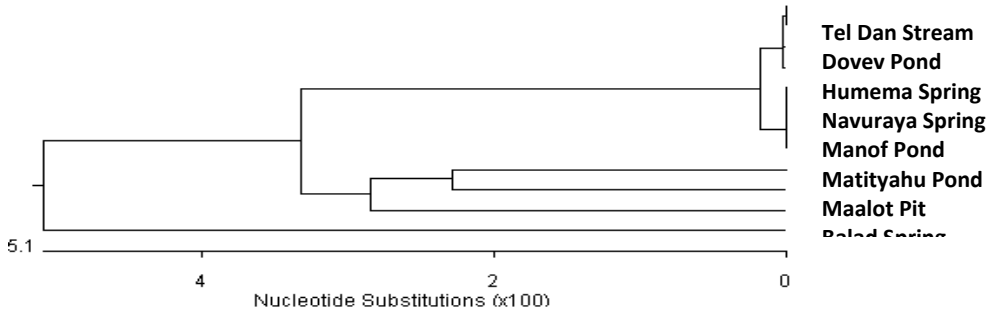


Figure .4 Unrooted phylogenetic tree of the partial cytochrome *b* fragment based on nucleotide sequences of the genus Salamandra. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. The phylogenetic tree was constructed using the MegAlign program (DNASTAR) by the CLUSTALW method. The branch length represents the evolutionary distance.

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Emergence de la fièvre catarrhale ovine (FCO ou bluetongue) dans le nord de la France en 2006

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La fièvre catarrhale ovine (FCO) appelée aussi « bluetongue » est une arbovirose transmise par un moucheron hématophage du genre Culicoides (genre Diptera, famille Ceratopogonidae). Elle se manifeste cliniquement surtout chez les moutons, rarement chez les bovins et les chèvres et se traduit dans la première espèce par une infection généralisée et grave.

Depuis sa réapparition en Europe en 1998, cinq sérotypes (1, 2, 9, 4 et 16) sur les 24 existants ont été recensés dans de nombreux pays du pourtour méditerranéen. En France, la Corse a été le lieu, depuis 2000, de 4 épizooties impliquant les sérotypes 2, 4 (2003) et 16 (2004).

Le 17 août 2006, les autorités vétérinaires hollandaises ont notifié des foyers de FCO dans le Sud Est du pays (commune de Kerkrade) dans une zone limitrophe de la Belgique et de l'Allemagne (Nordrhein Westphalie). Les zones de restriction prévues par la directive 2005/75/CE ont été mises en place, à savoir une zone d'observation d'un rayon de 20 km autour des foyers, puis une zone de protection de 100 km, complétée par une zone de surveillance de 150 km.

Pendant les mois d'août et de septembre 2006, ce virus qui a brusquement émergé dans cette région de l'Europe s'est répandu en Allemagne, en Belgique et en France.

Au 22 janvier 2007, le nombre de foyers était le suivant : 695 en Belgique, 914 en Allemagne, 459 aux Pays Bas, 8 au Luxembourg et 7 en France.

Les particularités de cet événement sont la zone géographique touchée, beaucoup plus au nord que l'aire considérée habituellement à risque (bassin méditerranéen), l'absence de mise en évidence de Culicoides imicola, considéré comme le vecteur principal, les espèces ayant exprimé des signes cliniques (les bovins ont été particulièrement touchés ce qui est très inhabituel), l'implication d'un sérotype (8) n'ayant jamais été isolé en Europe.

L'origine de l'infection n'est pas connue. Plusieurs hypothèses peuvent être émises (importation d'animaux, la contamination de vaccins comme ceci a été observé aux Etats-Unis il a quelques années, la contamination de semence bien que ce mode de transmission soit très rare).

Outre les données virologiques (notamment les données moléculaires) relatives au virus de sérotype 8 isolés en France, les résultats des enquêtes sérologiques et entomologiques menées dans le nord du pays depuis le mois de septembre seront présentés.

Key Words: bluetongue, emergency, France

Morphoclinical and histopathological aspects in a chicken encephalomalacy episode

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Chicken encephalomalacy seems to be the purest form of E hipovitaminose (1, 2, 3, 7). The disease appears in chicks (chicken and turkey) and in duck chicks about 1-8 weeks of age, with maximum incidence between 15 and 30 days (Scott, 1974 cit. de 7).

In the evolution of many natural episodes of encephalomalacy, besides lack of E vitamin in the forages, and the excess of the no saturated fatty acids can interfere gastrointestinal lesions and malasorbtion produced by eimeriosis (1, 3, 4, 6, 8).

These processes, irrespective to their origin and extension, they are affecting bird stability in station or by walking, inducing balance disorders which can lead to astasy and one desynchronisation between different group muscles, one disharmony between flexors and extensors, with the same consequences regarding balance (1, 3).

Key Words: encephalomalacy, chicken, histology

Materials and methods

The researches have been made in Avicola Giarmata, Timis county, were has been diagnosticated one encephalomalacy episode. In the farm, chicken suspected of encephalomalacy have been separated from the healthy ones, in a cage were they have been observed from the clinical point of view. The day of the appearance of the nervous symptoms, most important clinical manifestations and the day of the death and the one of the sacrifice of the chicken have been observed and noted.

After the sacrificing an exterior examination of the body regarding apparent mucous, the feathers and the down around the anus, was made. After the complete examination of each tissue or organ, we took samples from the encephalon, cerebellum, and the bulb.

For the histopathological examinations the fragments from the encephalon were fixed in formaldehyde 10%, included in paraffin, divided at 6 micrometeres and colored by (HEA) trichromic method , and Giemsa.

Results and discussions

First clinical signs have appeared at the age of 19 days. The disease was suspected on the account of the deaths and the nervous symptoms. Histopathological examination from the cerebellum have identified characteristical lesions. We put the enceplalomalacy diagnosis and we pursue the treatment with Nutril-SE from the LEK firm. The histopathological examinations were negative.

The lost from mortalities began to be higher from 19.08.2005 (70 chicken), and have raised since that moment daily until 163 chicken in 23.08.2005. Further, during a week, the mortality stays flat with about over 150 chicken lost daily.

The necropsy of the dead chicken showed discrete lesions in the encephalon and more obvious in the cerebellum as punctiform hemorrhages and even micro necrosis. Visceral lesions are less important, as anemia, and sometimes small hemorrhages.

Table 1

Shows the evolution of the lost from mortalities on each cicle of production.Evolution mortalities in farm

Nr.crt.	Data	Mortalities			Balance(g)	Obs.
		Morning	Evening	Absolute		
1.	1.08.2005	21	20	41		
2.		27	9	29		
3.		19	3	22		
4.		2	3	5		
5.		7	5	12		
6.		2	3	5		
7.		2	10	12		
8.		5	14	19		
9.		13	12	25		
10.	10.08.2005	12	16	28	228	
11.		9	15	24		
12.		15	14	29		
13.		13	10	23		
14.		14	12	26		
15.		8	13	21		
16.		10	12	22		
17.		11	12	23		
18.		16	13	29		
19.		54	16	70		
20.	20.08.2005	59	68	127		Diagnosis Treatment Nutril-SE
21.		24	74	98		
22.		53	104	157		
23.		62	101	163		
24.		48	86	134		
25.		59	97	156		
26.		52	79	131		
27.		27	85	112		
28.		39	65	104		
29.		42	70	112		
30.	30.08.2005	36	49	85	1.872	
31.		25	46	71		
32.	1.09.2005	18	27	45		
33.		13	28	41		
34.		17	16	33		
35.		12	18	30		
36.		16	21	37		
37.		11	17	28		
38.		15	16	31		
39.		5	14	19		
40.		6	14	20		
41.	10.09.2005	14	19	33		
42.		15	18	33		
43.		18	50	68		
44.		54	22	76	2266	Outlet

The suspected chicken (18 cases) of encephalomalacy have been separated from the health ones and isolated in a cage were they have been observed from the clinical, anathomopathological and histopathological point of view.

In the cited literature after GUARDA, 1983, cited by 7, the encephalomalacy on the histostructural way shows three distinct forms: acute, vasculo proliferrative and schlerotic.

From the three known forms in the studied area only acute encephalomalacy form developed.

The main cause of the apparition of the encephalomalacy episode was the change of the combinated forage supplier for chicken, who used one low quality zoofort forage (with low vitamin E contain).

E hipovitaminosis develops the raise of the encephalic capillaries permeability , as a result of the incorporation of some abnormal metabolic compounds (hyaline, etc), and a raise of the permeability of the membranes of the cellular organists , with the installation of the dystrophic lesions at the nervous cells level, cerebral or cerebellum edema. The suddenly, circumcise or diffuse growth of the cerebral volume produces intra cranial hypertension, rarely reversible. Other times, the edema leads to sudden death by the compression of the bulb (2, 3, 7).

In the acute form in the structure of the cerebellum we observed: the spongiest aspect of the Purkinje cells layers from the cerebellum; the complete lysis of the Purkinje cells (neuronolysis), the rarefaction of the granular and molecular cell layers ; hyalinization of the cerebral and cerebellum capillaries; edema and hemorrhagic infiltrations at different dimensions in the nervous system (Fig. 1, 2, 3, 4).

The lesions that we found are in line with the cited literature, regarding the acute form (2, 7).

The other two forms of the encephalomalacy evolution, vasculo- proliferrative and sclerotic, have not been observed, because of the quick establishment of the diagnosis and in the same time with the adequate treatment, leading to the fact that the regressive phenomena are followed by reparatory modifications.

Conclusions

The encephalomalacy developed as an explosive episode determined by the change of the combined forage supplier.

The disease appeared in this institution at chicken about 19 days old with the maximum incidence between 20-30 days. The lost from mortalities and necessity sacrifications have been of 14,44%.

Morphoclinical it manifested through astasy, locomotory ataxia, tremors, lurch, head balance, incoordinations, tendency to drop dorsolateral, clonic contraction of the members, cahectisation.

The **necropsic** path shows cerebral or cerebellum edema and punctiform hemorrhages lumped in the cerebellum mostly in the vermis.

The **histostructural** segment most affected from the nervous system was the cerebellum, followed by the cerebral hemispheres and the bulb. From the three known histopathological form of evolution: acute, vasculo-proliferrative and sclerotic, in this institution has been detected only the acute one.

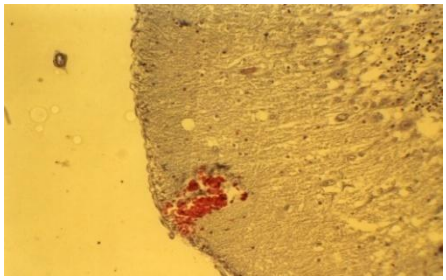

Fig. 1. Cerebellum-hemorrhages in the molecular layer
Col. HEA, 10 x 10

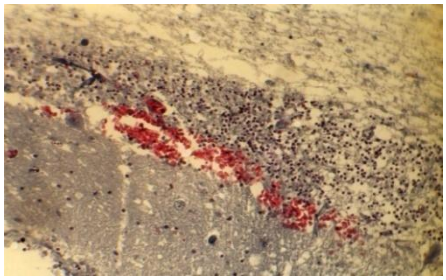

Fig. 2.Cerebellum – hemorrhages in the Purkinje cells layer
Col. HEA, 10 x 10

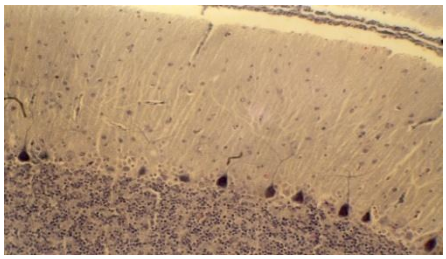

Fig. 3. Cerebellum – hemorrhages in the white matter
Col. HEA, 10

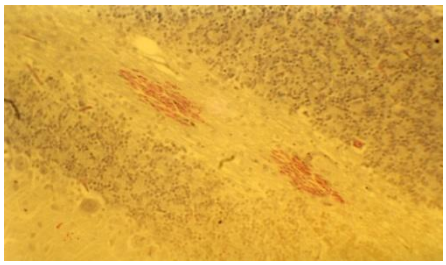

Fig. 4. Cerebellum – degenerated Purkinje cells
Col. HEA, 10 x 10

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The morphological relation between the dimensions of the coronoid process and mandible angle at some carnivours

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The study was made through the comparison of the skulls of the different carnivorous species. The dimension of the coronoid process is direct and same relation with the developing of the surface of the temporal fosse that offers insertion place for the strong temporal muscle in carnivores. At the big carnivores, the exaggerated developing of the temporal muscle is made through the modification of the muscle's volume, that is bulky, that leads to the enlarging of the temporal fosse. The elongation of the coronoid process is realized through the lowering of the mandible condyle near to the angular process of mandible.

Key Words: carnivorae, skull, choronoid process, morphology

Anatomicaly differences of the head muscles at the *Capreolus* and the *Cervus*

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The study was performed with the dissection method on three Cervus and six Capreolus – both males and females.

Unlike the Cervus, the Capreolus presents a masseter muscle with three different pieces on three superposed levels. The superficial piece has an almost horizontal orientation and it is playing the role of the mandible's propeller.

At the Capreolus, the head's platysma muscles have two retracting muscles of the lips' commisure from which the ventral is represented by the labial insertion of the sternomandibular muscle. The important caudal retraction of the lips' commisure facilitate the Capreolus's easy caching of the top leafs. The roedeer's auricular musculature is more developed than the one of the roebucks (Capreolus).

Key Words: Cervus, Capreolus, head muscles

The morphological diferential aspects about the tronk muscles at the *Myocastor coypus* and *Ondatra zibethica*

COȚOFAN V., HRIȚCU Valentina, SPĂTARU C.
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The study was made by the classic method of the dissection on seven cadavers of Myocastor and eight of the muskrat. The real morphological differences, comparatively emphasized in comparison with the most of the mammals justify the motivation of this research. The most important morphological differences are represented by the synsarcotic and abdominal muscles. The cervical muscles of the muskrat have some differences compared to the muscles of the other good swimming rodents. These differences are represented first of all, by the distinct relation established in the development of the muscular abdomens, by the more restricted and more extended insertions and origins of the muscles, all these factors modifying significantly the contribution of each muscle to the movements of the neck and the head. The muscles of the body are represented by the superficial muscles, which besides assuring the bond between the forelimbs and the body they also have an essential role in moving in the water by swimming or on the ground in digging galleries.

Key Words: Myocastor coypus, Ondatra zibethica, anatomy, trunk muscles

Morphological aspects regarding suprarenal glands in dog

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Adrenal glands harvested from 4 dogs, fixed in Susa-Heidenhain, were processed with standard histological techniques and the 6 micrometer-thin slices were stained in tricromic Masson-Goldner dyes.

Macroscopically, the adrenal glands in dog did not show particular aspects apart from the normal known anatomical aspects.

The histological study revealed a thick capsule, with a great number of cells and also glandular formations- well round defined or pediculate with eratical disposition. In the pericapsular tissue autonomous nervous ganglia with various dimensions were observed and at the cortico-medular boundary- filled with conjunctive tissue- an enormous number of blood vessels with quite large lumen were identified.

Vascularisation and innervation on such an extent in adrenal glands suggests an intense metabolic activity, most probably in direct connection with the dog's strength and resistance and high adaptation possibilities for this species.

Key Words: dog, suprarenal glands, morphological investigations, vascularisation, innervations

Introducere

Suprarenalele sunt glande endocrine care intervin direct în coordonarea a numeroase mecanisme, printre care şi cele legate de adaptare şi stres.

Unul dintre animalele nevoite să se acomodeze la schimbări dese şi de multe ori majore, este câinele. Câinele este un animal aparte, format în condiţii de concurenţă dură şi folosit la activităţi de o foarte mare diversitate. Datorită acestor aspecte el a fost şi mai este încă, nevoit să se adapteze la condiţii foarte variate şi uneori cu schimbări bruşte de la o situaţie la alta.

Acestea sunt motivele pentru care am considerat oportun să facem investigaţii morfologice asupra glandelor suprarenale, responsabile de procesele de adaptare, pentru observarea eventualelor particularităţi structurale, care să sugereze o funcţionalitate mai aparte, la această specie foarte solicitată şi obişnuită să intre în alertă în timp foarte scurt, la factori perturbatori, de multe ori discreţi.

Material şi metode

Lucrarea s-a efectuat pe glandele suprarenale provenite de la 4 câini (2 masculi şi 2 femele). Animalele au fost injectate intravenos (vena safenă) cu soluţie suprasaturată de sulfat de magneziu în laboratorul de Anatomie comparată a Facultăţii de Medicină Veterinară Cluj-Napoca. După sacrificare, animalele au fost aşezate în decubit dorsal şi s-a practicat o incizie a peretelui abdominal de la apendicele xifoidian până la pubis. Pentru crearea accesului în cavitatea abdominală s-au practicat incizii laterale în peretele abdominal (paralele cu coastele) (fig.1).

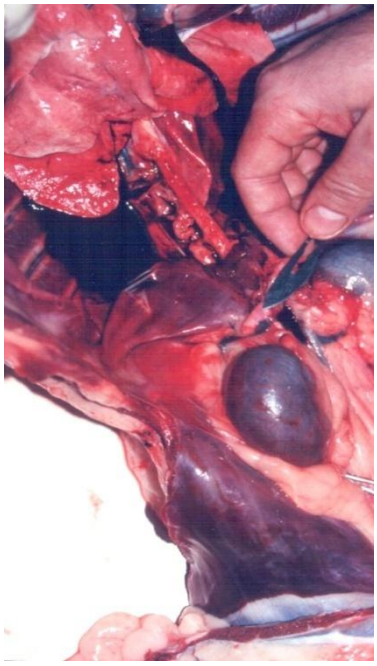


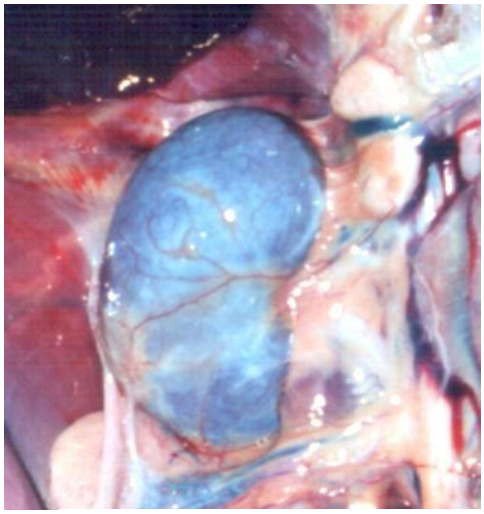
Fig.1. Topografia glandelor suprarenale

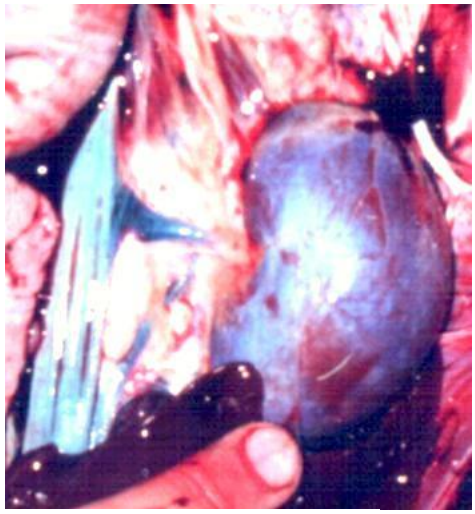
S-a trecut imediat la recoltarea glandelor suprarenale care au fost secționate în două cu un bisturiu foarte fin şi au fost introduse apoi, imediat, pentru fixare, în amestec Susa-heidenhain pentru 15 ore. După încheierea fixării, piesele au fost incluse în parafină prin procedeul: deshidratare cu alcool etilic, clarificare cu alcool amilic, penetrare cu parafină la 56°C, turnare în parafină. După întărirea parafinei, piesele au fost debitate în secțiuni cu o grosime de 6 micrometri şi colorate prin metoda tricrom Masson, modificată de Goldner. Principali timpi s-au derulat astfel:

- deparafinare cu benzen;
- hidratare cu alcool (alcool absolut, alcool 96°, alcool 70°, apă distilată);
- eliminarea precipitatelor mercurice, cu alcool iodat;
- colorare 7 minute, cu hematoxilină Groat;
- colorare 5 min, cu soluție apoasă de fuxină acidă - ponceau de xylidină;
- diferențiere, cu o soluție apoasă de acid fosfomolibdenic - Orange G, până la decolorarea țesutului conjunctiv (aproximativ 5 minute);
- colorare timp de 5 minute, cu o soluție apoasă de verde lumină, urmată de clătire cu apă distilată, deshidratare, clarificare şi montare, ca în tehnica precedentă.

Rezultate şi discuţii

Examenul anatomic al glandelor suprarenale la câinii luați în studiu a evidențiat faptul că ele sunt mai mari la femele, decât la masculi. În cazul fiecărui animal s-a constatat că suprarenala dreaptă este mai voluminoasă decât cea stângă. Există diferențe între suprarenala dreaptă şi cea stângă şi în privința formei. Astfel, suprarenala dreaptă are formă aproximativ triunghiulară, iar cea stângă se aseamănă cu o clepsidră. Culoarea glandelor suprarenale este gălbuie spre galben





brun ***glanda suprarenală stângă*** ***glanda suprarenală dreaptă*** ı-roşcat.

Fig. 2. Aspectul glandelor suprarenale

Suprarenalele câinelui au suprafața brăzdată, neregulată şi străbătută de numeroase reliefuri (fig.2).

Din punct de vedere histologic, suprarenala la câine, prezintă aceleaşi componente ca la alte specii de mamifere: **capsulă, corticală** şi **medulară**.

Capsula, care acoperă la exterior suprarenalele, este relativ groasă. Ea conține numeroase lame conjunctive colagene cu dispoziție paralelă cu suprafața organului. Treimea externă a capsulei are aspect predominat fibros, iar cele două treimi interne conțin pe lângă fascicule groase de colagen un număr mare de celule (fig.3). Majoritatea acestora sunt fibroblaste şi fibrocite. Pot fi identificate şi rare fibre musculare. Din capsulă se desprind travee conjunctive foarte subțiri, ce pătrund pe alocuri, până la limita cortico-medulară (fig.4).

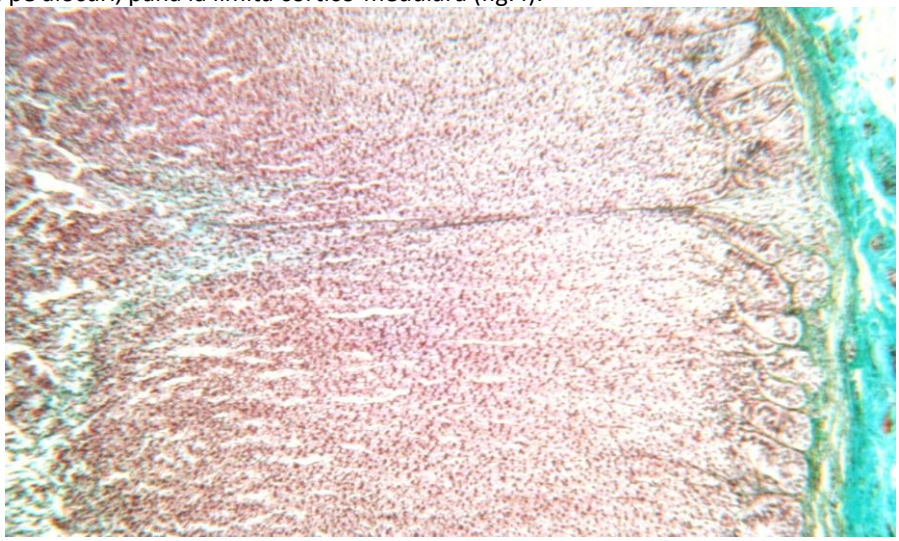


Fig. 3. Aspectul histologic al capsulei (col. tricromică Goldner, 500x)

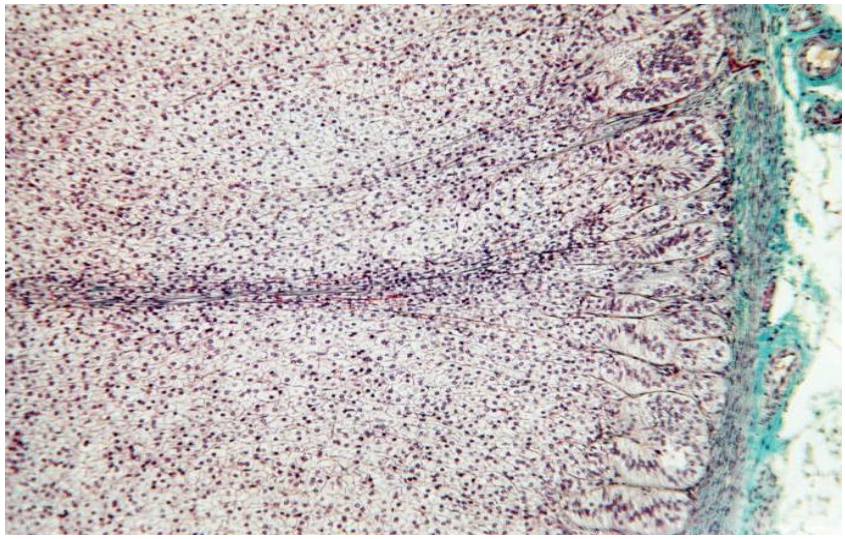


Fig. 4. Travee conjunctive fine (col. tricromică Goldner, 100x)

Spre exterior, capsula este îmbrăcată într-un țesut adipos a cărui grosime variază de la o zonă la alta (fig.5). În țesutul pericapsular se află ganglioni vegetativi de diferite forme şi dimensiuni (fig.6).

Corticosuprarenala apare cu cele trei zone caracteristice - glomerulară, fasciculată şi reticulată, bine delimitate (fig.7). Cea mai groasă dintre ele este zona fasciculată, urmată îndeaproape de zona reticulată, pe când zona glomerulară apare ca fiind cea mai subțire.

În zona glomerulară aspectul este caracteristic, formațiunile existente aici, au la câine, formă arcuată. Aceste formați sunt net exprimate şi de dimensiuni mari, ceea ce face ca zona glomerulară să fie mai dezvoltată la câine decât la alte specii. Formațiunile arciforme sunt alcătuite din celule prismatice foarte înalte, dispuse pe un singur rând (fig.8). Formațiunile alcătuite din celule de acelaşi tip sunt relativ frecvent întâlnite în afara zonei glomerulare - dispunere eratică. Ele pot fi sesizate în grosimea capului şi apar fie bine circumscrise (fig.9) sau cu aspect pediculat (fig.10). Formațiuni similare au fost descrise şi la alte specii, dar la câine numărul lor este relativ mai mare.

Zona fasciculată este formată ca şi la celelalte specii, din cordoane celulare lungi şi paralele. Celulele din această zonă au aspect tipic, clar, specific spongiocitelor (fig.11). Deşi este cea mai dezvoltată dintre cele trei zone ale corticosuprarenalei diferența de grosime dintre ea şi celelalte două zone - glomerulară şi reticulată, nu este la fel de mare ca la alte specii.

Zona reticulată apare uşor mai întunecată şi este alcătuită din cordoane celulare evidente, bogat anastomozate, între care se află vase de sânge de calibru mai mare decât în celelalte două zone (fig.12).

Limita dintre corticosuprarenală şi medulosuprarenală apare clară, aproape liniară. La acest nivel este prezent un țesut conjunctiv străbătut de un număr impresionant de vase de sânge. Grosimea acestei zone de trecere, nu este uniformă, fiind pe alocuri relativ subțire, pentru ca, în alte zone să fie foarte groasă (fig.13).

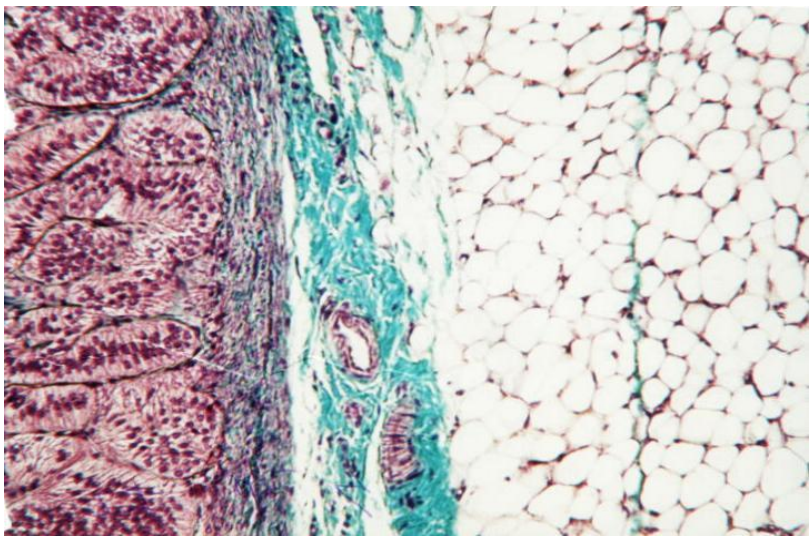


Fig. 5. Țesut adipos pericapsular
(col. tricromică Goldner, 150x)

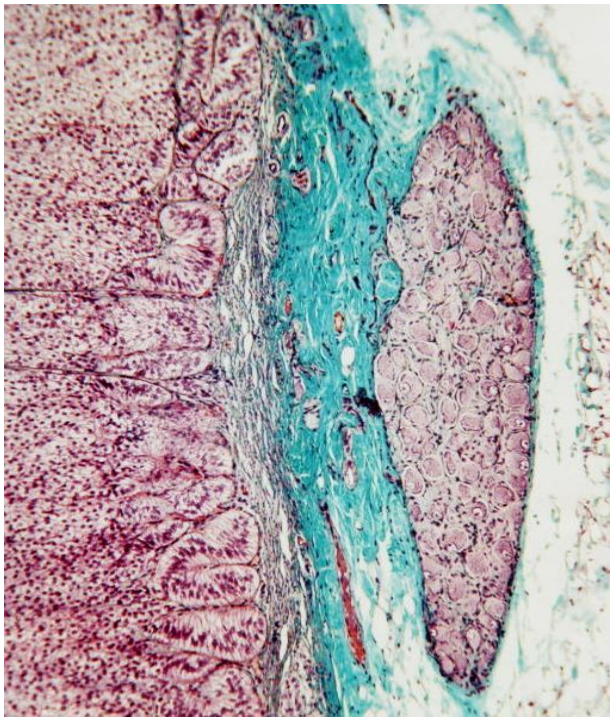


Fig. 6. Ganglion vegetativ pericapsular (col. tricromică Goldner, 100x)

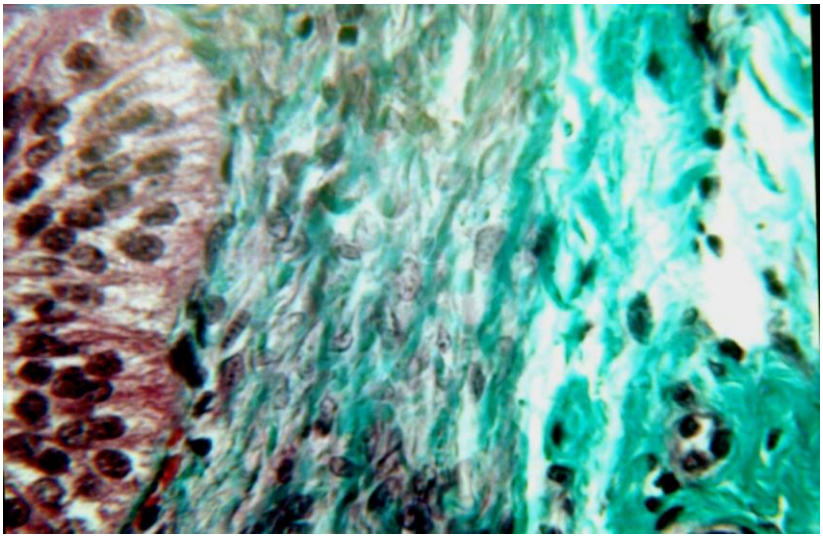


Fig. 7. Aspectul histologic al corticosuprarenalei (col. tricromică Goldner, 60x)

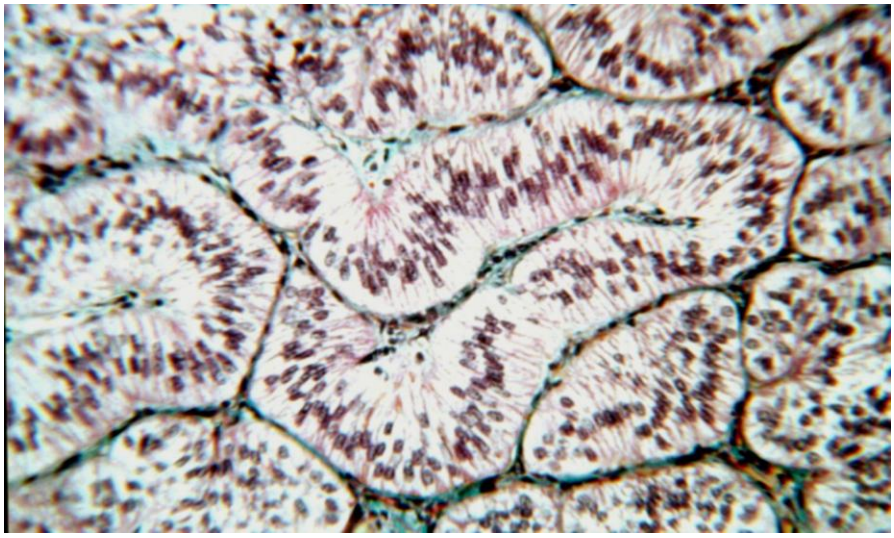


Fig. 8. Formațiuni arciforme din zona glomerulară
(col. tricromică Goldner, 200x)

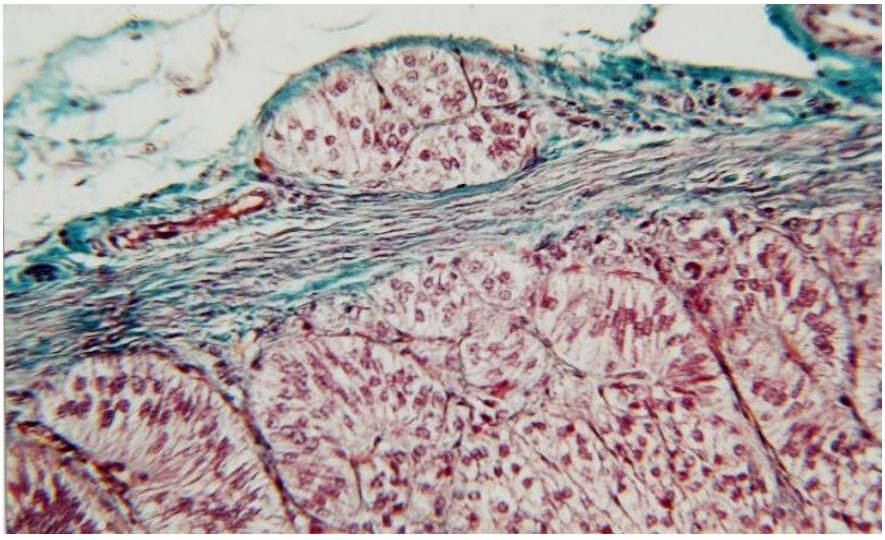


Fig. 9. Formațiune glandulară circumscrisă cu dispunere eratică
(col. tricromică Goldner, 200x)

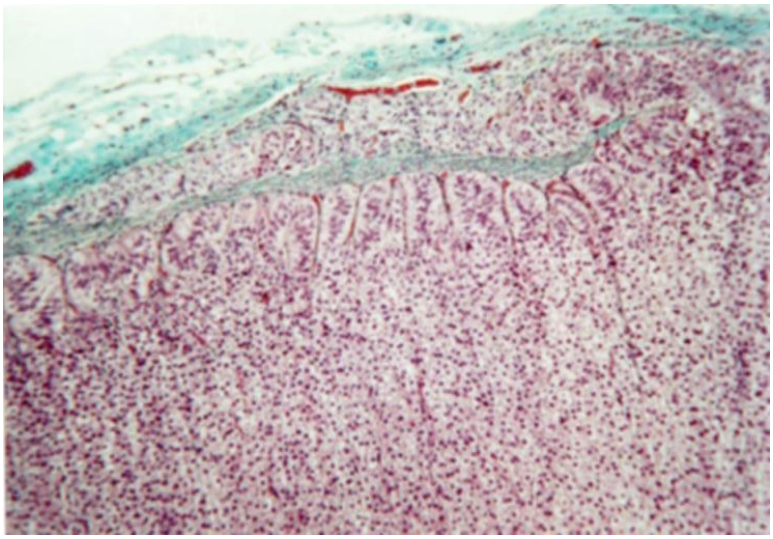


Fig. 10. Formaţiune glandulară pediculată cu dispunere eratică (col. tricromică Goldner, 100x)

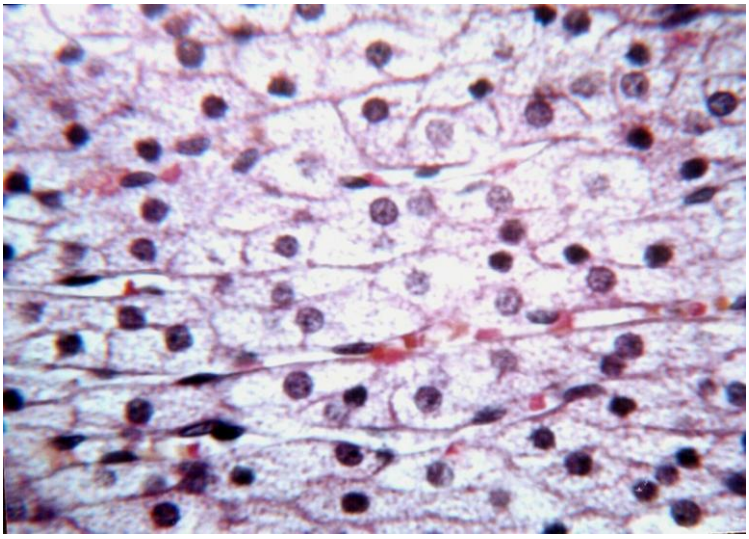


Fig. 11. Cordoane de spongiocite din zona fasciculată (col. tricromică Goldner, 500x)

Zona medulară este formată din celule mari, cu nuclei ovalari, care conţin cromatină fin granulară. Se pot deosebi două tipuri celulare, care se disting uşor unele de altele. Prima categorie este reprezentată de celule mari prismatice, dispuse în cordoane groase cu aspect de fişicuri. Ele pot fi observate pe toată suprafaţa medularei, dar predomină net în zona periferică a acesteia. Astfel de cordoane celulare sunt dispuse în relaţii intime cu vase de sânge de calibru ceva mai mare (fig.14).

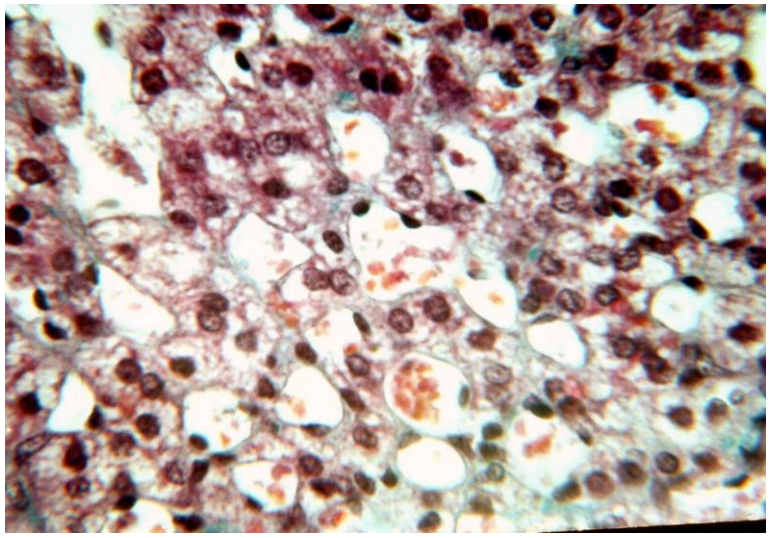


Fig. 12. Cordoane celulare ramificate din zona reticulată (col. tricromică Goldner, 500x)

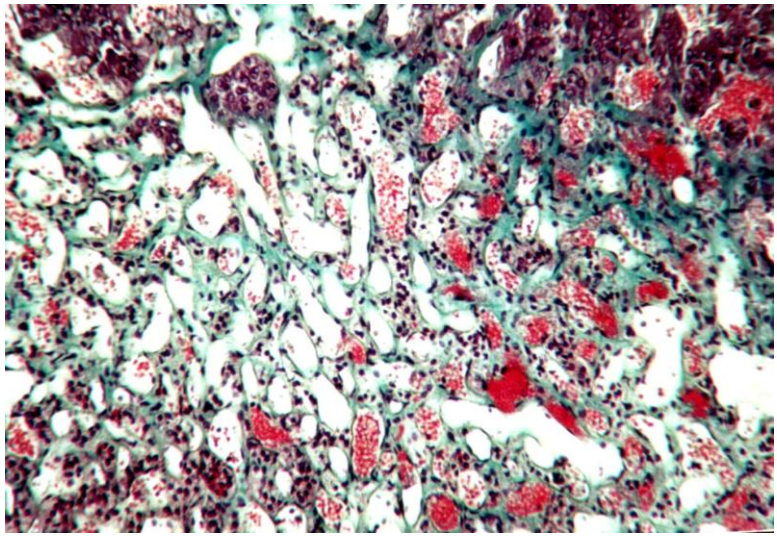


Fig. 13. Țesut conjunctiv bogat vascularizat de la limita cortico-medulară (col. tricromică Goldner, 150x)

A doua categorie este reprezentată de celule poliedrice uşor rotunjite, de talie mai mică, în comparație cu precedentele (fig.15). Aşezarea lor se face cu precădere, sub formă de cuiburi rotunde. Acestea se află în relații intime cu vase de sânge de calibru mic.

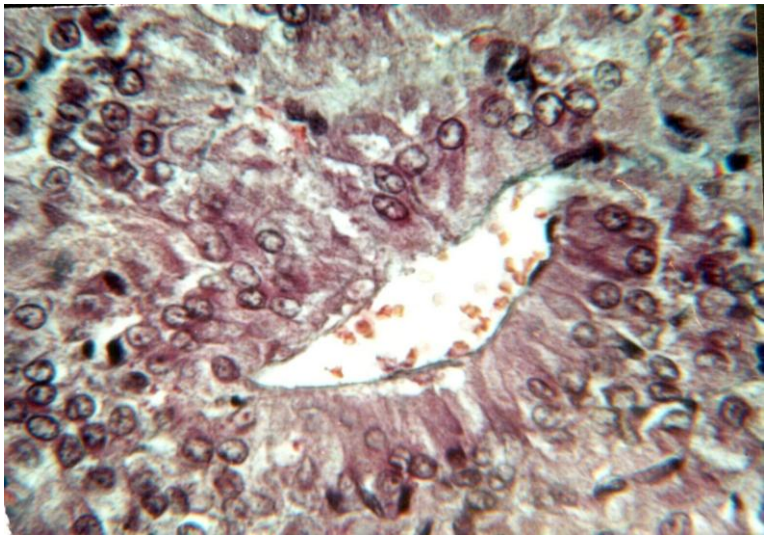


Fig. 14. Celule prismatice dispuse sub formă de cordoane Perivasculare col. tricromică Goldner, 500x)

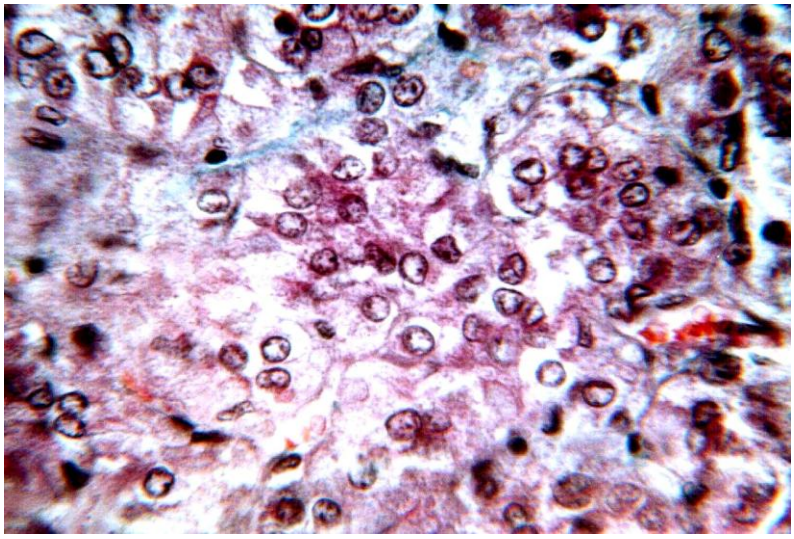


Fig. 15. Celule poliedrice de la nivelul medulosuprarenalei (col. tricromică Goldner, 500x)

Concluzii

Din punct de vedere anatomic, glandele suprarenale la câine, nu au relevat aspecte particulare, demne de a fi reținute, comparativ cu ceea ce se cunoaşte la această specie.

Studiul histologic însă, a scos în evidență anumite particularități după cum urmează:

- Capsula este groasă şi conține un număr mare de celule, precum şi formațiuni glandulare - circumscrise sau pediculate, cu dispunere eratică.
- În țesutul conjunctivo-adipos pericapsular au fost identificați ganglioni vegetativi de diferite forme şi mărimi, ceea ce evidențiază faptul că organul este foarte bine inervat.
- Corticosuprarenala apare cu cele trei zone bine delimitate, iar ca mărime pe primul loc se află zona fasciculată, urmată de zona reticulată şi zona glormerulară.
- Deşi există diferențe de grosime între cele trei zone ale corticosuprarenalei, ele sunt la câine, la fel de mari ca la alte specii.
- Zona glomerulară are un aspect particular, fiind alcătuită din celule foarte înalte dispuse în cordoane încurbate cu aspect arcuat.
- Dispunerea sub formă de cordoane arciforme înalte face ca zona glomerulară să aibă o grosime ceva mai mare decât la speciile la care celulele alcătuiesc formațiuni glomerulare rotunde sau ovale.
- Limita cortico-medulară este foarte netă şi este ocupată de un țesut conjunctiv care conține un număr impresionant de vase de sânge.
- Numărul şi calibrul vaselor de sânge este mare şi la nivelul medulosuprarenalei, astfel încât în ansamblul ei, glanda suprarenală este mai bine vascularizată la câine, în comparație cu alte specii.
- Vascularizația şi inervația deosebită a glandelor suprarenale sugerează o activitate intensă a acestora, care ar putea fi în relație directă cu rezistența deosebită a câinelui şi cu posibilitățile lui mari de acomodare la cele mai diverse medii şi situații.

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Blood and wool copper concentration following suplimentary feed copper administration in sheep

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In order to observe the clinical and toxicological modifications, 42 adult, not gestating Tsigai ewes were studied, during a period of six weeks, after treatment with supplementary copper doses in feed.

Primary objectives of the research were to observe toxicological modifications at blood and wool level as a consequence of overdosing copper products in sheep from two different copper sources supplementary administered into feed (copper sulfate or copper proteinate).

Blood copper level was within normal limits for sheep independent of copper dose or source administered supplementary in feed. A significant increase was observed only in the immediate period after initial administration, but also this concentration could not elicit the hemolysis.

Wool copper concentrations increased proportionally with copper dose and period of administration. Higher wool copper levels were observed in ewes fed an organic copper source, suggesting a better bioavailability of copper from proteinate compared to copper sulfate.

Key Words: copper, sulfate, proteinate, sheep

Sheep are unique in their way of metabolizing copper. Copper is an essential element for all farm animals; however it has a potentially toxic effect, sheep being the most sensitive animals to copper toxicity. Therefore the use of copper in ovine nutrition is complicated being essential and toxic in the same time.

The pathogenesis of copper intoxication has two distinct phases: first phase is the accumulation in tissues and the second one is an acute phase due to the hemolytic crisis [4].This morbid entity may be unobserved until stress factors intervene such as transport, lactation, effort, or diet modification [7]. During this stage of the intoxication there is a massive hepatic copper release, generating a sharp increase of blood copper level and haemolysis, accompanied by severe gastroenteritis, jaundice, dehydration, lack of appetite, hemoglobinuria.

During the last years there is a large increase in mineral supplements containing copper, while the use of chelated mineral become very popular due the better absorption of chelated trace elements compared to inorganic compounds. [1, 2, 6].

Copper based treatments are supplementary added into feed or are parenteral administered mainly for the prevention of hypocuprosis

The use of organic or inorganic copper may have advantages as well as disadvantages concerning the administration but mainly regarding the development of intoxications [5].

Although the bioavailability and absorption of organically bound copper was demonstrated there is little information on induced intoxication in ovine due to organically bounded copper from feed supplements.

The aim of this study was the measurement of the copper blood and wool concentration as a result of supplementary copper feed in sulfate or proteinate form.

Material and methods

In order to observe the clinical, pathological, hematological, blood biochemical and toxicological modifications, 42 adult, not gestating Tsigai ewes were studied, during a period of six weeks, after treatment with supplementary feed copper doses. The ewes were divided by body weight in seven groups. Six groups were randomly assigned to dietary treatments by copper source supplementary administered into feed (copper sulfate or copper proteinate) fed at one of three dietary levels (10, 20 or 30 mg/kg), and one was the control group.

The groups were conventionally noted:

- control group – L.M.
- group no. 1 – L.O.1 – copper sulfate 10 mg/kg;
- group no. 2 – L.O.2 – copper sulfate 20 mg/kg;
- group no. 3 – L.O.3 – copper sulfate 30 mg/kg;
- group no. 4 – L.O.4 – copper proteinate 10 mg/kg;
- group no. 5 – L.O.5 – copper proteinate 20 mg/kg;
- group no. 6 – L.O.6 – copper proteinate 30 mg/kg;

During the study, the ewes were closely examined clinically, and blood samples were collected after well settled intervals to determine blood copper levels (weeks 2, 4 and 6). Also, wool samples were collected on weeks 2, 4 and 6, to determine copper levels.

Blood and wool levels of copper were determined by atomic absorption spectrometry.

Animal care and use was in concordance with ethic requirements from the international practices and regulations that are mentioned in national legislation of Romania about ethical problems in research that include regulations on experiments and research, also on animals.

The data were statistically analyzed using MINITAB software.

Results

Blood copper concentration were significant for the treated ewes compared to the control ($P<0.05$), with increased values of copper concentration in blood disregarding the copper compound or doses, remaining within normal limits for ovine.

The blood copper levels are presented in tables 1-7 and graphs 1-2.

The control group had a mean of blood copper level of 65.27 µg/dl ranges from 45.71 to 87.90, lightly lower than the normal values.

There was an increased blood copper concentration in the samples collected from the animals receiving copper sulfate supplemented feed in doses of 20 and 30 mg/kg compared to the concentration in the blood samples of sheep receiving the minimal dose of copper sulfate.

Thus the mean cupremia of L.O.1 was 100.87 µg/dl compared to 122.58 µg/dl for L.O.2 ($P=0.006$) and 110.85 µg/dl, for L.O.3 ($P=0.049$).

During the following weeks of the study significant results were obtained for L.O.1 and L.O.2 (105.95, compared to 114. 90 µg/dl, $P=0.027$).

There was a significant difference between the blood copper level of ewes in L.O.1 and L.O.4, which received the lower copper doses of both copper chemical forms during the second ($P=0.014$) and the forth week of treatment ($P=0.005$) with increased values for the animals receiving copper proteinate. However there was no significant difference between the two animal groups during the last week of the treatment ($P=0.621$) with the low doses.

The rest of the animal groups receiving 20 mg/kg and 30 mg/kg, there were no significant differences between the averaged values (P>0.10) with one exception (P=0.009) for the L.O.3 and L.O.6, during the second week of the experiment (110.85 compared to 125.25 µg/dl).

For this study the blood copper level in ewes fed with supplementary copper proteinate there were no significant differences between the mean values obtained for each group (P>0.10), disregarding the doses and time of exposure.

Therefore it may be concluded that the values for blood copper level were within the specie characteristic limits, with no significant increased values over the maximum normal value, and with no eritocitary damage or haemolysis initiation.

Table 1. Blood copper levels (µg/dl) in control group (L.M.)

	L.M.
1	45.71
2	52.74
3	54.76
4	86.06
5	87.90
6	64.43
Average	***65.27***
Min.	45.71
Max.	87.90

Table 2. Blood copper levels (µg/dl) in L.O.1

	week 2	**week 4**	**week 6**
1	89.30	106.90	101.30
2	95.70	102.80	106.50
3	106.20	119.00	97.50
4	113.30	100.20	113.80
5	100.10	101.60	124.80
6	100.60	105.20	111.70
Average	***100.87***	***105.95***	***109.27***
Min.	89.30	100.20	97.50
Max.	113.30	119.00	124.80

Table 3. Blood copper levels (µg/dl) in L.O.2

	week 2	**week 4**	**week 6**
1	106.40	109.70	86.60
2	121.50	112.80	89.70
3	112.60	111.60	100.90
4	128.90	118.10	113.30
5	139.30	121.30	111.10
6	126.80	115.90	105.60
Average	***122.58***	***114.90***	***101.20***
Min.	106.40	109.70	86.60
Max.	139.30	121.30	113.30

Table 4. Blood copper levels (µg/dl) in L.O.3

	week 2	week 4	week 6
1	111.80	108.70	117.40
2	107.70	129.10	94.90
3	123.90	106.40	109.00
4	105.10	86.40	114.30
5	106.50	101.30	103.10
6	110.10	92.60	101.30
Average	***110.85***	***104.08***	***106.67***
Min.	105.10	86.40	94.90
Max.	123.90	129.10	117.40

Table 5. Blood copper levels (µg/dl) in L.O.4

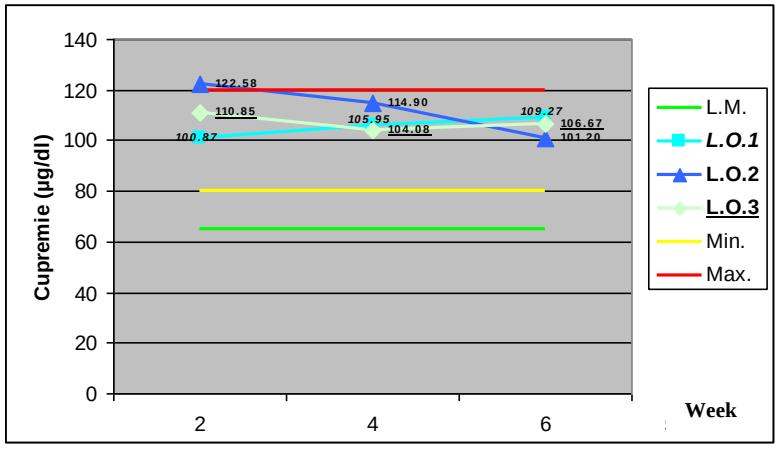
	week 2	week 4	week 6
1	105.40	116.80	122.70
2	118.50	132.90	100.20
3	127.90	118.80	114.30
4	119.30	110.40	119.60
5	110.80	124.50	108.40
6	109.60	129.80	106.60
Average	***115.25***	***122.20***	***111.97***
Min.	105.40	110.40	100.20
Max.	127.90	132.90	122.70

Table 6. Blood copper levels (µg/dl) in L.O.5

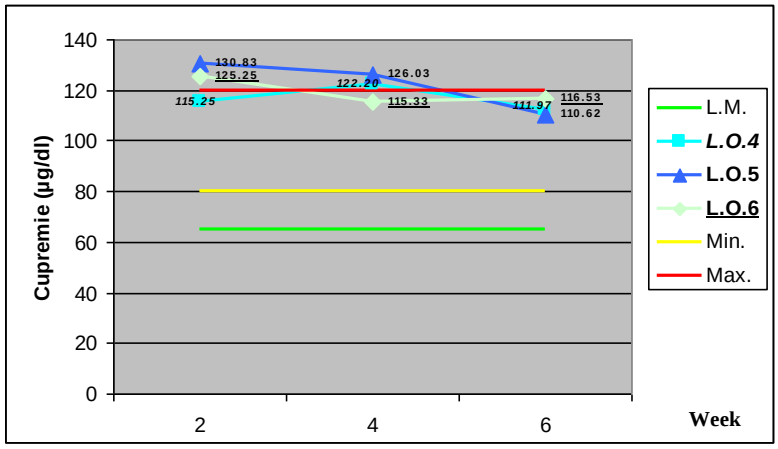
	week 2	week 4	week 6
1	121.30	104.40	109.70
2	105.20	138.50	121.20
3	149.50	130.80	111.10
4	147.50	112.80	102.80
5	139.60	136.90	110.40
6	121.90	132.80	108.50
Average	***130.83***	***126.03***	***110.62***
Min.	105.20	104.40	102.80
Max.	149.50	138.50	121.20

Table 7. Blood copper levels (µg/dl) in L.O.6

	week 2	week 4	week 6
1	137.90	114.70	112.80
2	129.30	121.50	126.90
3	120.80	102.80	112.80
4	119.60	118.90	104.40
5	115.40	122.80	118.50
6	128.50	111.30	123.80
Average	**125.25**	**115.33**	**116.53**
Min.	115.40	102.80	104.40
Max.	137.90	122.80	126.90



Graph 1. Dynamics of blood copper average values in L.O.1, L.O.2 and L.O.3



Graph 2. Dynamics of blood copper average values in L.O.4, L.O.5 and L.O.6

The values of the copper concentration in wool are presented in tables 8-14 and graphs 3-7.

The copper concentrations in wool after six weeks were significantly different for the control group compared to the groups receiving the copper sulfate treatment ($P<0.05$), and showed no significant differences after four weeks of treatment ($P>0.10$).

For the groups of animals receiving supplementary feed copper proteinate there was a significant difference ($P<0.05$) between copper concentration in control group wool compared to the groups receiving treatments regardless the treatment dose and time of exposure.

There was a significant difference ($P<0.05$) between the copper wool concentrations in ewes receiving the maximum / minimum doses of copper of different chemical forms regardless the duration of exposure. However the individuals from the groups receiving the intermediate doses showed no significant differences regardless the chemical form of feed copper supplement over the entire duration of exposure ($P>0.10$).

There were significant differences for wool copper concentration ($P<0.05$) between groups receiving copper proteinate regardless the doses in the second week of treatment, although the concentrations of copper in wool were not significant increased during the study.

It may be concluded that the copper concentration in wool increased proportionally with copper feed doses, and the higher increase correspond to the organic copper compound feed additive.

These data suggests that the copper in the organic form is more readily available compared to the copper sulfate.

Evans (1973) suggested that one of the possible roles of cerulopasmine could be of transport protein releasing copper for the tissues [3].

The correlation between the blood copper level and the copper concentration in wool suggests that the copper transport to the hair follicle was more intense inducing an increased accumulation in wool for the animals fed organic copper.

Table 8. Wool copper concentrations (ppm) in control group (L.M.)

	L.M.
1	4.511
2	2.937
3	6.958
4	3.285
5	3.516
6	3.875
Mean	**4.180**
Min.	2.937
Max.	6.958

Table 9. Wool copper concentrations (ppm) in L.O.1

	week 2	**week 4**	**week 6**
1	5.113	6.002	7.013
2	5.032	5.028	6.065
3	3.852	4.069	5.975
4	6.864	7.119	8.138
5	4.072	6.016	6.248
6	3.820	6.052	6.117
Average	**4.792**	**5.714**	**6.593**
Min.	3.820	4.069	5.975
Max.	6.864	7.119	8.138

Table 10. Wool copper concentrations (ppm) in L.O.2

	week 2	**week 4**	**week 6**
1	3.605	4.097	4.866
2	4.698	5.128	5.897
3	5.921	7.116	8.009
4	6.289	8.181	8.331
5	5.393	6.121	6.811
6	6.268	7.116	7.560
Average	**5.362**	**6.293**	**6.912**
Min.	3.605	4.097	4.866
Max.	6.289	8.181	8.331

Table 11. Wool copper concentrations (ppm) in L.O.3

	week 2	**week 4**	**week 6**
1	4.118	5.087	5.174
2	4.077	5.291	5.949
3	5.239	5.642	5.901
4	5.051	6.480	6.743
5	6.065	6.130	6.310
6	4.101	4.926	5.134
Average	**4.775**	**5.593**	**5.869**
Min.	4.077	4.926	5.134
Max.	6.065	6.480	6.743

Table 12. Wool copper concentrations (ppm) in L.O.4

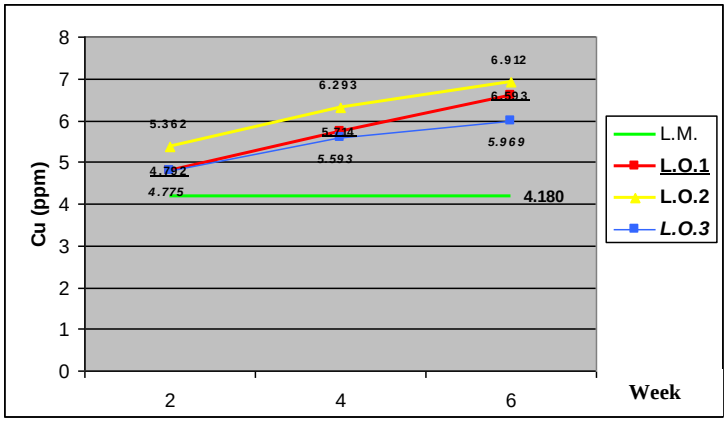
	week 2	**week 4**	**week 6**
1	6.054	7.168	7.227
2	6.185	7.329	8.002
3	5.779	6.688	7.143
4	6.193	7.104	7.596
5	6.108	7.245	7.840
6	6.096	7.298	7.663
Average	**6.069**	**7.139**	**7.579**
Min.	5.779	6.688	7.143
Max.	6.193	7.329	8.002

Table 13. Wool copper concentrations (ppm) in L.O.5

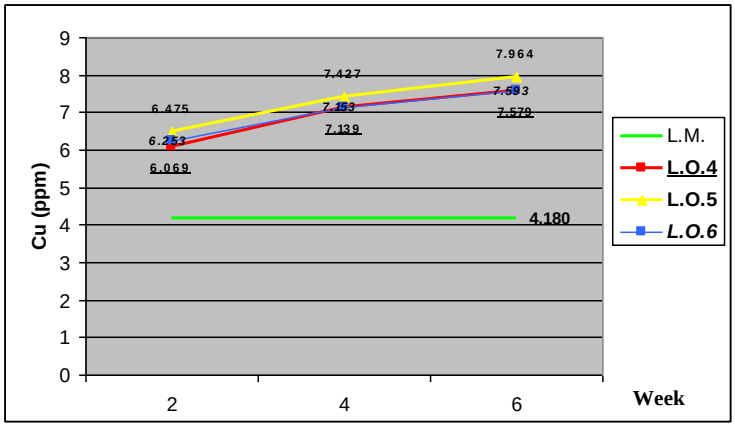
	week 2	**week 4**	**week 6**
1	6.213	7.044	7.970
2	7.052	8.385	8.821
3	6.495	7.308	7.811
4	6.475	7.128	7.625
5	6.396	7.368	7.704
6	6.219	7.328	7.854
Average	**6.475**	**7.427**	**7.964**
Min.	6.213	7.044	7.625
Max.	7.052	8.385	8.821

Table 14. Wool copper concentrations (ppm) in L.O.6

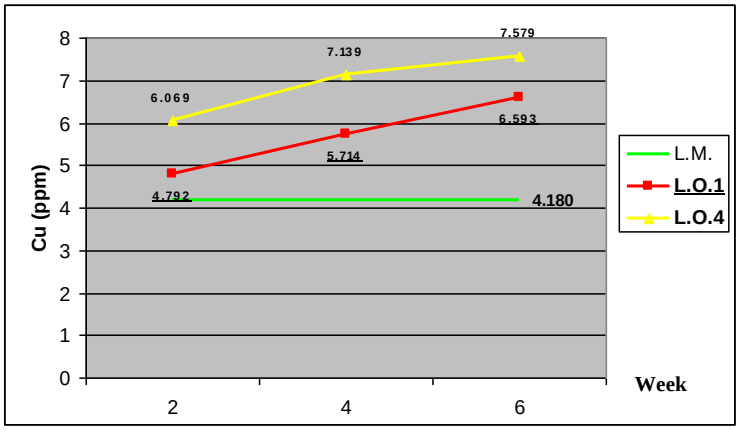
	week 2	**week 4**	**week 6**
1	6.379	7.147	7.285
2	6.293	7.215	7.269
3	6.208	7.028	7.824
4	6.196	7.189	8.044
5	6.154	7.228	7.895
6	6.285	7.113	7.238
Average	**6.253**	**7.153**	**7.593**
Min.	6.154	7.028	7.238
Max.	6.379	7.228	8.044



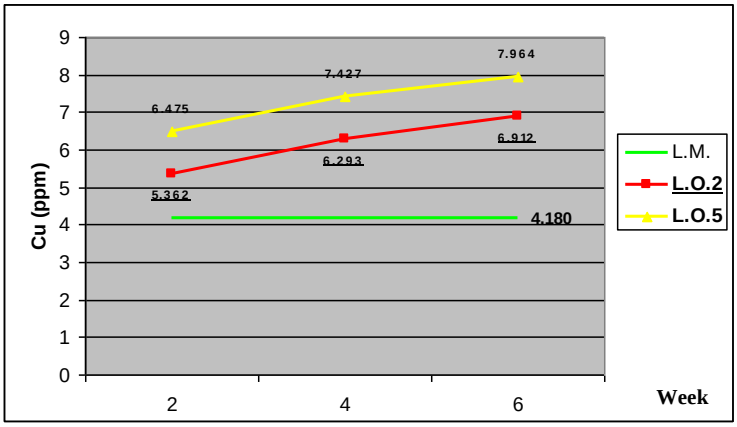
Graph 3. Dynamics of wool copper average levels in L.O.1, L.O.2 and L.O.3



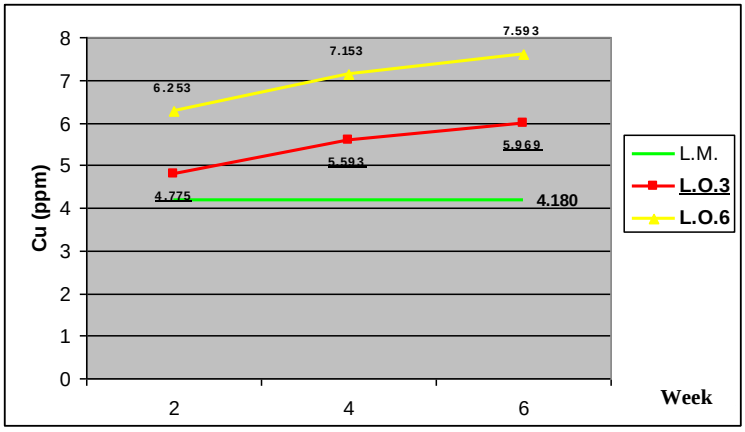
Graph 4. Dynamics of wool copper average levels in L.O.4, L.O.5 and L.O.6



Graph 5. Dynamics of wool copper average levels in L.O.1 and L.O.4



Graph 6. Dynamics of wool copper average levels in L.O.2 and L.O.5



Graph 7. Dynamics of wool copper average levels in L.O.3 and L.O.6

Conclusions

Following the statistical analyses toxicological laboratory tests of blood tissues and wool samples it may be concluded that:

1. Blood copper levels for ewes were in normal limits regardless the form of feed copper additives.
2. Blood copper level significantly increased being positively correlated to the copper doses during the first week of the study for the treatments using copper sulfate. However the increase of the blood copper level during the rest of the study was not significant.
3. Blood copper level was significantly increased for the treatments using the maximum or minimum concentration of organic copper additives in feed compared to the treatments using inorganic copper only for the first week of the treatment.
4. The blood copper level did not exceed the maximum accepted levels for sheep regardless the copper supplement used or the duration of administration. The doses used for this study did not influence the erythrocyte status or induce haemolysis.
5. The copper concentration in wool increased regardless the treatment doses of feed copper sulfate or the duration of exposure, being lower compared to the values due to the organic copper feed supplement only for the minimum and maximum treatment doses.
6. The copper concentration in wool increased being positively correlated with the copper sulfate added into feed regardless the duration of exposure, but being lower than the values obtained due to the organic feed copper supplement, demonstrating the higher bioavailability of the organic copper feed supplement.

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The biocompatibility and regenerative properties of polyurethaneurea doped with silver nanoparticles

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The biocompatibility study of silver nanoparticles doped polyurethaneurea by subcutaneous implants on mice revealed that these materials are well accepted. The microscopically investigation of histological sections revealed a mild inflammatory reaction. The material do not adhere to cutaneous tissue and promote a fast skin regeneration, that comprise even hair follicles.

Key Words: silver, polyurethaneurea, hair follicles, regeneration

Previous works of our team made on rabbits were revealed that the polyurethanes, witch are high biocompatible materials (1, 2, 4, 5, 6, 7, 8), when are treated with Ar ion beams stimulates proliferation of the collagen material and the material strong adhere to cutaneous tissue (3, 9), and when are doped with silver nanoparticles promote a very rapid cicatrisation (10). In the present work we evaluated on the mouse the biocompatibility and regenerative properties of polyurethaneurea doped with silver nanoparticles, by analyzing the local reactions at skin level.

MATERIALS AND METHODS

The investigations were performed on 7 adult mice, race SWISS, on which 2 subcutaneous implants (0,5 x 0,5 cm^2) were performed (observing the animal protection rules) in the lombosacral region. One of the implant was the simple polyurethaneurea and the other was the doped material with 20 ppm silver nanoparticles (2-10 nm diameters). After two weeks the implants were extracted together with the tissue next to the cicatriceal tissue. The tissular samples and the poly(urethaneurea)s samples were first preserved in neutral formol (15%) and then the samples were included in paraffin and cut in slices of 5 µm thickness, perpendicularly on the implant and also on the skin surfaces. The slices were colored by hematoxylin-and-eosin method.

RESULTS AND DISCUSSIONS

The silver doped polyurethaneurea determined a fast cicatrisation of the operatory plague, phenomenon that was observed for the silver doped polyurethaneurea implants performed on rabbits too (10). Tissue adhesion was not observed in case of the polyurethane doped with 20 ppm silver nanoparticles (2-10 nm diameters). The microscopically investigation, in case of the non doped polyurethane implant, shows an inflammatory reaction of moderate intensity at skin level, and a small quantity of inflammatory exudation was observed (fig 1).

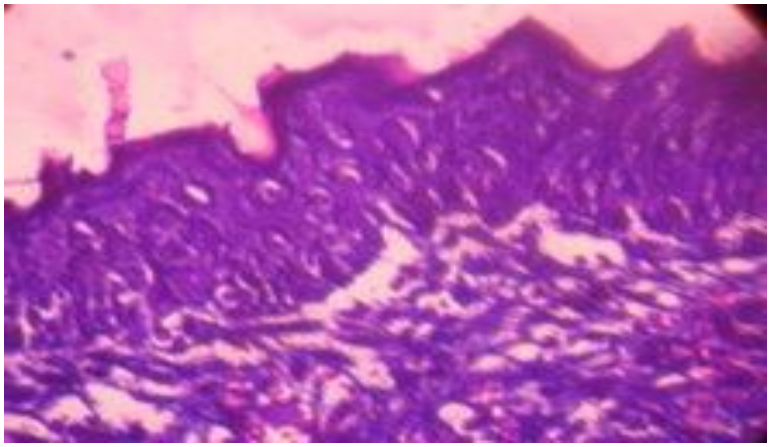

Fig. 1. Moderate inflammatory reaction at skin level. Histological section (H.E. – ob. 20x).

The simple polyurethan samples showed a small quantity of inflammatory exudates present inside the pores. A fibroblasts adhesion and collagen synthesis was observed on the material surface (fig. 2).

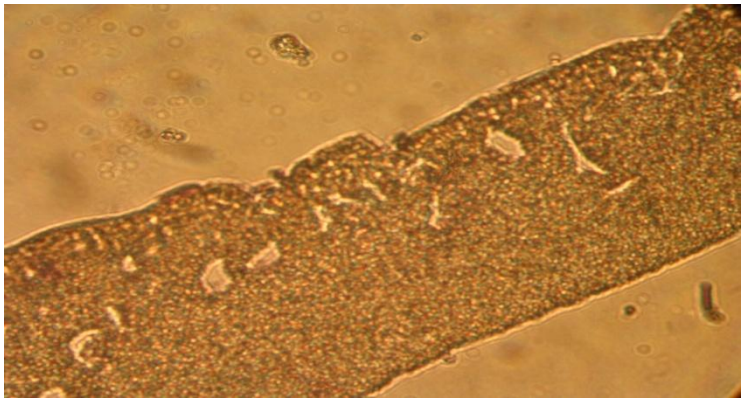

Fig. 2 Transversal cut through a poly (urethaneurea) film implant (H.E. - Ob.10x).

The tissue prelevated from the cicatriceal area around the implant of polyurethane doped with silver nanoparticles showed a low inflammatory reaction (revealed by a small quantity of exudates) and a very good epidermal regeneration with low derma proliferation. Those aspects are also observed in some treatments with ionic silver, but at higher concentrations (11). An amazing phenomenon observed was the regeneration of hair follicles (in case of simple polyurethane implants, the skin regenerated without hair follicles) (fig. 1, 3, 4).

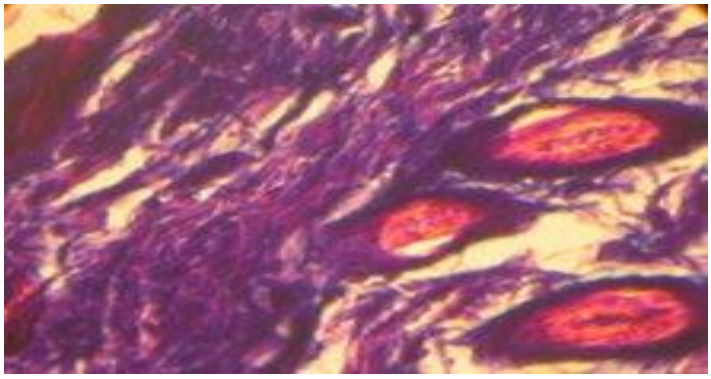

Fig.3. Low inflammatory reaction at skin level. Good regeneration, even for hair follicles. Histological section (H.E. – ob. 10x).

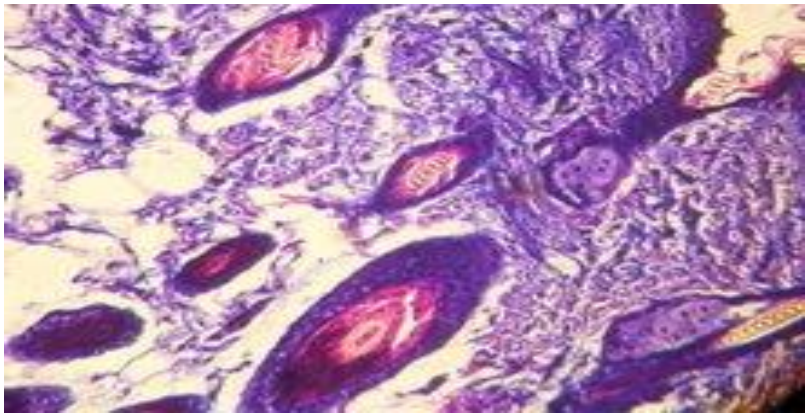

Fig.4. Hair follicles of normal aspect. Histological section (H.E. – ob. 10x).

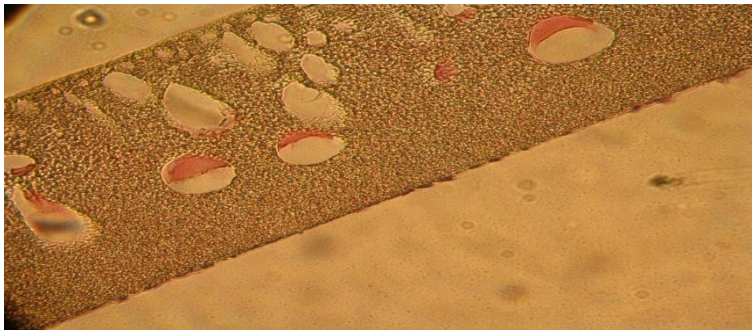

Fig. 5. Transversal section through poly (urethaneurea) (H.E. – Ob. 4x).

The transversal sections of the silver doped polyurethaneurea implants showed that inside pores the inflammatory exudate is almost absent and there are no polymorphonuclear neutrophyles or macrophages (which are arguments for the very low inflammatory reaction observed) and also a fibroblast adhesion and collagen synthesis on the surface, but in very small quantity. (fig 5).

CONCLUSIONS

1. The implant of polyurethaneurea doped with silver nanoparticles determines a very low inflammatory reaction at the level of mouse skin.
2. The polyurethaneurea doped with silver nanoparticles did not adhere to tissue.
3. This material favorise a rapid and good quality skin regeneration including the hair follicles.

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The urine test after the administration of the nonionic radiologic contrast substance Ultravist 370 to the cat

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The use of ionic iodine contrast substances (Odistan) for the cat, was up until recently quite problematic, casting a lot of doubts since this animal is highly sensitive to iodine, which in clinical terms is expressed by a varied and severe symptomatology, which eventually leads to the loss of the animal. These adverse reactions are based upon the important factor of the high osmolarity of the ionic iodine substances and on the sensitivity of each patient.

The discovery of the nonionic low osmolarity contrast substances, enables their use in the urology of the cat.

A complete examination of the urinary tract is comprised of both the study of the capacity of the kidney to function and the urine test.

Key Words: cat, radiology, urology, nonionic contrast substances

Material and Method

At this stage in the study, the urine test meant an analysis taking a physical and biochemical test both before the administration of the nonionic contrast substance (Sample 1) and after the administration of the substance at 1 hour (Sample 2), 3 hours (Sample 3), 6 hours (Sample 4) and 24 hours (Sample 5).

The urine was sampled from every patient; this process involved taking the samples into sterile trays from which the urine was sucked in sterile seringes and sent out to be examined.

The optimal sampling procedure was represented by cateterism for the males, the later being exposed to a mild tranquilization, and by bladder massage for the females as they represent the short urethra.

Results and discussions

For the physical test of the urine, the amount of urine collected at each moment in time, the color and transparency were taken into account. The collected amount of urine was approximately 10 ml, a lower amount of urine being collected 1 hour after the administration of the contrast substance and the initial sampling.

Taking into account the fact that the cat produces 100–200 ml of urine over a 24 hour period, it is possible to say that the volume of urine is approximately the same suffering no major changes after the administration of the contrast substance.

The color of the urine was different at different points in time after the administration of the contrast substance.

Before the administration of the contrast substance Ultravist 370 the collected urine had a slightly light yellow color. After the administration the color changed. The most noticeable change occurred 1hour after the administration of the substance.

Table 1 – Urinal test before the administration of Ultravist 370 to the cat

Den-sity	Leuco-cytes	Nitrates	pH	Erythro-cytes	Prote-ins	Glu-cose	Ascorbic acid	Ketonic bodies	UBG	BIL
1.025	75++	Neg.	7	Neg.	Neg.	Nor-mal	Neg.	Neg.	Nor-mal	1 mg/dl

Urine sediment test: - over 50 leucocytes/field
- frequent epithelial cells
- mucus filaments

A radiography of the urinary bladder done 15 minutes after the administration of the substance highlights the presence of the contrast substance, but in a low concentration.

Therefore the attempt to collect a urine sample at this time did not yeld any results.

The smell of the urine did not show the presence of any change, as it was the one characteristic to this species.

The samples were collected 1hour, 3 hours, 6 hours and 24 hours after the administration of the contrast substance.

Table 2 – Urinal test 1 hour after the administration of Ultravist 370 to the cat

Density	Leucocytes	Nitrates	pH	Erythrocytes	Proteins	Glucose	Ascorbic acid	Ketonic bodies	UBG	BIL
1.015	Neg.	Neg.	7	Neg.	Neg.	Nor-mal	Neg.	Neg.	Normal	Neg.

Urine sediment test: - 2-3 leucocytes/field
- rare erythrocytes
- Calcium oxalate microcrystals

An hour after the administration of the contrast substance, a slight decrease of the urine density as well as the presence of the leucocytes and erythrocytes in the urine sediment become noticeable.

The urine test three hours after the administration of the contrast substance highlights a rapid decrease in density as well as the presence of the epithelial cells and erythrocyctes in the urine sediment.

Table 3 – Urinal test 3 hours after the administration of Ultravist 370 to the cat

Density	Leucocytes	Nitrates	pH	Erythrocytes	Proteins	Glucose	Ascorbic acid	Ketonic bodies	UBG	BIL
1.005	Neg.	Neg.	8	10+	Neg.	Nor-mal	Neg.	Neg.	Normal	Neg.

Urine sediment test: - epithelial cells
- rare leucocytes
- ammonium magnesium phosphates
- 10-12 erythrocytes/field

Six hours after the administration of the contrast substance, the urine density is still lower than the previous values and the leucocytes re-appear in larger numbers.

Table 4 – Urinal test 6 hours after the administration of Ultravist 370 to the cat

Den-sity	Leuco-cytes	Nitrates	pH	Erythro-cytes	Proteins	Glucose	Ascorbic acid	Ketonic bodies	UBG	BIL
1.000	25++	Neg.	8	Neg.	100 mg/dl	Normal	Neg.	Neg.	Normal	Neg.

Urine sediment test: - 20 – 25 leucocytes/field
- rare calcium oxalate crystals

The urine density returns to the initial values (thevalues it had before the administration of the contrast substance) in 24 hours as erythrocytes, epithelial cells and microbial flora reappear as a result of the methods for collection.

Table 5 – Urinal test 24 hours after the administration of Ultravist 370 to the cat

Density	Leuco-cytes	Nitrates	pH	Erythro-cytes	Proteins	Glucose	Ascorbic acid	Ketonic bodies	UBG	BIL
1.030	75++	Neg.	7	Neg.	Neg.	Normal	Neg.	Neg.	Normal	1 mg/dl

Urine sediment test: - 2 – 3 erythrocytes
- epithelial cells
- microbial flora

General conclusions

1. In order get conclusive results for the urine exam, techniques for collecting it, (regarding the sex of the animal) as well as proper storage must be respected;
2. The administration of the contrast substance does not affect the amount of urine produced in 24 hours as it remains constant.
3. The urine physical test regarding the color reveals a change in the first hours after the administration, but the urine returns to its normal color in 24 hours.
4. The lab test of the urine highlights oscillations in urine density especially in the first 6 hours after the administration of the substance, but without any influence on the working state of the urinary tract;
5. The presence of the erythrocytes in the urine sediment might be explained by a minimal irritation of the urinal bladderas coming into contact with the contrast substance, or they might be a result of lesions provoked by the methods of collecting the urine (bladder massage, urethral probing);
6. The influence of the contrast substance on the urine is minimal, the substance not having a negative influence either on the filtering process or on the urine elimination.

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The study of some plasma minerals components (Na+, K+, Cl-, Ca++, and Mg++) in Wistar rats inoculated iv. with Walker 256 ascitic carcinoma correlated with daily deuterium depleted water intake

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The goal of this experiment was the achievement of an evaluation of the plasma level of some plasma mineral components (Na^+, K^+, Cl^+, Ca^{++}, and Mg^{++}) in Wistar rats inoculated iv. with Walker 256 ascitic carcinoma, correlated with the daily deuterium depleted water (DDW) intake. The experiment was achieved on twenty Wistar rats, distributed in four groups, each of them containing five adult animals. The results obtained permit us to come to the conclusion that, comparatively with the reference groups, the greatest differences regarding the plasma mineral components measured affect the group four, which was inoculated iv. with Walker 256 ascitic carcinoma and which consumed daily the deuterium depleted water. The way of the differences recorded in plasma samples were, predominantly, negatives and affected all the mineral components investigated. Thus, in group four (W 256 + DDW), comparatively with the reference groups one (R), two (DDW) and three (W 256), the percentage differences were negative for Ca^{++} (-7,90%, -1,17%, -21,13%), Cl^+ (-6,25%, -18,97%, -10,93%), K^+ (-14,87%, -21,61%, -36,21%) and positive for Na^+ (21,04%, 48,9%, 24,16%).

Key Words: deuterium depleted water, Walker 256, Na+, K+, Cl+, Ca++, Mg++

Introduction

The importance of the deuterium depleted water in cell biology, came first in the 80's.The deuterium depleted water can be produced by boiling the water many times or rather with electrical fission. In this process a lot of hydrogen gas spring up, which must be burned. Deuterium, an isotope of hydrogen, is part of the water in the nature and it is very important in human and animal organism. (SANETAKA SHIRAHATA, 1997) But about this the scientists was knowing, till some years ago, just very few things. The only exception is the isotope-phenomenon, which has been researched by decades (ALTERMATT, 1990). This means that the biochemical reactions in animals or humans are slower in deuterium rich fluid, maybe because the connection between the deuterium atoms. The research workers thinks that in absence of deuterium cells get some kind of shock, but while the healthy ones can adapt very fast to the new situation, the tumorous cells can't, so they will die (LAMPRECT, 1990).

"Life made a very quick and super regulating system for deuterium. This hydrogen isotope has a main role in cell division. Deuterium surplus or depletion blow over this control system" (SOMLYAI G, 1993, 1998). Deuterium depleted water can block the cell division (mitosis), but this can be harmful for the healthy cells too (ALTERMATT, 1990). Researches with mice in which human tumors was transplanted proved that, while they were drinking deuterium depleted water, the tumor became smaller or even disappeared. The mice in the control group died. The replacement

of tap water with deuterium depleted water in a drinking water for the mice diminishes the growth rate of the tumors, and the slight increase in the deuterium concentration stimulates tumour cells proliferation (BERDEA si col., 2001). But there is another problem with this type of water, because in the proccess of destillation (making deuterium depleted water) the very important minerals and trace elements disappears (KUSHNER and col, 2001). This experiment is proposed to investigate if the daily deuterium depleted water intake, for a period of six months, affect significantly the balance of some essential plasma minerals in healthy and Walker 256 ascitic carcinoma bearing rats (Na^+, K^+, Cl^-, Ca^{++}, and Mg^{++}), minerals which play a very important role in normal and tumor cells biology, affecting their capacity of proliferation and mitosis.

Materials and methods

Were made up four groups of adults rats, each of them containing 5 animals per group. Group 1 (n = 5), was used as reference group, to obtain the reference values for some clinical, haematological and biochemical parameters investigated, and had at the beggining of the experiment a body weight of 145 ± 2,5g/animal. Group 2 (n = 5) with a initial body weight of 140 ± 2,5g/animal, was used for the evaluation of the biological effects of deuterium depleted water intake. Group 3 (n = 5) with a body weight at the beggining of the experiment of 148 ± 3g/animal, was inoculated iv. with 2 ml of Walker 256 ascitic carcinoma. Group 4 (n = 5) with a body weight at the beggining of the experiment of 126 ±2,5g/animal, was inoculated iv. with 2 ml of Walker 256 ascitic carcinoma and was drinking the deuterim depleted water for the whole period of the experiment. During the experiment, all the animals were evaluated from the clinical point of view, by weekly measuring of body weight, tumour volume and aspects of behaviour. In the period of experiment the animals were nourished by standard fodder (MARCUS, 2004). After six months from the beggining of the experiments, the animals were killed, under narcose with ether, and the blood samples were gathered by heart punction. From the plasma samples obtain by blood centrifugation were measured the mineral components investigated (Na^+, K^+, Cl^-, Ca^{++}, and Mg^{++}), by means of semiautomatic devices STAT-FAX 1904 Plus. The results obtained were statisticaly processed, by determination of the arithmetical mean, standard deviation, the percentage differences (%) and the „t" student test.

Results and disscutions

In the table no.1 are presented the statistical processing of the results obtained with respect to the plasma mineral components investigated.

Table no. 1

Statistical processing of the results obtained in the experimental groups with respect to the plasma mineral components measured at the end of the experiment

P Group	Statistics	**Na** (mmol/l)	**Cl** (mEq/l)	**K** (mEq/l)	**Ca** (mg/dl)	**Mg** (mg/dl)
Group 1 (M)	**Mean**	***136,6***	***95,2***	***3,476***	***10,098***	***2,084***
	DevSt	*18,875*	*8,105*	*0,783*	*1,074*	*0,106*
Group 2 (DDW)	**Mean**	***88,4***	***106,6***	***3,68***	***9,468***	***2,04***
	DevSt	*19,424*	*5,029*	*0,372*	*1,150*	*0,866*
	D%	-54,524	10,694	5,543	-6,653	-2,156
	Ftest 1	*0,957*	*0,377*	*0,179*	*0,898*	***0,001***
Group 3 (W 256)	**Mean**	***131,2***	***99,4***	***4,122***	***11,336***	***2,358***
	DevSt	*19,162*	*5,594*	*0,588*	*0,754*	*0,547*
	D%	-4,115	4,225	15,672	10,920	11,620
	D%	32,621	-7,243	10,722	16,478	13,486
	Ftest 1	*0,957*	*0,377*	*0,179*	*0,898*	***0,001***
	Ftest 2	*0,977*	*0,490*	*0,592*	*0,510*	***0,007***
Group 4 (W 256 + DDW)	**Mean**	***173***	***89,6***	***3,026***	***9,358***	***2,288***
	DevSt	*30,975*	*1,140*	*0,418*	*0,384*	*0,423*
	D%	21,040	-6,25	-14,871	-7,907	8,916
	D%	48,901	-18,973	-21,612	-1,175	10,839
	D%	24,161	-10,937	-36,219	-21,137	-3,059
	t- test 1	*0,360*	***0,002***	*0,251*	***0,071***	***0,020***
	t- test 2	*0,388*	***0,013***	*0,828*	***0,056***	*0,193*
	t- test 3	*0,226*	***0,013***	*0,677*	*0,298*	*0,799*

Legend: M - reference group, DDW - duterium depleted water group, W 256 – Walker 256 ascitic carcinoma group, W 256+DDW - Walker 256 ascitic carcinoma + deuterium depleted water group. D% - percentage differences, DevSt – standard deviation.

One can notice that, comparatively with the reference groups 1, the greatest differences regarding the plasma mineral components evaluated (Na^+, K^+, Cl^-, Ca^{++}, and Mg^{++}) affect the group 4, which was inoculated iv. with Walker 256 ascitic carcinoma and which was drinking daily the deuterium depleted water.

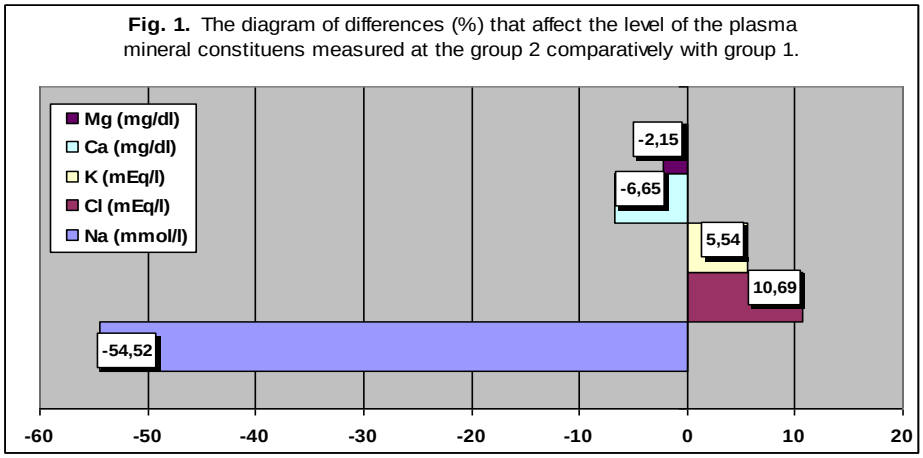


Fig. 1. The diagram of differences (%) that affect the level of the plasma mineral constituens measured at the group 2 comparatively with group 1.

The way of the differences recorded in the value of plasma minerals were, predominantly, negatives and affected almost all the components investigated.

Fig. 2. The diagram of the differences (%) that affect the value of the plasma mineral constituens at the group 3 comparatively with the groups 1 and 2 .

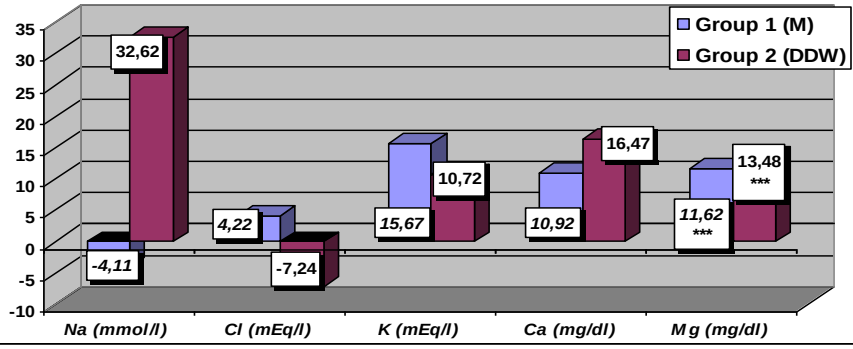
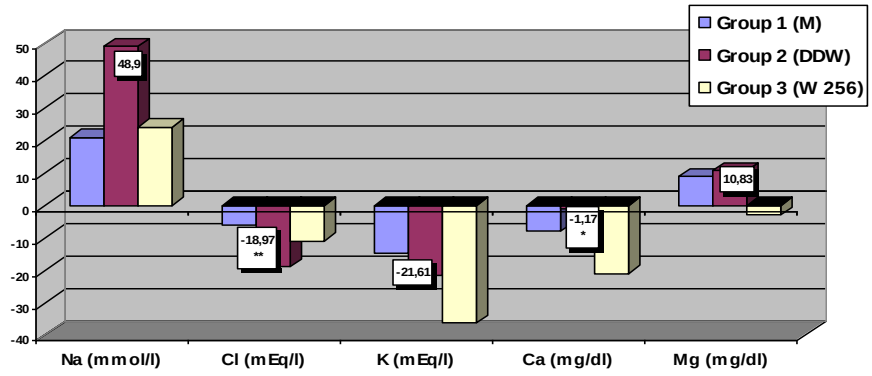


Fig 3. The diagram of the differences (%) that affect the value of the plasma mineral constituens at the group 4, comparatively with the groups 1, 2 and 3.



In the next figures 1, 2 and 3 are showed the diagrams of the percentage differences (%) between the groups that were drinking the deuterium depleted water and were inoculated with Walker 256 ascitic carcinoma and the reference group 1. One can see that, in the case of the group 2 (DDW), comparatively with the reference group 1, the main differences are negative, and affect Na (-54,5%), Ca (-6,65%) and Mg (-2,15%), but without statistical significance, excepting Mg ($p<0,001$). One the other hand, in the case of group 3 (inoculated with Walker 256 ascitic carcinoma) the differences recorded are inconstant and mainly possitive, affecting particularly the plasma level of Mg^{++}, whose percentage differences comparatively with the group 1 and 2, are of +11,62% and +13,48% ($p<0,001$).

Finally, the most significant differences were recorded in the case of the group 4 (inoculated with Walker 256 ascite and drinking the deuterium depleted water) and affected, comparatively with the groups 1, 2 and 3, the value of the Cl^- (-6,25%**, -18,97%**, -10,93%*), K^+ (-14,87%, -21,61%, -36,21%), Ca^{++} (-7,90%*, -1,17%*, -21,13%) and Mg^{++} (-3,05%* comparatively with the group 3). In the same time, all the percentage differences were strongly positive at the group 4 in the case of Na^+ (21,04%, 48,90%, 24,16%), even if they haven't any statistical significance.

Conclusions

1. All the values of the plasma mineral components (Na^+, K^+, Cl^-, Ca^{++}, and Mg^{++}) investigated at the animals from the reference group oscillated into the normal limits specified by speciality literature (MARCUS, 2004).
2. No significant percentage differences for the plasma mineral constituents investigated at the group 2 (deuterium depleted water), excepting Mg^{++}, whose plasma level decrease significantly, comparatively with the reference group 1.
3. Inconstant and without any statistical significance differences recorded at the group 3 (inoculated with Walker 256 ascite), comparatively with the groups 1 and 2, excpting again Mg^{++}, whose plasma level increase very significantly.
4. Significant negative percentage differences were recorded at the group 4 (inoculated Walker 256 ascite + DDW) for Cl^-, K^+, Ca^{++} and Mg^{++} and strongly possitive for Na^+, but without any statistical significance.
5. There is no any evidence that the daily deuterium depleted water intake influence the balance of the Na^+, K^+, Cl^-, Ca^{++}, and Mg^{++} in healthy animals, but disorders of this minerals were noticed at the animals inoculated iv. with Walker 256 ascitic carcinoma, correlated with the deuterium depleted water intake.

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Morpho-pathological changes in the anaerobic enterotoxaemia in lambs and aspects concerning the histopathological diagnose of this disease

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In lambs, enterotoxaemia is caused by C. perfrigens B-type and scarcely by A, C, D-types. Enterotoxaemia with B-type (lamb anaerobic dysentery) may be frequently observed in 2-12 day old animals and rarely in three-week old, when it may be identified especially at the end of the endemic state. This disease appears especially in well-grown edacious lambs, from sheep with much milk; in some flocks, the disease may affect the lambs with congenital hypotrepsia, from badly maintained mothers. This disease burst and evolution is favored by bad pedological-climatic, maintenance and feeding conditions, and also by the parasitism state (2, 4). The morpho-pathological image ranges according to age, toxotype and alimentation and maintenance. It is rather characteristic, especially in acute and sub acute evolutions, being expressed by fibrin-hemorrhagic, respectively fibrin-necrotic enteritis. The intestine content is sanguinolent (1, 3, 5, 6).

Key Words: lamb, enterotoxaemia, C. perfrigens, histology

MATERIALS AND METHODS

Our researches have been performed during February – May 2006, through the necropsy of 4-5 week-old 12 young sheep bodies (lambs), males and females, the breed Turcana and Tigaie, taken from households from the county Timis, in order to elucidate the death cause; at the necropsy examination, the corpses presented lesions specific to the anaerobic enterotoxaemia (dysentery).

After evisceration, we have performed the profound macroscopic examination of the organs taken into study. We have taken samples from stomach, lung, heart, liver, kidney, lymph and renal nodes and encephalon for histopathological examination. He samples have been processed with the help of the paraffin technique, colored with the methods HEA and Giemsa. The histopathological preparations have been examined microscopically, and the changes observed are presented iconographically.

RESULTS AND DISCUSSIONS

At the external **body examination**, we have noticed the dehydration state and the lack of skin elasticity.

Most cases presented a bad maintenance state, enolftalmia and paleness of the apparent mucous. The anal region was dirty, with brown faeces and disgusting smell.

At the **internal examination**, we have remarked the presence of a yellowish liquid in the thoracic cavity (serous exudation). Within the pericardial cavity, we have noticed the presence of a yellowish, friable mass, adherent to epicardium in some locations (fibrinous pericarditis). Within the abdominal cavity, we have observed the existence of 120 ml citrine liquid (in 8 cases) – ascitis. The entire small intestine was lax, red-black, containing numerous gas bubbles under serous and along the mesentery.

The macroscopic examination has revealed changes of the physical-structural particularities (shape, size, color, aspect, lobular design, consistence, etc.) in stomach (rennet), intestine, lung, heart, liver, kidneys, lymph nodes and encephalon.

The stomach (rennet)

Necropsically, we have opened along the great curve and examined the content, mucous and sectioned wall (aspect, color and width).

Microscopically, we have observed necrosis and desquamation of the covering and glandular epithelium; desquamative catarrh (mucocellular) within the lumen of the glandular tubes; degenerative changes of variable intensities of the covering and glandular epithelial cells; mucous hypertrophy, successive to the edema congestion and leukodiapedesis; sub mucous congestion and edema – catarrhal gastritis.

Small intestine

Because the pathological process was localized especially within the jejunum and ileum, it has determined their opening on the small curve, in longitudinal axe, along the mesentery and the mesenteric lymph nodes insertion.

Macroscopically: tumid intestine wall, with edema; it presented gas bubbles within the serous, and a brown-red color on the section; the content was semi-fluid, red-black, and we could notice, after its removal, the brown-red mucous, which has maintained successive to the washing, too – diffuse hemorrhagic enteritis (Fig. 1).

Microscopically, we have observed: the presence of a fibrin-hemorrhagic exudation at mucous surface and within the lumen; the necrosis of the covering and glandular epithelium; humid necrosis of the lymphoid follicles and of the neighbor tissue; massive leukocyte infiltrate at mucous base; gas bubbles within the sub mucous; hypotrophies in the muscle tissue; the presence of numerous germs within the mucous structure – fibrin-hemorrhagic jejunitis (Fig. 2, 3).

Lung

Microscopically: hyperemia of the alveolar capillaries, with the presence of the joint red-black erythrocytes within the lumen; most pulmonary alveoli had a reduced lumen, adjacently there were numerous areas with compensatory alveolar emphysema. In other microscopic areas, we have noticed numerous alveoli full with a basophilic liquid, which determine lumen diameters, so that they change their architectonic from hexagonal into oval. These modified structural particularities define the passive pulmonary congestion and the stasis pulmonary edema.

Heart

Microscopically, we have noticed: a fibrin network (containing micro- and macrophages in its loops), attached by the tumid epicardium with edema, partially or totally desquamated mesothelium, sub-epicardial mesenchymal reaction; myocardium in transversal section. Within the myocardocyte cytoplasm, we have identified numerous basophilic granulations, determining the aspect of turbid intumescence – fibrinous pericarditis and protidic myocardosis (Fig. 4).

Liver

Microscopically, the hepatocytes from most lobules presented numerous optically-empty vacuoles, of different shapes and sizes, which have determined compressions upon the nucleus (small, edgy or absent). These vacuoles represent the lipid quartering place, which through the paraffin technique were solubilized in organic solvents (toluene, xylene, benzene) and so the place remained empty. Some hepatic lobules present scarce small achromatic areas, with a higher density around the centro-lobular vein.

At big magnification (x20, x40), we could observe hepatocytes with nucleus vacuoles and other serious alterations; reduced in volume, of various shapes; hyper-chromatic located marginally, absent in other locations. These lesions are characteristic for the hepatic steatosis (Fig. 5).

Kidneys

Microscopically, we have observed: interstitial and glomerular emphasized hyperemia; accumulation of proteinaceuous material within the glomerular area and the presence of the hyaline microthrombs within capillaries; tumid nephrocytes and their necrobiosis on large areas – passive renal congestion.

At the examination of the samples taken from the seven cases with protidic nephrosis, at small magnification (x6, x10), we have observed numerous uriniferous tubes with numerous vacuoles within the nephrocyte membrane (mitochondrial swellings), optically empty, without delimiting membrane; renocyte nuclei presented necrobiotic changes; numerous uriniferous tubes with hypertrophied epithelium, decreased lumen or even destroyed; some nephrocytes had a granular cytoplasm – renal granular-vacuolar degeneration (Fig. 6).

Mesenteric lymph nodes

Microscopically, at small magnification, we have observed red areas, on a grey-blue background, subcapsular, perifollicular and peritrabecular; at big magnification (x20, x40), we have observed peritrabecular perifollicular and subcapsular erythrocytary infiltrates – hemorrhagic lymporeticulitis-lymphonodulitis in focuses.

Encephalon

Microscopically, we have noticed: vascular ectasia, perivascular and perineuronal edema; neurono-dystrophies and neuronolyses – cerebral edema.

CONCLUSIONS

The anaerobic enterotoxaemia was macroscopically and microscopically diagnosed in 12 4day-5 week old lamb bodies.

Macroscopically, we have noticed characteristic lesions: diffuse hemorrhagic jejunal-ileitis / fibrinous hemorrhagic; unspecific lesions: catarrhal gastritis, congestion and stasis pulmonary edema, protidic-lipidic cardiac-hepatic-renal dystrophies; hemorrhagic lymphonodulitis in focuses and cerebral edema.

The microscopic examination of the intestinal, pulmonary, cardiac, hepatic, renal, lymph nodular and encephalon lesions confirm the macroscopic aspects. The enteric lesions reflect themselves upon the whole organism, affecting most organs, especially, finally, the kidneys, heart, lungs, mesenteric lymph nodes and the central nervous system.

The diagnostic of anaerobic enterotoxaemia in lambs was established histopathologically through the identification of the hemorrhagic jejunal-ileitis / fibrinous hemorrhagic, of numerous germs of *Cl. perfrigens* within the mucous structure and through the presence of numerous gas bubbles within the intestinal submucous and the mesentery structure.

Fig. 1. Diffuse hemorrhagic enteritis and gas bubbles within the subserous

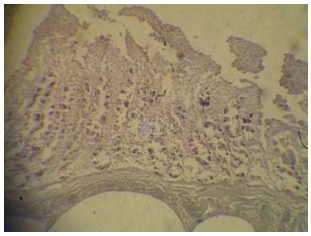

Fig. 2. Fibrinous hemorrhagic jejunitis: fibrinous-hemorrhagic exudate in lumen, at mucous surface and in the lamina propria, gas bubbles within the subserous

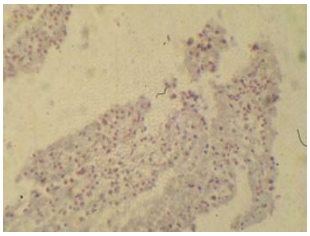

Fig. 3. Fibrinous hemorrhagic exudates and numerous germs (Cl. perfrigens) within the fibrinous hemorrhagic exudates from the lamina propria

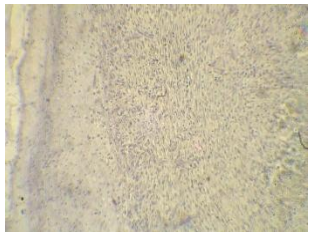

Fig. 4. Fibrinous pericarditis and protidic myocardosis

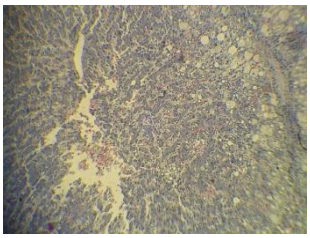

Fig. 5. Exolobular steatosis and hepatic centrolobular necrosis

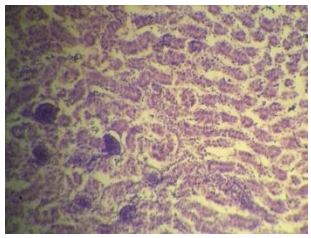

Fig. 6. Lamb enterotoxaemia – parenchymatous tubulonephrosis

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Morphopatological aspects of distrophic nephropaties in dogs

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After the examination of 127 dog bodies, 93 cases of distrophic nephropaties were selected. After histopatological exams they were classified in lipidic protidic, pigmentar and mineral nephropaties.

The nephrosis were the result of either progressive alterations of urinary filtration or the disease states with systemic effects and of course renal ones.

Key Words: dog, kidney dystrophy, histology

MATERIAL AND METHODS

Between 2002 and 2007 , the study material consisted of kidneys prelevated from 127 dead or euthanized dogs suffering from several organopathies, out of the 127 cases, 93 were diagnosed with dystrophic nephropathies of various intensities.

After necropsic examination, organ samples were prelevated for histopathological investigations. Each case was prelevated kidney fragments, as well as fragments of different organs, physiologically closely related (heart, liver, lung, intestine, spleen, etc.).

Organ samples were fixated in formaldehyde 10%, then paraffin - included. The histological sections measuring 5 µm were stained Haematoxilin - Eosin (HE), Haematoxilin - Eosin - Methyl Blue (Tricromic - Masson, HEA), Haematoxilin - Eosin - Safran (HES), Periodic- Acid-Schiff (PAS) and Periodic - Acid - Schiff - Light green (PAS-light green) and a general coloration with Mayer Haematoxilin.

RESULTS

The research made followed mainly the histopathological aspects of kidney's dystrophies was realized through the identification and localization of the metabolites involved due to the tinctorial affinities of histopathological colourings.

The etiopathogenesis of the lesions was the result of corroboration of anamnetic date, clinical observation papers and histological lesions observed in other examined organs.

The systematization of kidney dystrophies was realised after the localization of the metabolite (glomerular dystrophies, tubular and interstitial ones) and after the biochemical belonging to the involved metabolit (lipids, protids, pigments, minerals) (11, 15).

Trigliceridosis (steatosis) of the kidney was identified at a large number of cases with tubular intraepithelial localization and in association with other protidic dystrophies (granular and hyaline dystrophy)(13, 14).

The dystrophy was noticed throughout the triglicerides from the tubular epithelium, in all the parts which, in the preparates obtained through the paraffin inclusion is presented with unequal vacuoles, mostly big, spheric and in general with well defined contours, placed at the apical regions of the cells pushing the nuclei with different stages of necrobiosis at the bottom of nephrocytes.

The repartition of steatosis suggests that at the origin of this pathological process stay either different toxic substances secretated preferential in the distal parts of the urinary tubes or the nephrotical syndrome installed next to glomerulonephritis **(Fig. 1, 2, 3, 4).**

From the category of hidroprotidic dystrophies were noticed the granular and hydric dystrophy.

Granular dystrophy was noticed as simple primary or secondary lesion to other kidney lesions, confirming the existing data in the speciality literature(6, 7, 17).

Histological aspect corresponded to the classical description, the lesion being more frequent in the proximal tubes whose cells appear swell to the perishing of the tubular lumen **(Fig. 5)**.

Granulations, fine blue or red in HEA, correspond to the mithocondrial swell determined by the absorbtion and coagulation of proteins.

The lesson was correlated to the anoxy determined by vascular spasms secondary to the septicemic stages, the nephrotoxic substances action (excessive use of aminoglicozides - gentamicine).

Hydric dystrophy was noticed at dogs with tubular localization through hiperhydratation and vacuolization of tubular epithelial cell cytoplasma.

Hydropic (osmotic) nephrosis with tumefied cells with fine cytoplasma and nuclei in necrobiosis as well as basal vacuolization, extracelular through accumulation of water between the basal area of the nephrocytes and basal membrane, with the dislocation of epithelium under the form of a cellular ring thar suffers degeneration and necrosis **(Fig. 6, 7, 8).**

This form of hydroprotidic dystrophy was noticed at cahectic and dehydrated dogs with severe gastroenteritis expressed through diarrheea and vomit, excessively hydrated.

Kidney intracellular hyalinosis was noticed in proximal tubes under the form of unequal spheres, homogenous, with various dimensions, glassy, bright red in HEA and intensively PAS+ **(Fig. 9, 10).**

Intracelular spheres are giant heterofagolysosomes that were formed when the absorbtion mechanism of filtrate was saturated or overloaded(3).

After the rupture of the apical pole, the spheres gather in the lumen under the form of hyaline spheres, made of albumines and mucoproteins as homogenous bodies, glassy, red or pink **(Fig. 11).**

Hyalin cylinders were noticed also in the collector system from the medulla under the form of big, round bodies. The increased density of these bodies can produce the blocking of collector system (1, 2, 8, 9)**(Fig. 12).**

Focalized hyalinizations were noticed at the basal membranes of the capillaries and glomerular capsula and also in the media of the interlobular artheries **(Fig. 13, 14, 15).**

Renal amyloidosis was identified at Malpighi corpuscles and the interstitium of kidney medulla.

Diffuse glomerular amyloidosis consisted upon the deposit of amyloid in the whole kidney glomerul. Amyloid deposits were noticed in the afferent and efferent artheries wall, metaartheries, but also mesangium, transforming between the glomerules in a spheroidal mass of amorph material in wich are disseminated the picnotic nuclei of local cells (4, 12) **(Fig. 16, 17).**

In the interstitial medular localization, the amyloid was deposited on the conjunctive stromal fibres, leading to the compression of urinary tubes **(Fig. 18).**

Kidney haemosiderosis was identified at dogs with haemoragipar syndrome, the ferruginous pigment being under the form of a granular material, brown in HEA at the tubular epithelium level **(Fig. 19).**

Colemical bilirubinic nephrosis or icterical one was noticed at dogs diagnosed with contagious hepatitis and babesiosis.

Macroscopically, the mucosa, skin, subcutaneous tissue and organs showed a yellow orange colour. Histologically, in HEA, were noticed yellow-brown deposits in the tumefied tubular epithelium as well as in the intraluminal biliar cilindres **(Fig. 20, 21, 22).**

Biliary pigments were noticed in other tissues: the macrophagic - monocitary cell system(10).

Crystalin dystrophy was noticed at three cases in the antifreeze intoxication. Calcium oxalate was noticed in the urinary tubes but also in the interstitium under the form of yellow crystals with radiary disposition and rephringent (14)**(Fig. 23, 24).**

Distrophic calcification was noticed mainly in the cortical at the tubular level.

Tissular epithelial necrosis followed by the constitution of necrotic detritus in the basal membrane determined the fixation of calcium ions. Necrotic detritus mineralized had granular aspect, intensive haematoxilinical and PAS+ **(Fig. 25).**

Metastatic kidney calcification at one dog was correlated with hyperparatiroidism.

So, as a consequence of hypercalcemia and hypercalciuria with paratiroidian origin, mineralizations included tubular epitheliums, basal, glomerular and tubular membranes and even interlobular artheries intima **(Fig. 26, 27, 28).**

Lesional table of methastatic calcification was completed by diffuse mineralization of myocardium, endocard and gastric mucosa.

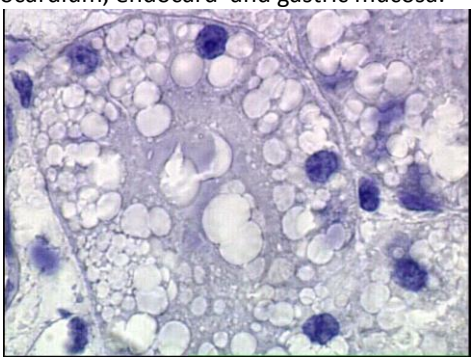

Fig. 1 Kidney. Tubular steatosis
Col. Hematoxiline Mayer, x 1000

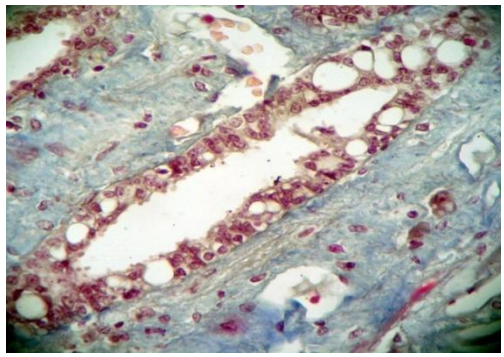

Fig. 21 Kidney.Colector tubes steatosis
Col.HEA, x 1000

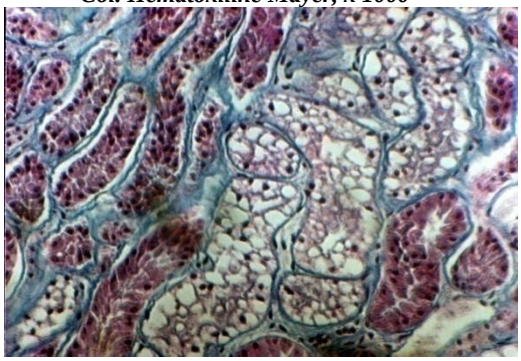

Fig. 3 Kidney. Tubular steatosis
Col. HEA, x 200

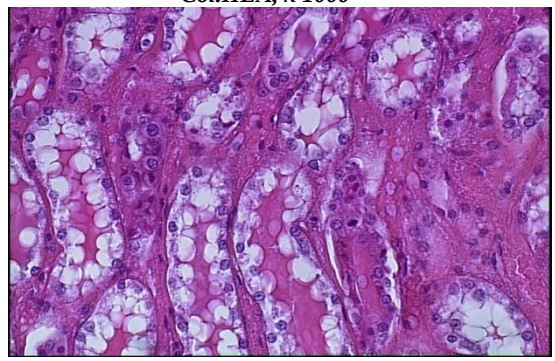

Fig. 4 Kidney. Tubular steatosis
and hyalinosis
Col. HE, x 200

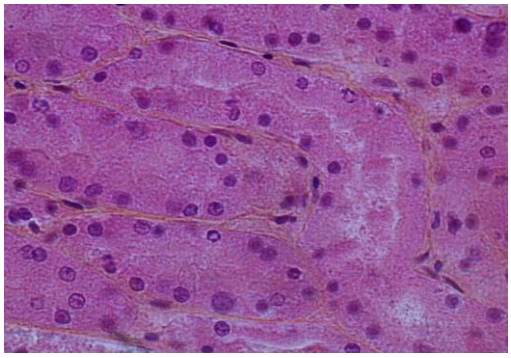

Fig. 5 Kidney. Granular tubular distrophy
Col. HES, x 400

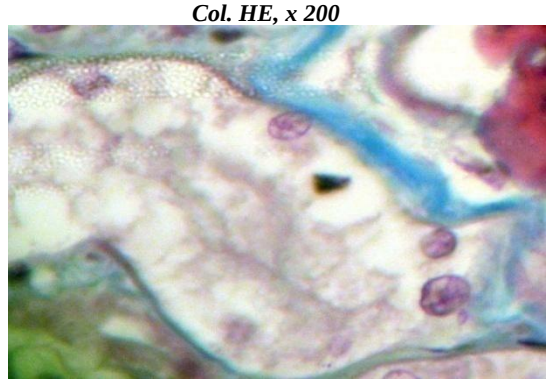

Fig. 6 Kidney. Hidric tubular dystrophy
Col. HEA, x 1000

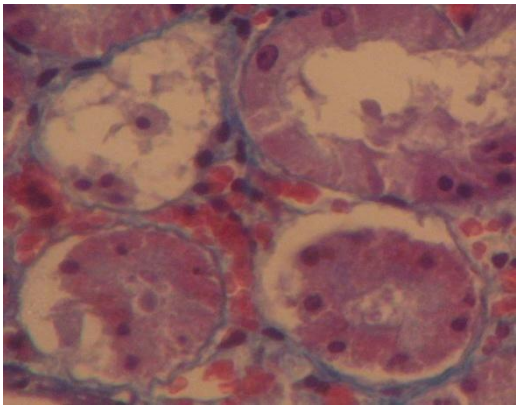

Fig. 7 Kidney. Hidropic tubular distrophy
Col. HEA, x 200

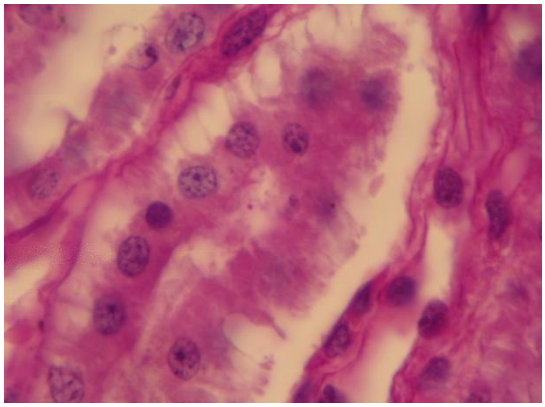

Fig. 8 Kidney. Hidropic tubular dystrophy
Col. PAS, x 1000

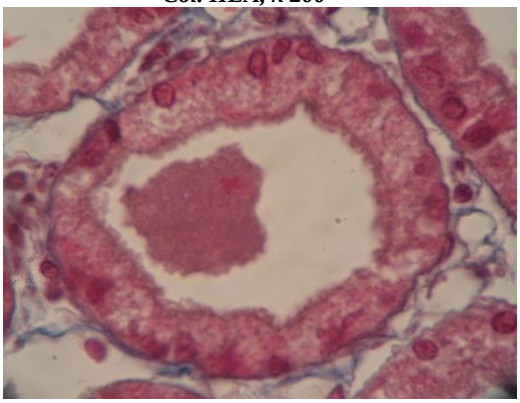

Fig. 9 Kidney. Tubular hyalinosis
Col. HEA, x 400

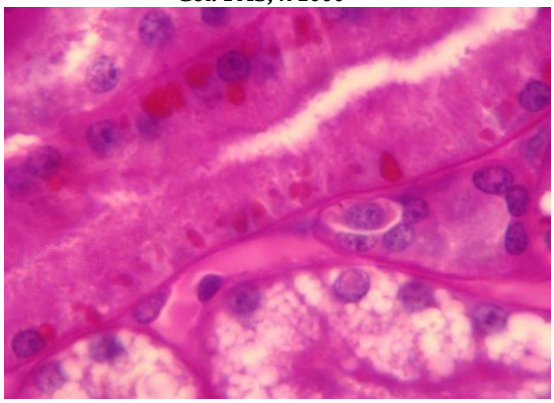

Fig. 10 Kidney. Tubular hyalinosis
Col. PAS, x 1000

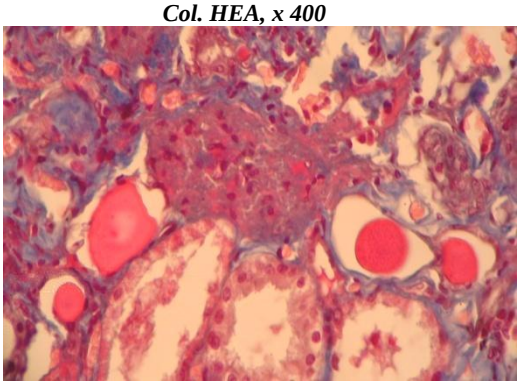

Fig. 11 Kidney. Hyaline cilindres
Col. HEA, x 400

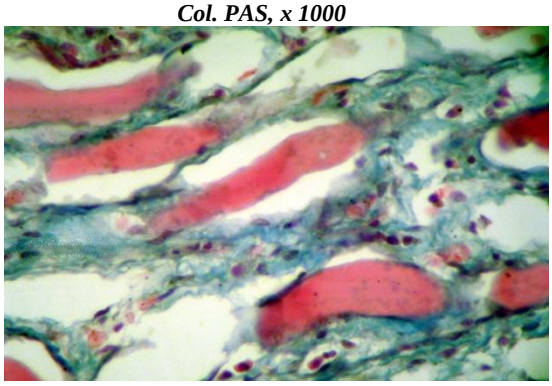

Fig. 12 Kidney. Hyaline cilindres
in the collector tubes
Col. HEA, x 400

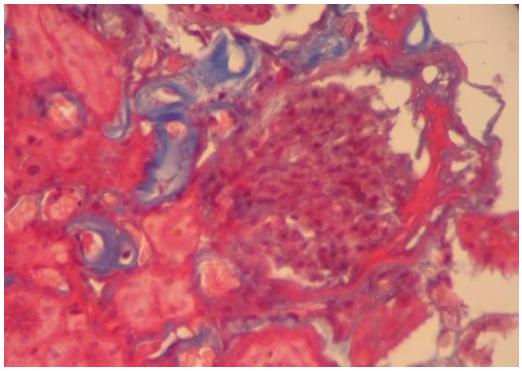

Fig. 13 Kidney . Hyalinisation of capsule basal membrane
Col. HEA, x 200

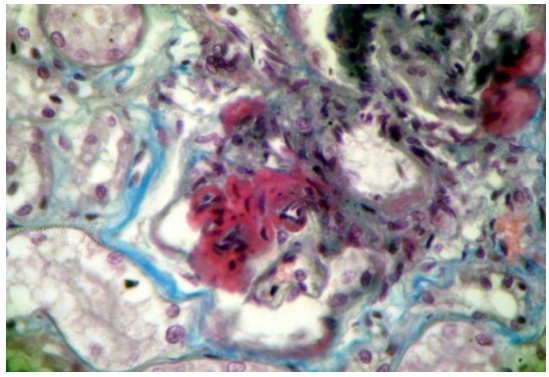

Fig. 14 Kidney. Hialinisation of capillary membranes
Col. HEA, x 200

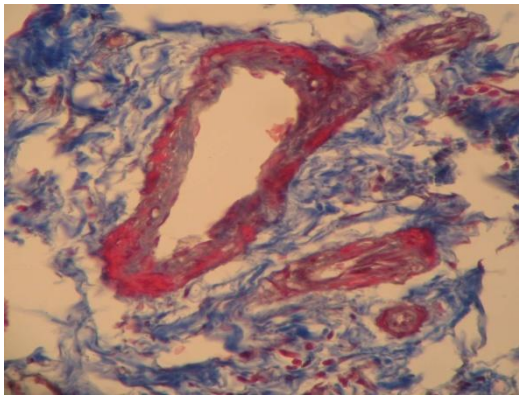

Fig. 15 Kidney. Hyalinisation of the interlobular artheries media
Col. HEA, x 200

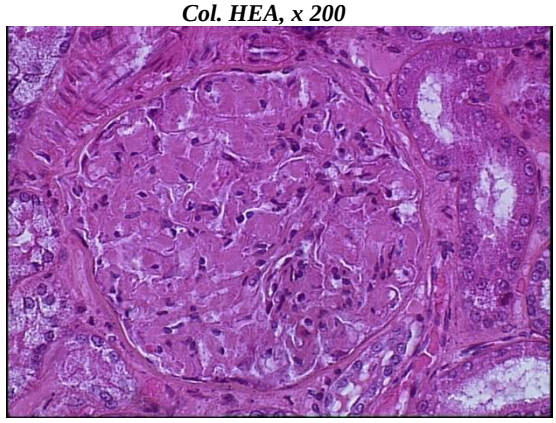

Fig. 16 Kidney. Glomerular amiloidosis
Col. HE, x 400

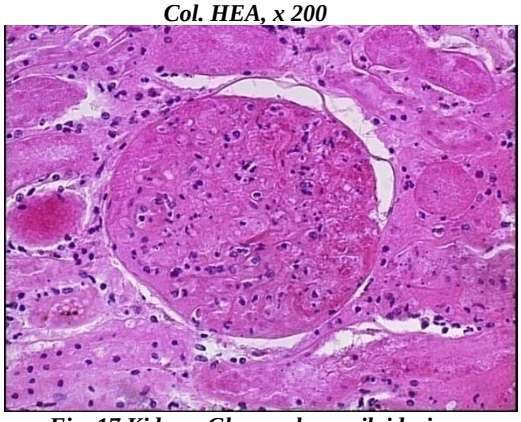

Fig. 17 Kidney. Glomerular amiloidosis
Col. HE, x 200

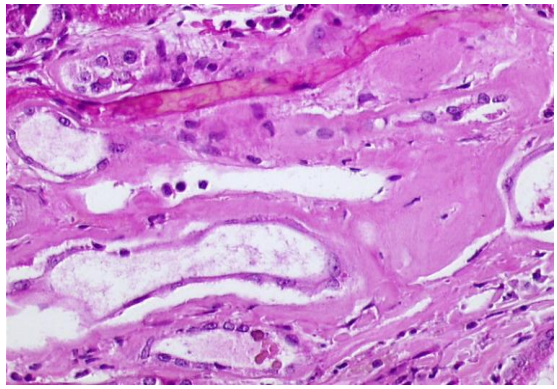

Fig. 18 Kidney. Interstitial amiloidosis
Col. HE, x 400

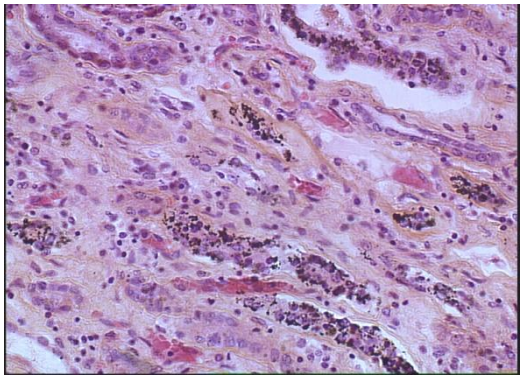

Fig. 19 Kidney. Tubular epithelial haemosiderosis Col. HES, x 400

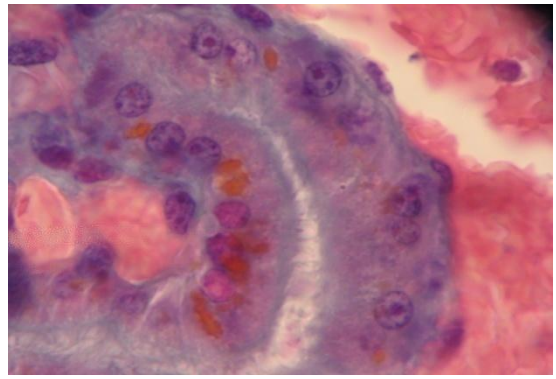

Fig. 20 Kidney. Colemical tubular distrophy Col. HEA, x 1000

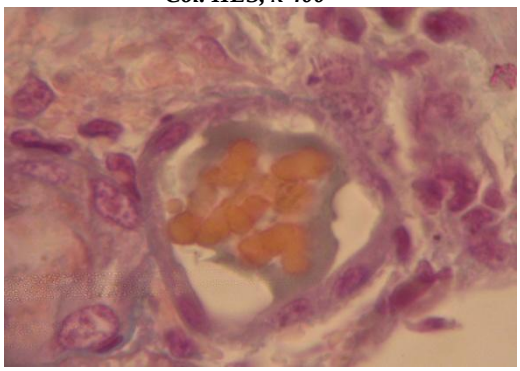

Fig. 21 Kidney. Colemical tubular distrophy Col. HEA, x 1000

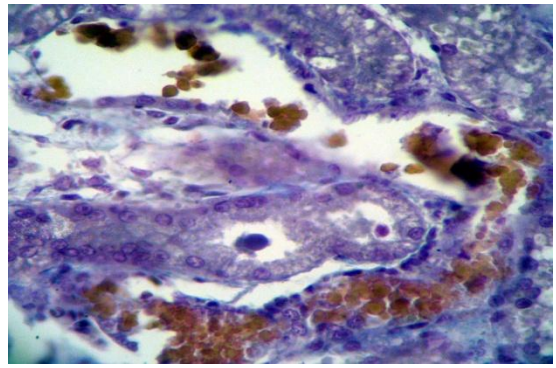

Fig. 22 Kidney. Colemic cilindres Col. HEA, x 400

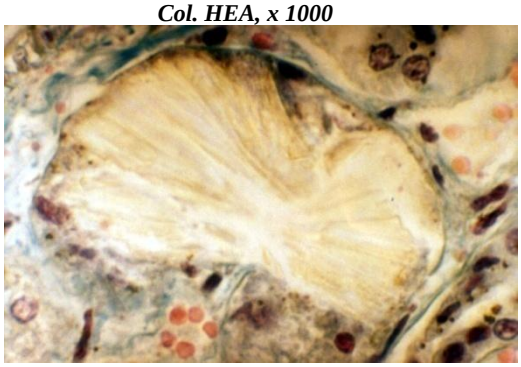

Fig. 23 Kidney. Oxalic tubular nephrosis Antigel intoxication Col. HEA, x 1000

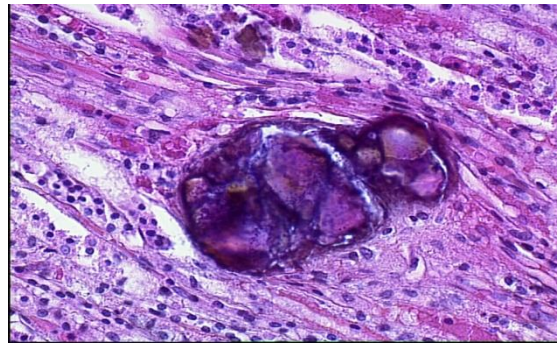

Fig. 24 Kidney. Oxalic interstitial nephrosis Antigel intoxication Col. HEA, x 1000

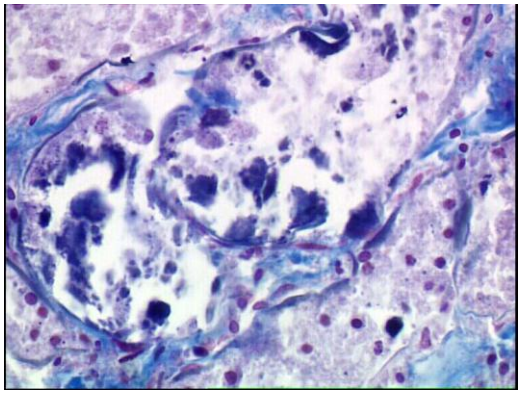

Fig. 25 Kidney. Tubular distrophic calcification Col. HEA, x 400

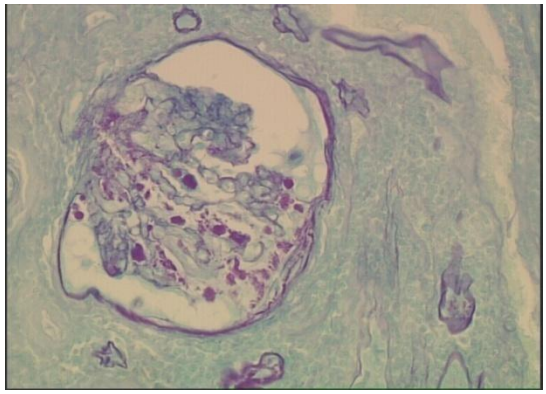

Fig. 26 Kidney. Metastatical calcification Hyperparatiroidism Col. PAS light green, x 400

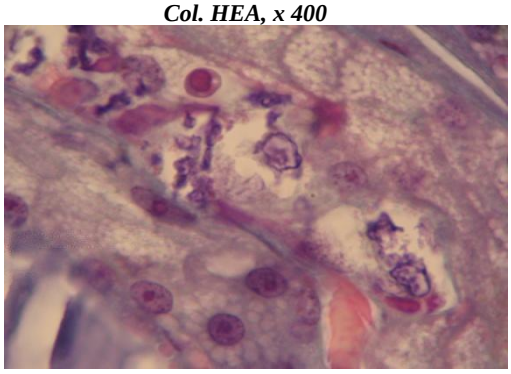

Fig. 27 Kidney. Metastatical calcification Hyperparatiroidism Col. HEA, x 1000

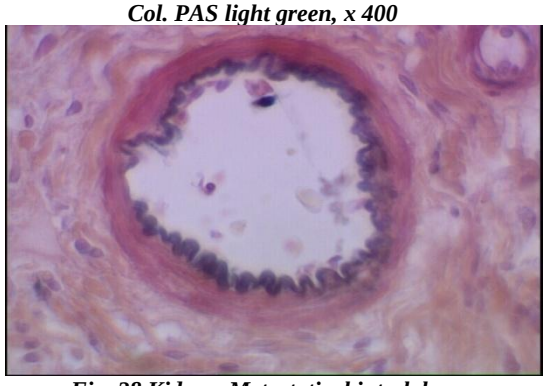

Fig. 28 Kidney. Metastatical interlobar intimal calcification Hyperparatiroidism Col. HES, x 200

CONCLUSIONS

Hydroprotidic nephrosis was presented through the granular dystrophy and through hydric dystrophy, changes correlated with hydroelectrolitical balance and nephrocyte permeability.

Hyalin intracellular nephrosis and cilidruria showed the supersaturation and overload of the tubular absorbtion mechanism.

Amyloid dystrophy was mainly diffuse at the level of kidney glomerul.

Colemical nephrosis was histologically showed in HEA colouring through yellow-brown deposits in the tumefied tubular epithelium as well as intraluminal biliar cilindres.

Metastatic kidney calcification associated with the mineralization of myocard, endocard and gastric mucosa was showed at a dog with hyperparatiroidism.

Antifreeze intoxication at 4 dogs was showed at kidneys through the tubular and interstitial crystalin dystrophy.

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Influence of preserving temperature on the biochemical transformations of trout musculature

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In order to assess the biochemical transformations in trout musculature preserved by cold for different periods of time, at different values of the storing temperature, it was performed the sarco-plasmatic protein extraction from muscular tissue samples. The extracts were analyzed for determination of the protein content, of the electrophoresis distribution of protein and enzymatic activities of superoxid dismutase.

There were taken 20 samples of trout which were divided in 4 lots of 5 samples. Lot no 1was formed by samples refrigerated for 48 hours at 2-4°C, while the second lot was represented by samples of trout frozen continuously for 1 week. The third lot is formed by samples of trout which was frozen continuously for 4 weeks, and lot no 4 was frozen for 4 months.

It was observed a supplementary protein strip only for protein extracts obtained from the trout frozen for 4 months. We have to mention that this fraction presented the greatest electrophoretic mobility. The cleavage of protein observed at the frozen fishes demonstrates the impossibility of annulment of proteolysis in fish meat at a temperature of -18…-20°C.

Determination of the SOD activity revealed significant differences between the refrigerated trout and the frozen one, due to the partial inactivation of the enzyme by freezing. The investigations revealed the smallest activity for the trout frozen for 4 months. There weren't registered significant differences between SOD activity in the extracts obtained from refrigerated muscular tissue and muscular tissue frozen for 1 week.

Key Words: Lipid peroxidation, quality, nourishing value, fish

Fish meat, as well as farm animal meat forms an important food category for humans, representing a rich source of nitrates substances. It brings a great intake of iron and represents the second fluoric source after tee, it contains great quantities of phosphorus (200-350 mg %), and the vitamins which are found in great amount are represented by niacin or PP vitamin, riboflavin, pyridoxine, panthotenic acid, folic acid, B_{12} vitamin, A vitamin (1000-2000 UI%), D vitamin (1500-4000 UI%).

The advantages of the fish consumption are determined by the specific dynamic activity of intensification of the metabolism, the increase of the defense action of the organisms against infections and different toxic substances.

Material and methods

In order to observe the type and the volume of lipid peroxidation at the fish musculature level, there were investigated the modifications suffered at antioxidant enzymes level.

There were sampled a number of 20 trout samples which were divided in 4 lots of 5 samples each, as it is represented in table no 1. Lot no 1 was formed by samples refrigerated for 48 hours at 2-4^{0}C, while the second lot was represented by samples of trout frozen continuously for 1 week. The third lot is formed by samples of trout which was frozen continuously for 4 weeks, and lot no 4 was frozen for 4 months.

Table no 1

Period and temperature of preservation of the trout sample lots analyzed

Lot no	Period	Temperature (^{o}C)	Musculature area	Termic behaviour
Lot 1	2 days	2-4	Dorsal musculature	Continuous refrigeraration
			Abdominal musculature	
Lot 2	1 week	-18	Dorsal musculature	Continuous freezing
			Abdominal musculature	
Lot 3	4 weeks	-18	Dorsal musculature	Continuous freezing
			Abdominal musculature	
Lot 4	4 months	-18	Dorsal musculature	Continuous freezing
			Abdominal musculature	

In order to assess the biochemical transformations it has been done the extraction of sarcoplasmatic proteins by sampling muscular tissue which was homogenized in a Porter homogenizer. The extraction of sarcoplasmatic proteins was accomplished with physiologic serum in a 1:4 rate (m/v), at 4^{o}C. After centrifugation, the extracts were analyzed to determine the protein content, the electrophoretic distribution of proteins and enzymatic activities of SOD.

Superoxiddismutase inhibits the forming of formazan by decomposing the superoxid radicals generated by the photo reduction of riboflavin. The resulted color intensity varies inversely proportional with the activity of enzymes.

For 2 ml solution formed by *para*-nitroblue tetrazolium (NBT) 75 µM, 100nM EDTA and 2µM riboflavin in 50mM tampon phosphates at pH 7,8 there were added 0,05ml of protein extract. The tubes were exposed to a light source and it was determined the absorbance at 560nm. Parallely was formed a testimony sample. A unit of enzymatic activity was defined as the quantity of enzyme necessary for inhibition of 50% of the photo reduction process (Iordăchescu, D. and Dumitru, I. F., 1988).

Results and discussions

Electrophoresis in poliacrilamid gel in non-denaturizing conditions revealed the high degree of molecular heterogenity of the extracts obtained for each type of muscle. The distribution of the proteins for abdominal muscles was different from the one obtained for dorsal musculature (Figure no 1). There weren't signalized major differences between extracts obtained from refrigerated trout musculature and extracts obtained from frozen trout musculature.

It was observed a supplementary proteic strip only for the protein extracts obtained from trout frozen for 4 months. We mention that this fraction presented the greatest electrophoretic mobility.

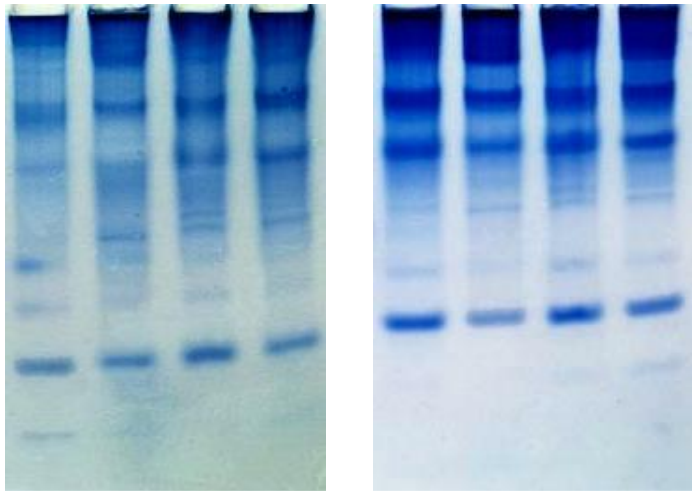

Figure no. 1
PAGE for sarcoplasmatic proteins in dorsal (A) and abdominal (B) muscles.
1. Trout frozen 4 months; 2. Trout frozen weeks 3. Trout frozen 1week; 4. Trout refrigerated 48 hours

The results obtained at the electrophoresis in poliacrilamid gel demonstrate the cleavage of some sarcoplasmatic proteins during the process of preserving by freezing. These proteins can be substantially oxidized during freezing, under the influence of oxygen reactive species, oxidations that can forego the protein cleavage and determine the increase of susceptibility towards proteolysis. Consequently, we have to say that oxygen reactive species have an important role in conditioning the meat preserved by freezing. The cleavage of some proteins observed in frozen fish demonstrates the impossibility of annulling the proteolysis in fish meat at temperatures of -18…-20^{0}C.

Also, the appearing of supplementary protein strips in extracts obtained from trout frozen 1 week, comparative with the ones obtained from the refrigerated trout, demonstrates the deteriorating of membranes of sub-cellular protein fractions and the flow of proteins (enzymes) contained by those fractions.

This phenomena allows us to say that the electrophoretic profile of the sarcoplasmatic proteins can be utilized at differentiation of the refrigerated trout musculature from the frozen for different periods of time musculature.

For the analyzed lots the electrophoresis in denaturizing conditions (SDS) revealed different distributions for the extracts obtained from frozen fish and from refrigerated fish.

This way, for the dorsal musculature it was observed the cleavage of the protein fraction corresponding to the most abundant protein in more strips. The process was more obvious in case of trout frozen for 4 months. The same modifications were signalized also for abdominal musculature. (*Figure no 2*).

The electrophoretic profile of sarcolpasmatic proteins extracted from refrigerated trout musculature was different from the one obtained from the musculature of trout frozen for 1 week. For the sarcoplasmatic proteins extracted from trout musculature that was frozen for 1

week it was revealed the existence of protein supplementary strips, represented by proteins localized in sub cellular fractions.

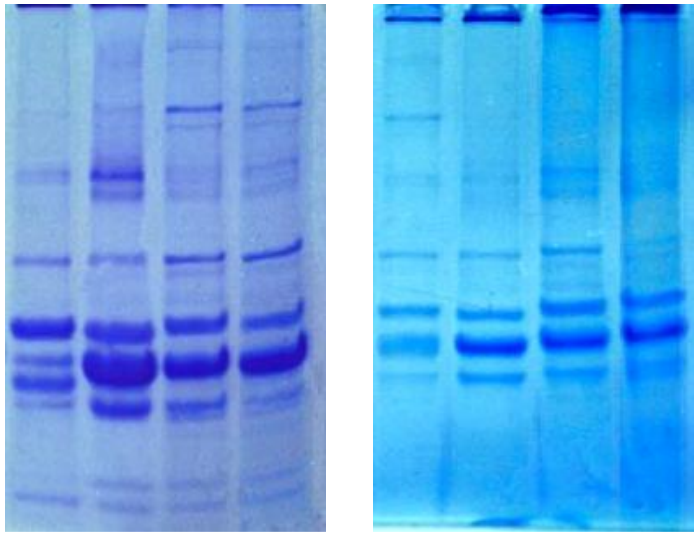

Figure no. 2
PAGE-SDS for sarcoplasmatic proteins in dorsal (A) and abdominal (B) muscle.
1.Trout frozen 4 months; 2. Trout frozen 4 weeks 3. Trout frozen 1 week; 4. Trout refrigerated 48 hours.

Although in our country there are not available bibliographic data untill now, these results confirm the possibility of using the electrophoretic profile of the sarcoplasmatic proteins as a differentiation criteria of the refrigerated musculature of fish from the frozen and defrozen one.

The determination of SOD activity revealed significant differences between refrigerated and frozen trout, given to the partial inactivation of the enzyme by freezing.

There were sampled a number of 20 trout samples which were divided in 4 lots of 5 samples each, as it is represented in table no 1. Lot no 1 was formed by samples refrigerated for 48 hours at 2-4^{0}C, while the second lot was represented by samples of trout frozen continuously for 1 week. The third lot is formed by samples of trout which was frozen continuously for 4 weeks, and lot no 4 was frozen for 4 months.

The determinations revealed the lowest activity for the trout frozen for 4 months. There weren't noticed significant differences between the SOD activities for the extracts obtained from the refrigerated and the frozen during 1 week musculature.

The results obtained from investigation of SOD izoenzymes confirmed the quantitative analysis made. This way, the electrophoretic analysis of SOD izoenzymes revealed the releasing of the mitochondrial izoenzyme Mn-SOD due to the freezing process, as well as the global decrease of SOD activity due to the denaturizing processes suffered by the protein macromolecules.

The decrease of SOD activity by freezing could be explained by modification of the polypeptidic chain at the level of the radicals in the hydrophobic middle of the enzyme molecule (oxygen reactive species affects these positions) with the forming of carbonyl derivates, accompanied by the total or partial inactivation of the enzyme.

Conclusions

1. The preserving of trout at temperatures of -18 to -20^{0}C determines the showing of some processes that affect the proteins and enzymes, these processes being directly proportional with the storing period;
2. Given the obtained results we may affirm that in the future there is a possibility of using the electrophoretic profile of sarcoplasmatyc proteins as differentiation criteria of the musculature of refrigerated trout from the frozen and defrozen one;
3. The freezing doesn't cancel the activity of endo-peptidases localized in lyzozomes (catepsines), responsible for cleavage of the proteic macromolecules.
4. By freezing the SOD activity decreases. In premiere was signalized the appearing of the mitochondrial izoenzyme (Mn-SOD) in the extracts obtained from the frozen fish, this being able to be utilized as a marker of differentiation of the refrigerated musculature from the musculature submitted to freezing.

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Observations concerning morphological pecularities of chickens skin

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Skin histology and skin associated lymphoid tissue (SALT) was studied in 4 chicken embryos of 18 days and 3 groups of chickens each of 5 individus, killed at 5, 14 and 20 days of life. In embryos skin the epidermis consists of a single layer of ectodermic cells situated under the peridermis. In basal layer of the epidermis, lipid spots joins in a smaller spot in the intermediary layer and in corneous layer, and together with keratine form a impermeabile (water proof) layer, covering the skin and feathers.

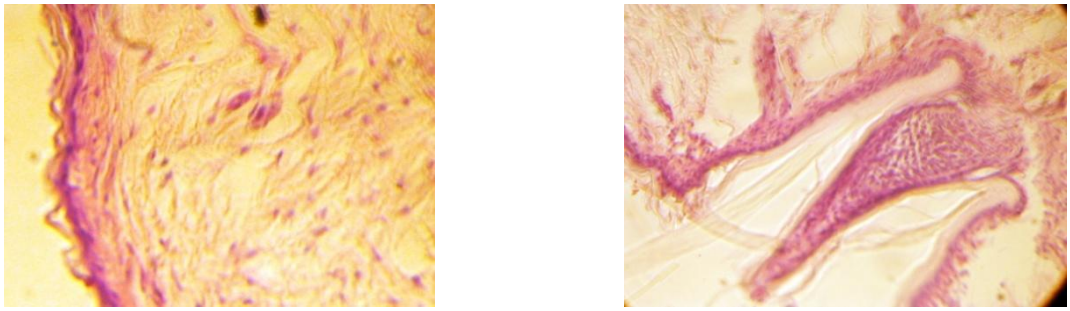
Feather smoth muscles are arranged around the plumiferous follicle adhering to the adjacent follicle in cross shape, like X letter. This arrangement of the muscles alow the complex motion of the feathers. Tight fibres are associated, up to 10 with a contour feather and seem to be sensorial detectors, being closely associated with sensorial terminations and Herbst corpuscle.

Key words: chicken, skin histology, feather, plumiferous follicle

Structura de bază a pielii la păsări este asemănătoare cu cea de la reptile şi mamifere. Spre deosebire de acestea pielea la păsări este mai elastică, transparentă în unele zone iar în altele este diferențiată în teritorii îngroşate (la picioare şi cioc) (1,4).

Investigațiile morfologice s-au efectuat pe 20 pui de găină, crescuți în sistem intensiv, începând cu embrioni de 18 zile şi pui în vârstă de 5, 14, 20 zile în urma recoltării fragmentelor de piele din următoarele regiuni: cap, stern, axilară, regiunea coccigienă împreună cu glanda uropigenă. Pielea recoltată a fost intactă, fără leziuni vizibile macroscopic şi de la pui clinic sănătoşi. Piesele au fost prefixate în formaldehidă 10% soluție apoasă, timp de 12 ore, apoi în fixator Bouin timp de 48 de ore, apoi incluse în parafină şi secționate la 5µm. Au fost efectuate colorații HEA, Acid Periodic Fuxină Schiff, Giemsa pe țesuturi şi Pappenheim. S-au urmărit particularitățile morfologice ale pielii de pasăre, glandelor, structura foliculilor plumiferi.

La embrion, epiderma este formată dintr-un singur strat de celule ale ectodermului care se divid mai târziu într-un strat germinativ de celule cuboide situate sub ***periderm***. Peridermul protejează embrionul în timpul creşterii din perioada eclozională, fiind format din celule moarte cheratinizate. Stratul format din celule cuboidale suferă diviziune mitotică şi formează epidermul, diferit de cel posteclozional prin faptul că este mai subțire şi nu are toate straturile constituite (4). ***(fig. 1)***.



a ***b***

Fig 1. Piele embrion de pui (a). Folicul plumifer la pui de 5 zile. Col PAS x100.

Epiderma prezintă stratul generator la bază, cu celule aflate în mitoză, care sunt împinse în stratul intermediar, omolog stratului spinos de la mamifere. Celulele prezintă joncțiuni de tip desmozomi în pată, dar spațiul dintre ele este permanent ocupat de lichid tisular. Aceste celule mature sunt împinse spre suprafața pielii pentru a forma un strat de tranziție care ocupă aceeaşi poziție în epidermă ca şi stratul granular de la mamifere*. La păsări granulele de cheratohialină din interiorul acestor celule nu pot fi văzute la microscop optic.* Odată aplatizate celulele epidermice devin cheratinizate, mor şi formează stratul cornos. În zonele acoperite cu pene acest strat este format din 2-3 rânduri de celule. Descuamarea se face ca la mamifere însă scuamele însoțesc sebumul provenit de la glanda uropigenă şi se deplasează împreună ajungând la pene. În alte zone cum ar fi ciocul şi picioarele (aria tarsometotarsiană) acest strat este foarte gros, se desprinde, astfel păsările pierd solzii tarsometatarsali periodic (1,4).

În stratul celulelor bazale se formează picăturile de lipide care converg într-o picătură mai mare în stratul intermediar, apoi în stratul cornos ***(fig 2 a)***. *Lipidele din stratul cornos formează împreună cu keratina cea mai importantă barieră împotriva apei, care acoperă pielea şi penele.* Astfel întreaga suprafață a pielii păsărilor devine acoperită cu un strat de grăsime. Aceste picături de grăsime sunt îndepărtate în timpul procedeelor histologice convenționale. Cantitatea de lipide eliberată creşte în spațiul interdigital şi la nivelul feței plantare a degetelor (2,4,5).

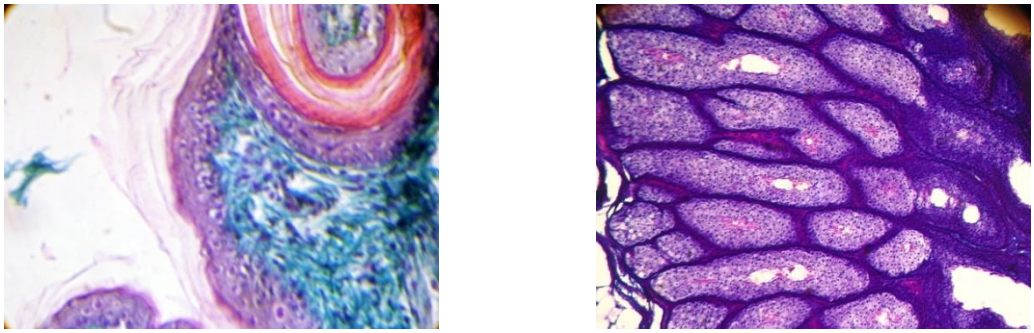
Epiderma şi derma au un aranjament similar la toate păsările (2,3).

Sub epidermă se află dermul divizat în două straturi: unul superficial mai subțire şi altul profund mai gros. Rețeaua de colagen este organizată în structuri largi romboidale, numite macrofibrile cu diametru de 5-20µm. şi lungime de 20-100µm.

Glandele cutanate pot fi: glande mici secretoare de cerumen în conductul auditiv extern şi glanda uropigenă (3,4).

Glanda uropigenă, este situată dorsal în zona pigostilului, fiind exocrină, holocrină, formată din doi lobi separați printr-un septum care se continuă cu capsula glandei. La unele specii este absentă (la struț -*Struthio camelus*, la papagali), iar la altele este foarte mare. La pelican poate avea mărimea unui ou (câțiva cm) (4).

Fiecare lob glandular este format din numeroase glande alveolare secretoare care se deschid în cavitatea centrală ***(fig 2b)***. Papila glandei uropigene are două deschideri căptuşite de un epiteliu cubic stratificat, câte una pentru fiecare parte a glandei, adesea înconjurată de un smoc de pene, care împiedică eliminarea secreției. Alveolele glandulare sunt furnizoare de grăsime conținută de celulele epidermice modificate, care nu suferă cheratinizare ci lipogeneză, iar la maturare celula devine plină cu picături de lipide urmată de eliberarea celulelor sub formă de secreție în canal. Funcțiile sebumului sunt: protecție hidrică a penelor şi pielii, păstrează suplețea pielii, are proprietăți antibacteriene, antifungice şi conține precursorii vitaminei D (1,3,4).



a ***b***

Fig.2. Piele şi glandă uropigenă la pui de 14 zile. Epiteliu pavimentos stratificat cheratinizat (a). Glandă uropigenă (b). Col HEA x200 (a); x100(b).

Păsările nu prezintă glande sudoripare. Penele sunt foarte bune termoizolatoare. Căldura este pierdută pe la nivelul aparatului respirator dar şi prin radiație de la suprafața penelor (1,2,3,4).

Penele se formează în ***foliculii plumiferi*** care sunt înconjurați de vase sanguine, muşchi netezi, terminațiuni nervoase periferice. Se formează ca produs a sintezei epidermei. Foliculul se termină cu un *bulb, în stratul profund al dermului,* de la nivelul căruia se produce creşterea şi elongația *sub forma unui con care se diferențiază prin B- cheratinizare şi se deplasează spre epidermă* ***(fig. 3a)***.

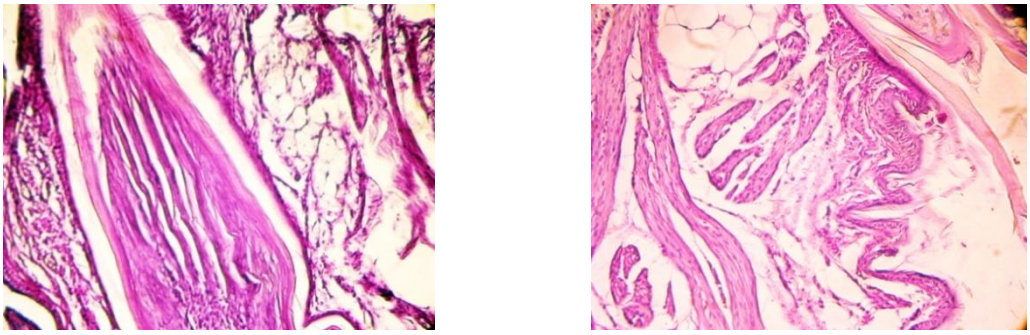


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Fig.3. Piele de pui în vârstă de 14 zile. Folicul plumifer cu aspect de con în derm(a) . Muşchi perifoliculari care se leagă la foliculul adiacent încrucişat, asemănător literei X(b). Col. PAS x100.

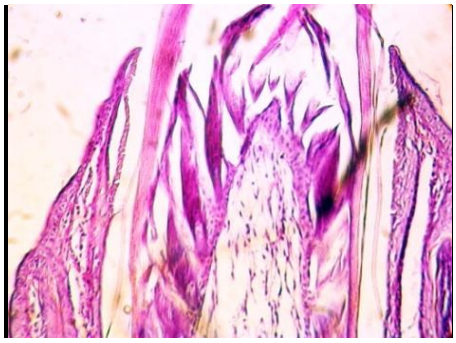
Muşchii penelor sunt aranjați în jurul foliculului şi aderă la foliculul adiacent încrucişat, asemănător literei X (***fig. 3b***.). Această aranjare a muşchilor permite ridicarea, coborârea, rotirea sau retragerea penelor (2,4). Există şi o presiune dată de corpusculii formați de adipocite dintre foliculi înconjurați de două teci elastice. Muşchii responsabili de aceste mişcări sunt netezi aflați sub controlul măduvii spinării şi a sistemului nervos vegetativ. Muşchii pteriali sunt compuşi din benzi de fibre musculare netede care sunt unite la capete prin minitendoane elastice.

Se cunosc mai multe tipuri de pene.

Puful este caracteristic puilor posteclozional, are un rahis scurt şi barbe pufoase şi lungi. Poate fi întâlnit şi la păsările adulte la baza penelor de contur, în funcție de specie.

Penele de contur formează majoritatea plumajului fiind divizate în pene pentru zbor (primare, secundare şi la unele păsări şi terțiare) şi penele corpului. Penele aripii se numesc retrice, ale cozii remige iar penele de contur care acoperă baza acestora se numesc tetrice. Fiecare pană are o tijă centrală- a cărei parte distală situată în grosimea dermului (*calamus)* se formează din papila dermică, înconjurată de o zonă *hyponema.* De pe tija centrală pornesc barbe care sunt divizate în

barbule proximale şi distale care păstrează legătura prin hamuli (cârlige). În jurul acestor foliculi se află numeroase terminaţiuni nervoase (2,4) care par să stimuleze mişcarea rahisului ***(fig.4 a***).



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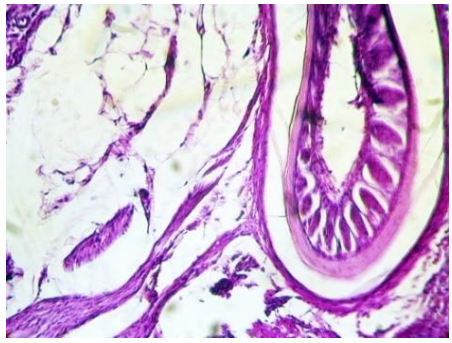


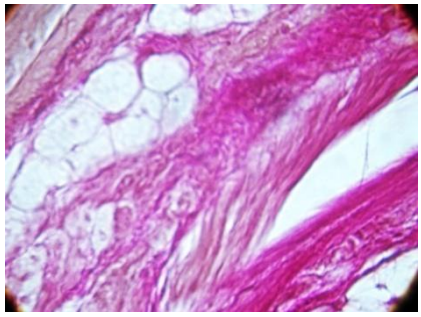
b

Fig 4.Piele pui de 20 zile.Folicul plumifer ai penei de contur surprinşi longitudinal (a), şi transversal (b). HEA x200(b,c).

Semiplumajul, este intermediar între puf şi pană, are un rahis mai lung decât barbele.

Fibrele subţiri, sunt prezente la aproape toate păsările. Au un rahis lung, barbe foarte scurte. Sunt asociate cu penele de contur şi par a fi detectori senzoriali deoarece sunt strâns asociaţi cu terminaţiuni nervoase senzoriale şi cu corpusculi Herbst cu dimensiuni de 124-168 µm. (omologi morfologici şi funcţionali ai corpusculilor Meissner de la mamifere). Aceste fibre subţiri sunt asociate până la 10 cu o pană de zbor (2,4).

Picioarele, respectiv aria tarsometatarsiană a acestora, sunt acoperite de solzi plaţi de cheratină numite şi scuturi***.*** Aceştia variază ca mărime de la foarte mici (*anulate*), mici (*reticula*), în zonele foarte solicitate şi foarte mari, în arii mai puţin solicitate. Morfologic, sunt formaţi din epidermă care dă naştere unui strat gros de cheratină. Printre scale apar şanţuri în care keratina are consistenţă moale (2,4).



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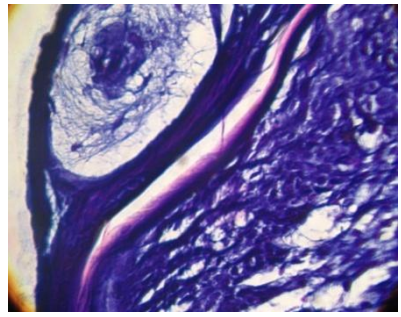

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Fig 4.Piele pui de 20 zile.Terminaţiuni nervoase şi corpuscul Herbst în vecinătatea unui folicul plumifer (c). Col HEA x100(a,b).

CONCLUZII

1. La embrion, epiderma este formată dintr-un singur strat de celule ale ectodermului situate sub periderm (care protejează embrionul în timpul creşterii din perioada eclozională),.
2. În stratul celulelor bazale se formează picături de lipide care converg într-o picătură mai mare în stratul intermediar, apoi în stratul cornos unde formează împreună cu keratina cea mai importantă barieră împotriva apei, care acoperă pielea şi penele.
3. Muşchii penelor sunt aranjați în jurul foliculului şi aderă la foliculul adiacent încrucişat, asemănător literei X. Această aranjare a muşchilor permite ridicarea, coborârea, rotirea penelor.
4. Fibrele subțiri sunt asociate până la 10 cu o pană de contur şi par a fi detectori senzoriali deoarece sunt strâns asociați cu terminațiuni nervoase senzoriale şi cu corpusculi Herbst.

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Observations concerning skin morphology and skin associated lymphoid tissue in chickens (*Gallus domestica*)

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Skin histology and skin associated lymphoid tissue (SALT) was studied in 4 chicken embryos of 18 days and 4 groups of chickens each of 5 individus killed at 5, 14 and 20 days of life. Morphology of skin and SALT was studied in different regions: head, sternum, axillar, coccigean and uropigeal gland.

The thickness of the skin progressively increase with the age. Diffuse lymphoid infiltrations appears firstly at 5 days of age. Lymphoid agglomerations in the dermis appear firstly in coccigian region, containing lymphocytes eosinophils, plasma cells and erythrocytes.

Key words: chicken skin, histology, skin associated lymphoid tissue

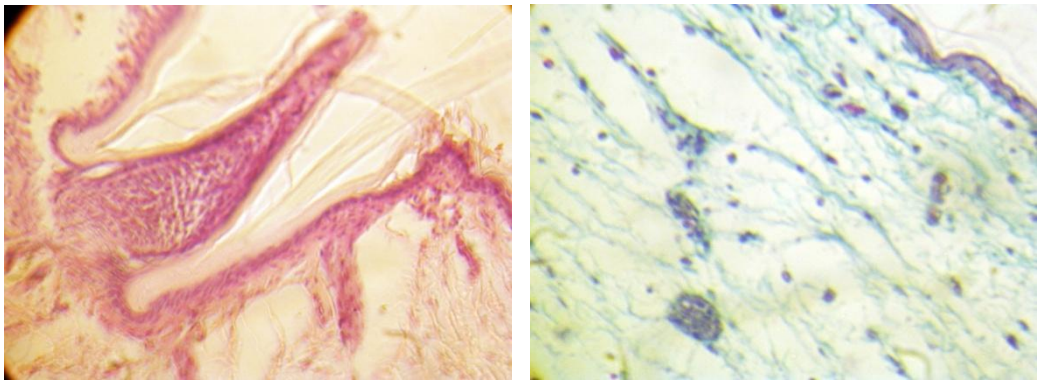
Pielea la păsări prezintă o serie de particularităţi morfostructurale. Scopul lucrării este de a le evidenţia şi de a prezenta dinamica infiltraţiilor limfoide în derm începând cu embrionii şă continuând cu puii de găină până la 20 zile.

MATERIAL ŞI METODE

Investigaţiile morfologice s-au efectuat pe 20 pui de găină, crescuţi în sistem intensiv, pe 4 loturi de câte 5 pui, începând cu embrioni de 18 zile şi pui în vârstă de 5, 14, 20 zile, în urma recoltării fragmentelor de piele din următoarele regiuni: cap, stern, axilară, regiunea coccigienă împreună cu glanda uropigenă. Pielea recoltată a fost intactă, fără leziuni vizibile macroscopic şi de la pui clinic sănătoşi. Piesele au fost prefixate în formaldehidă 10% soluţie apoasă, timp de 12 ore, apoi în fixator Bouin timp de 48 de ore, apoi incluse în parafină şi secţionate la 5µm. Au fost efectuate colorații HEA, Acid Periodic Fuxină Schiff, Giemsa pe ţesuturi şi Pappenheim. S-au efectuat investigaţii morfometrice şi histochimice urmărindu-se grosimea epidermei, dermului, infiltraţiile limfoide şi particularităţi morfologice ale pielii în funcţie de regiunea corporală.

REZULTATE ŞI DISCUŢII

La embrioni epiderma este foarte subţire, formată dintr-un strat de celule cubice, de 10-12 µm, acoperite de peridermă. Dermul este format din ţesut conjunctiv în care fibrele şi celulele sunt rare, predomină substanţa fundamentală. În regiunea capului, în derm apar rare limfocite ***(fig. 1)***.



a ***b***

Fig. 1. Piele eembrion de pui în vârstă de 18 zile, din regiune axilară (a) şi a capului (b). Infiltrație limfoidă difuză în derm în regiunea capului. Col. Giemsa x100.

La puii în vârstă de 5 zile epiderma din regiunea capului, sternului, axilară şi coccigienă are un diametru de 20-24 µm, iar dermul de 145-210 µm. Foliculii plumiferi sunt dispuşi oblic şi au un diametru de 98-150 µm. În derm apar limfocite dispuse difuz ***(fig. 2)***.

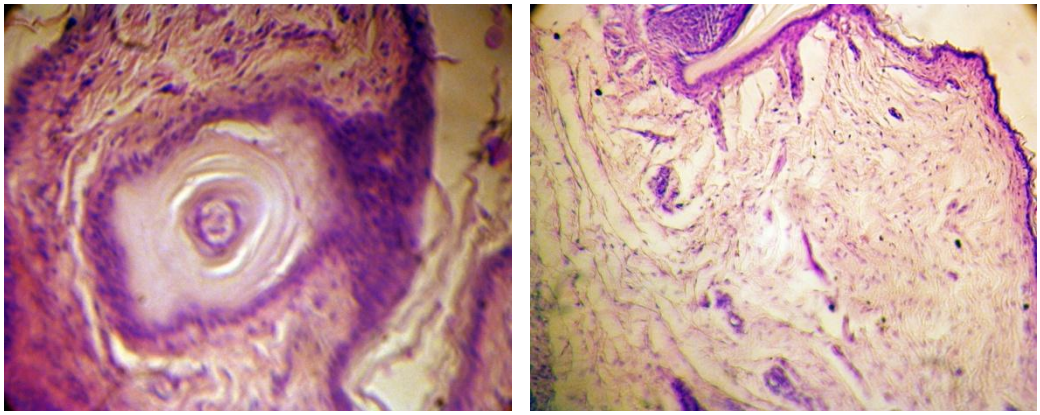


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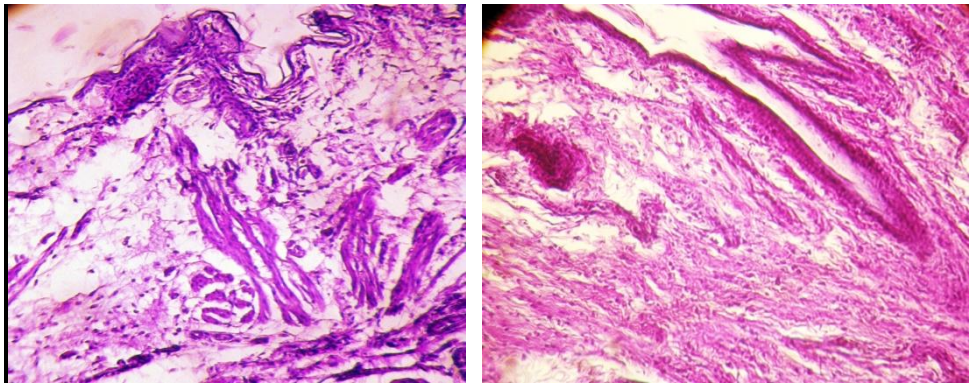
Fig. 2. Piele de pui în vârstă de 5 zile, din regiune axilară (a) şi a capului (b). Infiltrație limfoidă difuză în derm. Col. Giemsa x100.

Pielea la puii în vârstă de 14 zile din regiunea capului prezintă epiderma cu un diametru de 42-94 µm, iar dermul are o grosime de 246- 315µm.

Țesutul conjunctiv al dermului poate fi divizat în strat superficial şi profund. Stratul profund este format dintr-un strat compact şi unul mai puțin dens ataşat de o lamină elastică care realizează joncțiunea cu țesutul subcutanat. El conține fibre musculare netede şi paniculi adipoşi. Stratul compact este format dintr-o rețea de fibre de colagen şi fibre elastice, colagenul este aşezat orizontal (față de mamifere, la care este neordonat) (1, 3, 4, 5). Stratul superficial al dermului este format din multe fibroblaste, cu fibre de colagen mai puțin dense decât cele din stratul profund. Fibrele de elastină din derm sunt mai groase şi unite pentru a forma minitendoane (4, 6). Fiecare minitendon se direcționează către zona centrală a muşchilor. Aceştia se îndreaptă către derm interconectându-se cu muşchii care mişcă pana sau ai penei sau apterial care se leagă de tija penei (1,2,5).

Foliculii plumiferi au dispoziție oblică. Sunt subțiri şi au diametrul de 124 -228 µm. În stratul papilar al dermului se observă infiltrații limfoide difuze formate din limfocite, plasmocite, eozinofile, rare mastocite şi plasmocite.

Regiunea axilară se caracterizează printr-o piele mai subțire, în care epidermul şi dermul au o grosime de 198-240 µm. Epidermul este ceva mai subțire decât în regiunea capului 36-42 µm şi are o grosime de 162-180 µm. Dermul este foarte dens, bogat în fibre de colagen, fibre elastice şi fibre musculare netede. Foliculii plumiferi au un diametru de 820-260 µm. În derm au fost evidențiate infiltrații limfoide difuze formate din limfocite, plasmocite ***(fig.3)***.



a ***b***

Fig.3 . Piele de pui în vârstă de 14 zile, din regiunea sternului (a) şi axilară (b). Infiltrație limfoidă difuză în derm. Col. Gie msa x100; col HEA x200(b).

Epidermul din regiunea coccigienă şi a sternului are un diametru de 86-120 µm, iar dermul 182-250 µm. Foliculii plumiferi din regiunea coccigienă au un diametru de 1020-480 µm.

Glandele sebacee sunt reunite radiar în glanda uropigee.Această glandă prezintă un canal situat central. În lamina propria a acestui canal nu au fost observate infiltrații limfoide. In dermul pielii din regiunea coccigienă au fost observate infiltrații limfoide difuze mai numeroase decât în celelalte regiuni. Au fost surprinse şi aglomerări limfoide formate din limfocite, plasmocite, eozinofile şi eritrocite. Dimensiunile acestor aglomerări sunt variabile, de la 125-135 µm la 420-560 µm (***fig. 4***).

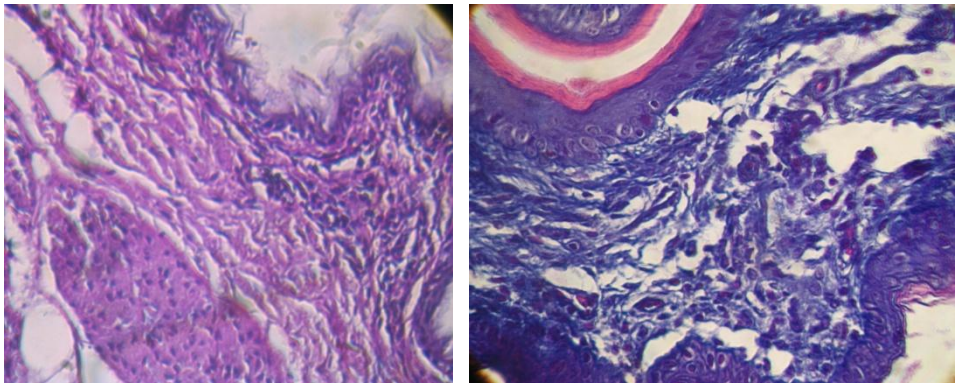


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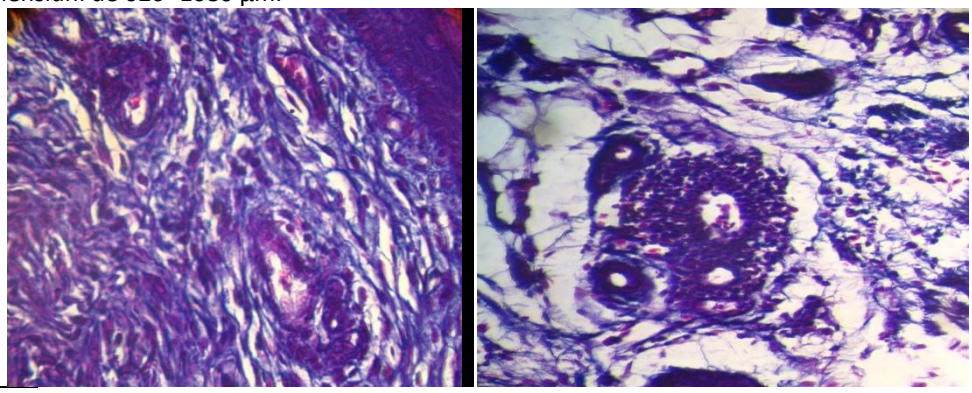
Fig.4 . Piele de pui în vârstă de 14 zile, din regiunea sternului (a) şi coccigienă (b). Infiltrație limfoidă difuză în derm. Col. Giemsa x100; col HEA x200(b).

Pielea din regiunea capului ***la puii în vârstă de 20 zile*** prezintă epiderma cu un diametru de 44 - 95 µm, iar dermul are o grosime de 240- 325µm. Foliculii plumiferi au diametrul de 124-268 µm. În stratul papilar al dermului se observă infiltrații limfoide difuze formate din limfocite, plasmocite, eozinofile, rare mastocite şi plasmocite.

Regiunea axilară se caracterizează printr-o piele mai subțire, în care epidermul şi dermul au o grosime de 198-240 µm. Epidermul este ceva mai subțire decât în regiunea capului 42-64 µm şi are o grosime de 162-240 µm. Dermul este foarte dens, bogat în fibre de colagen, rare fibre elastice şi fibre musculare netede. Foliculii plumiferi au un diametru de 810-264 µm. În derm au fost evidențiate infiltrații limfoide difuze formate din limfocite, plasmocite.

Epidermul din regiunea coccigienă şi a sternului are un diametru de 86-122 µm, iar dermul 182-250 µm. Foliculii plumiferi au dispoziție oblică şi

dimensiuni de 620- 1080 µm.



a ***b***

Fig.5 . Piele pui în vârstă de 20 zile, din regiunea sternului (a) şi coccigienă (b). Infiltrație limfoidă difuză în derm (a) şi aglomerare limfoidă (b). Col. Giemsa x100; col HEA x200(b).

Glandele sebacee sunt reunite radiar în glanda uropigee.Această glandă prezintă un canal situat central. În lamina propria a acestui canal nu au fost observate infiltrații limfoide. In dermul pielii din regiunea coccigienă au fost observate infiltrații limfoide difuze mai numeroase decât în celelalte regiuni .Au fost surprinse şi aglomerări limfoide formate din limfocite, plasmocite, eozinofile şi eritocite.. Dimensiunile acestor aglomerări sunt variabile, de la 115-145 µm la 320 - 524 µm (***fig. 5***).

CONCLUZII

1. Grosimea epidermului şi dermului variază în funcție de regiunea corporală şi vârsta puilor. Dimensiuni reduse se înregistrează la embrioni şi pui de 5 zile.
2. Tesutul limfoid asociat pielii este prezent inclusiv la embrionii de 18 zile sub forma infiltrațiilor limfoide difuze.
3. Aglomerările limfoide apar la puii de 20 zile în derm, în regiunea coccigienă, în vecinătatea canalului glandei uropigene.

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Inclusion complexes of Sulconazole with hydroxypropyl β-cyclodextrin: characterization in aqueous solution and in solid state

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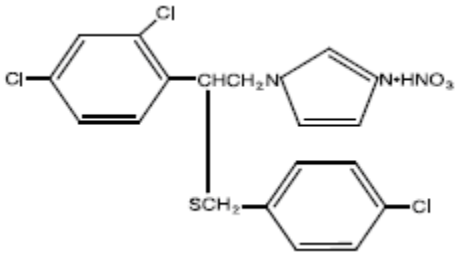
Complexation between sulconazole (SULC), an imidazole derivative with in vitro antifungal and antiyeast activity, HP-(-CD was studied in solution and in solid states. Complexation in solution was evaluated using solubility studies. In the solid state, differential scanning calorimetry (DSC),, scanning electron microscopy (SEM) and RX diffraction studies were used. Solubility studies suggested the existence of inclusion complex between SULC and HP- CD. DSC studies showed the existence of a complex of SULC with HP -CD. The RX studies confirmed the DSC results of the complex. Solubility of SULC in solid complexes was studied by the dissolution method and it was found to be much more soluble than the uncomplexed drug.

Key words: cyclodextrin, sulconazole, inclusion complex

Introduction

Cyclodextrins (CDs) and modified cyclodextrins act as host molecules to form inclusion complexes rather nonspecifically with a wide variety of guest molecules. Complexation of guest compounds with CDs or modified cyclodextrins can alter guest solubility, increase stability against the effects of light, heat, and oxidation, mask unwanted physiological effects, and reduce volatility [1]. The most common application of CDs in the pharmaceutical industry is to enhance drug solubility, dissolution rate, and bioavailability of poorly water soluble drugs. A large variety of drugs encapsulated through noncovalent interactions into unmodified or modified CDs (especially hydroxypropyl β-cyclodextrin (HP βCD)) cavity were described. [2].

Sulconazole nitrate is an imidazole derivative with in vitro antifungal and antiyeast activity called (+)-1-[2.4-dichloro-*b* -[(p-chlorobenzyl)-thio]-phenethyl] imidazole mononitrate. Sulconazole nitrate is a broad-spectrum antifungal agent intended for topical application.



Sulconazole nitrate is a white to off-white crystalline powder with a molecular weight of 460.77. It is freely soluble in pyridine, slightly soluble in ethanol, acetone, and chloroform: and very slightly soluble in water (1.9 mg/mL). It has a melting point of about 130°C.

Sulconazole is used to treat skin infections such as athlete's foot, jock itch, and ringworm. Like all azole antifungals, it inhibits the fungal cytochrome P-450 3-A dependent enzyme 14-alpha

demethylase, thereby interrupting the synthesis of ergosterol. Inhibition of this critical enzyme in the ergosterol synthesis pathway leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth [3]. Azole antifungals can also inhibit many mammalian cytochrome P450-dependent enzymes involved in hormone synthesis or drug metabolism [4]. Therefore, azole antifungals are particularly susceptible to clinically-significant drug interactions with other medications metabolized through the P450 pathway [5].

Sulconazole nitrate has a broad-spectrum antifungal activity that inhibits the in vitro growth of the common pathogenic dermatophytes including Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum and Microsporum canis. It also inhibits (in vitro) the organism responsible for tinea versicolor, Malassezia furfur. Sulconazole nitrate has also been shown to be active in vitro against Candida albicans and certain gram positive bacteria.

A modified Draize test showed no allergic contact dermatitis and a phototoxicity study showed no phototoxic or photoallergic reaction to sulconazole nitrate cream. Maximization tests with sulconazole nitrate cream showed no evidence of contact sensitization or irritation [3]. There were no systemic effects and only some cutaneous adverse sulconazole may cause side effects including: itching, rash, burning, irritation or stinging, redness.

There are no adequate and well controlled studies in pregnant women. Sulconazole nitrate should be used during pregnancy only if clearly needed. Sulconazole nitrate has been shown to be embryotoxic, it was not teratogenic in rats or rabbits. Sulconazole nitrate given orally to rats resulted in prolonged gestation and dystocia. Several females died during the prenatal period, most likely due to labor complications [6].

Complexation of sulconazole with cyclodextrin offers the possibility to improve the aqueous solubility of sulconazole without modification of its original structure. This may allow a homogeneous delivery system of sulconazole increasing its bioavailability. We synthesized hydroxypropyl β-cyclodextrin- sulconazole nitrate (HP βCD-SULC) inclusion complexes, in order to make it more available for the yeast metabolism, and to reduce consequently the dosage, the treatment period and the gravity of all possible side effects.

Materials and Methods

Materials

Sulconazole nitrate (SULC) (Fluka) it was used as given. HP βCD were obtained from Cyclolab. Double distilled water was used throughout the study.

Methods

Solubility studies

Solubility studies were carried out according to the Higuchi and Connors method [7]. HP βCD solutions of different concentrations (0,33 – 24,6 10^{-4}M) were added to a supersaturated solution of SULC and shaken at room temperature (22 ± 1^{o}C) for 24 hours. After reaching equilibrium, the solutions were filtered. The absorbance of solutions containing different mole fraction of the drug and HP βCD was measured by UV at 190 nm and the concentration of SULC in the each solution was determined with reference to a suitably constructed standard curve.

The apparent stability constant was calculated from initial straight portion of the phase solubility diagram using the Eq. 1 [7].

$$K_{1:1} = \text{slope}/S_o (1-\text{slope}) \quad (1)$$

where: S_o is the solubility of SULC in the absence of HP βCD

slope is the slope of the experimental phase solubility diagram for HP βCD-SULC.

Preparation of the solid complex

The inclusion complexes (C_s) were prepared by freeze drying method. An aqueous solution containing SULC and HP β-CD in a 1:1 molar ratio was frozen by immersion in liquid nitrogen and freeze-dried in a Martin Christ, ALPHA 1-2LD Freeze-Dryer. The aqueous solutions were obtained by dissolving $4{,}34x10^{-4}$ mol SULC and $4{,}34x10^{-4}$ mol cyclodextrin in 25 ml distilled water and stirring it at room temperature for 48 h.

Preparation of the physical mixture (Ph.M) was performed by mixing the powders in a 1:1 molar ratio in a ceramic mortar.

Complex Characterization

UV measurements were performed on a Analytik Jena Specord 200 Spectrophotometer.

Differential Scanning Calorimetry (DSC) DSC data were obtained using a Perkin Elmer-Diamond device. Each sample (2-6 mg) was exactly weighted in an aluminum pan and was heated at a rate of 10 ^{0}C/min between 30 to 330 ^{0}C, under nitrogen gas flow. Also, from DSC data it was calculated the inclusion ratio of sulconazole into CD cavity using Eq.2.

Inclusion ratio= $\Delta H_1 x100/ \Delta H_2$ (2.)

where: ΔH_1 represents drug discomposure enthalpy in the inclusion complex

ΔH_2 represents free drug discomposure enthalpy

ΔH_1 and ΔH_2 were obtained for the same amount of pure drug and complexed drug (2.5 mg).

Scanning electron microscopy SEM) SEM micrographs were obtained on a Tesla Scanning Electron Microscope.

X-ray diffraction (XRD) XRD patterns were obtained on a Bruker AXS D8 advance RX diffractometer.

RESULTS AND DISCUSSION

Phase solubility Studies

The solubility of SULC in water is very low, 1.9 mg.mL^{-1} at 25^{o}C, as described in literature [6]. In figure 1 it is shown the solubility curve obtained for SULC in presence HP βCD (Figure 1) in distilled water. As it can be seen, SULC solubility in water presents a linear growth, the resulting linear curve can be classified, in general, as an AL type (linear positive isotherm), as described in literature [7]. Since the slope of the diagame is less than 1 (0.1413 for SULC-HP βCD) was assumed that the stoichiometry of the complex is 1:1 according to Higuchi and Connors [7]. The apparent solubility constant, K1:1, of each complex was calculated from the correspondent curve of Fig.1. according to Eq. 1 and it was found to be 30.45 for SULC-HP βCD.

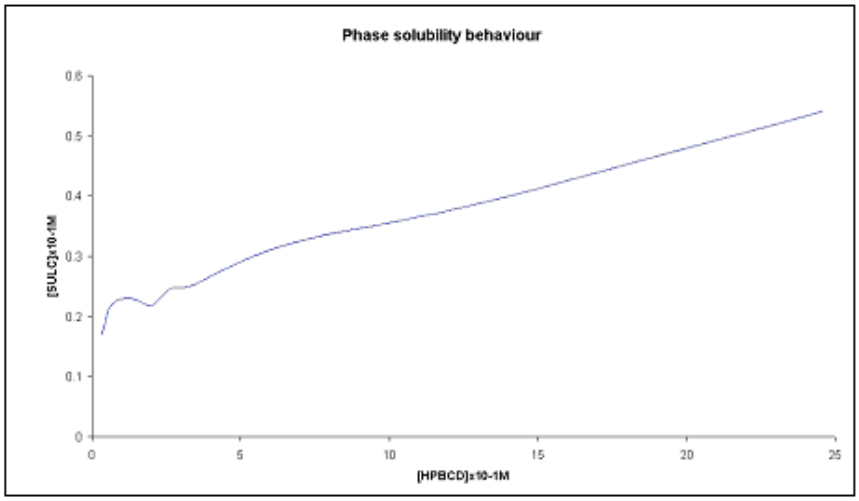


Figure 1. Higuchi phase solubility diagram of SULC in presence of and in presence of HP β-CD .

A significant increase in dissolution rate can be observed by analyzing Higuchi phase solubility diagrams, which can be explained due to the formation of the complex.

Differential Scanning Calorimetry

DSC reveales some information on solid-state interactions between drug and cyclodextrins. The DSC thermograms of pure drug and of SULC-HP-β-CD inclusion complexes are presented in Figure 2. The DSC curve of sulconazol is typical of a crystalline anhydrous substance, with a sharp fusion endotherm (T peak= 135°C). Liberation of crystal water from HP-β-CD is observed as a broad endothermal peak, at around 90°C. HPβ-CD is an amorphous material, and does not exhibit a melting point, as would be observed for crystalline materials. The characteristic thermal peak of the drug appeared around 137^{0}C, but it is strongly reduced in intensity and somewhat broadened in the SULC- HP-β-CD inclusion complexes due to the inclusion complexation process.

Analyzing decomposition enthalpies obtained for the pure and included drug the calculated inclusion ratio applying Eq.2. was to be 81.43 %.

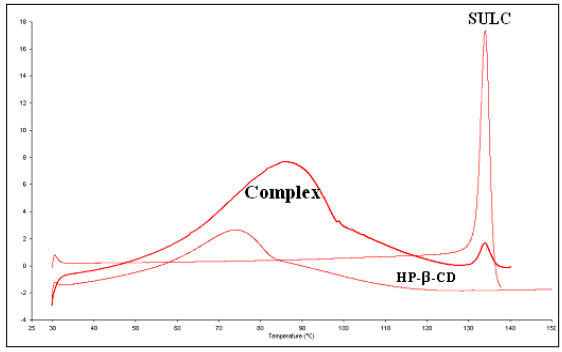


Figure 2. DSC thermograms of HP-β-CD, sulconazole and inclusion complex (complex)

Powder X-Ray Diffraction

X Ray powder, diffraction patterns of pure sulconazole and HP β-cyclodextrin, and corresponding solid inclusion complexes with CDs, are shown in Figure 3. In the X-ray diffractograms of sulconazole sharp diffraction peaks are present, indicating its crystalline state. By contrast the X-ray diffraction patterns of SULC-β-CD system were characterized only by large diffraction peaks, in which it is no longer possible to distinguish the characteristic crystallinity peaks of pure drug. These results indicate that sulconazole is no longer present as a crystalline material, and its CD solid complexes exist in the amorphous state.

The interplanar distances for the investigated samples are presented in Table 1. The data related to SULC can be seen from Figure 3 and Table 1 show also a crystalline structure. As can be seen from Figure 3 and Table 1, SULC-β-CD complex has different (individual) structure, showing almost complete amorphization of the drug and CD. We could not recognize any diffusion reflex. The formation of an amorphous state proves that the drug was dispersed in a molecular state with CD [8].

Tabel 1. Interplanar distances calculated from X ray pattern

Sample code	d (Å)
SULC	8.56 4.34 2.91 2.40 2.20 2.11 1.77 1.70 1.63 1.55 1.50 1.16
HP β-CD	7.77 4.94 4.74 4.52 2.06
SULC- HP β-CD	16.68 7.29 4.64 4.32 3.47 2.43

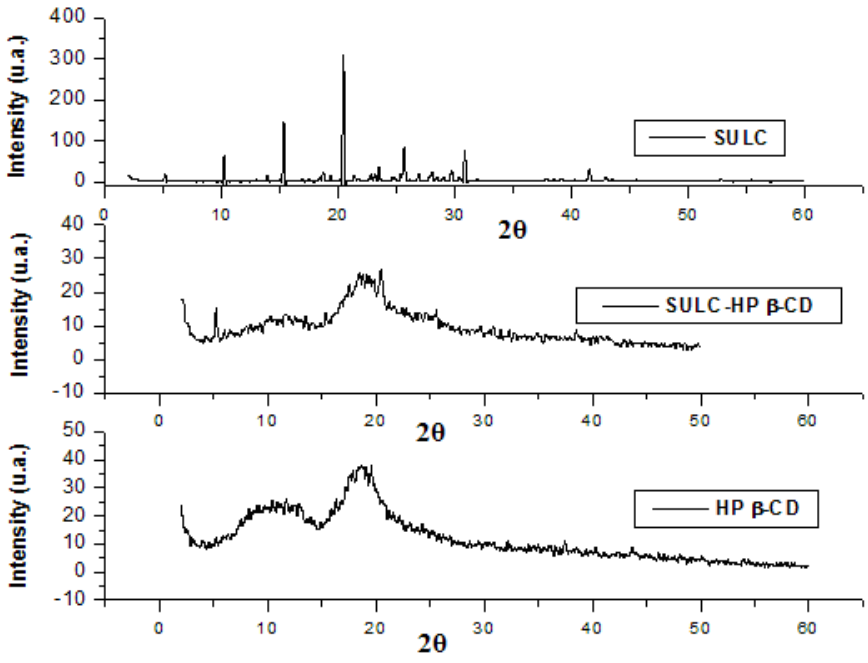
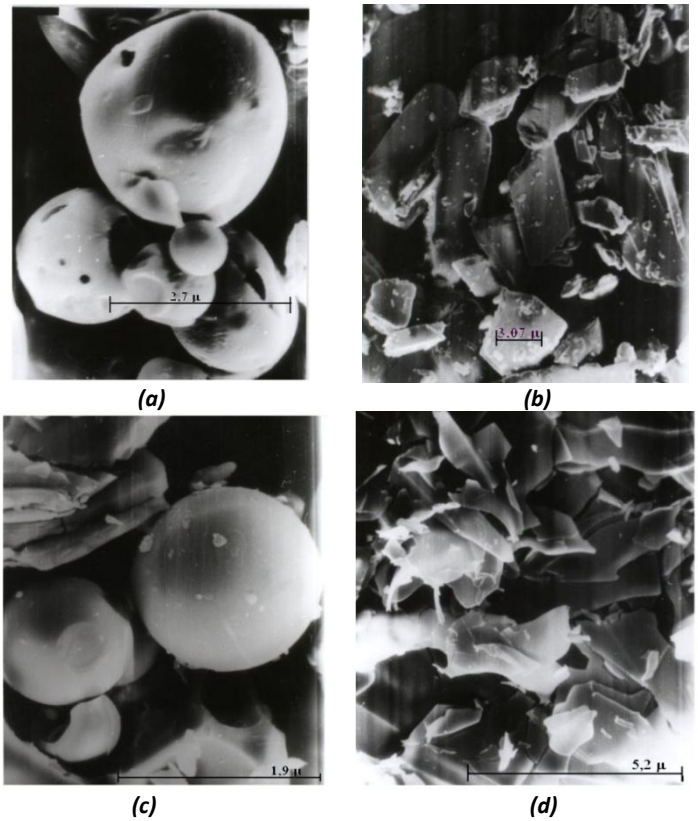


Figure 3. X-Ray Diffraction Patterns of: SULC, HP β-CD and SULC-HP β-CD inclusion complex

Scanning Electron Microscopy (SEM)

This method is not very conclusive method to confirm the formation of complex but it can give us useful informations about the efficiency of the inclusion process it helps to assess the existence of a single component in the complex.

The morphology of sulconazole, HP-β -cyclodextrin, and its physical mixtures, and solid complexes, can be seen in SEM photographs presented in Figure 4. Sulconazole appeared as crystaline crystals, tending to form aggregates. HP-β CD consisted of shrunken, cylindrical particles. The physical mixtures showed particles of HP-β CD and sulconazole, with the same morphology as the pure compounds. The complex presented a different morphology with plate-like crystals, revealing an apparent interaction in the solid state.



(a) *(b)* *(c)* *(d)*

Figure 4. SEM images of: HP β-CD (a); sulconazole (b); physical mixture of Hp β-CD and sulconazole (c); inclusion complex (d)

CONCLUSION

Inclusion complexes of sulconazole and HP- β-CD were prepared by freeze-drying method in a molar ratio 1:1.This was confirmed by DSC, SEM microscopy, X-Ray diffraction pattern. Complexation by inclusion increases sulconazole solubility and dissolution. Dissolution increasing is due to the low crystallinity of the complex.

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In vitro and in vivo study of the hypolipemic effect of some aminated polysaccharides polimers

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The aim of this study was to synthetize cationic dextran gels with aminic group wich contains an alkyl substituient with variable length (C2 -C12), to test in vitro the ability of these polymers to bind bile acids and to perform biochemical investigation of the synthesized compounds regarding their hypolipidemic action in normolipemic rats. In vitro study of the values of the binding constants determined for sodium cholate (NaCA) showes that physico-chemical properties of these polymers can influence their binding capacity in the following manner: B > A >Cholestyramin. In vivo results differ from the in vitro data, which demonstrate the following order of binding efficiencies: B > Cholestyramin >A. Polymer B(35% Ethyl, 25% dodecyl) was more effective in lowering the blood lipids and lipoprotein as compared to Polymer A(55% Ethyl, 0% dodecyl). The differences in the efficiency order for the binding of bile acids and the lowering of plasma lipid levels may be due to environmental variations in the digestive tract (pH, electrolytes, fatty acids). Aminated polysaccharides administered in rats do not affect the digestive system, liver or pancreas functions.

Key Words: rabbits, hypolipemic effect, aminated polysaccharides polymers

INTRODUCTION

The treatment of hyperlipidemias encounters difficulties due to their diverse origins. There are five types of genetically determined primary hyperlipidemias in addition to secondary ones caused by alcoholism, diabetes mellitus, hypothyroidism and hypopituitarism [18,21]. Each type requires a special treatment. Bile Acid Binding Polymers are polymers that contain amino groups and act by binding bile acid anions released in the small intestine after bile acid synthesis from cholesterol in the liver. The bound bile acids are eliminated from the body together with the insoluble and nonabsorbable polymer. Here, the enterohepatic balance of bile acids is disturbed, and to restore it the liver introduces new amounts of cholesterol into the bile. The result is a lowering of blood cholesterol level [4,29],

There are two basic anion exchange polymers that act according to this principle : cholestyramin (styrene-divinylbenzene copolymer with quaternary ammonium groups) and colestipol (condensation polymer of polyalkylene-polyamine and 1-chloro-2,3-epoxypropane). These polymers have been available for over 20 years, and they give good results in the treatment of hyperlipidemia [4,21].

These polymers have some drawbacks, e.g., unpleasant smell and taste, which are more evident with cholestyramin. Consequently, the manufacturers have changed the preparation process [26] by covering polymer particles with protective materials [8,9], or conditioning them with flavoring agents [22-23] to obtain products with improved palatability. The side effects recorded in the treatment with these polymers, such as abdominal pain, flatulence, vomiting and constipation, could not be completely eliminated.

Other polymers containing amino groups have been synthesized for the treatment of hyperlipidemia. Their pharmacological action is based on the same principle of binding bile acids. The aim was tc obtain hypolipidemic drugs with improved properties, i.e., lower tcxicity, better hypolipidemic action, and more acceptable organoleptic features. Synthetic or natural polymers with secondary, tertiary or quaternary amino groups were obtained.

There are numerous studies on the influence of Cholestyramin on blood lipid levels both in animals [19,20,28] and in humans [7,10,19,20,24]. These agents potentialy induce a lowering of the total cholesterol amount, along with the elevation of serum triglyceride content. To counteract this drawback, bile acid adsorbing polymers were combined with other hypolipidenic drugs, such as nicotinic acid [2,3], clofibrate [10], and fenofibrate [29].

In the United States, the long-term clinical trials were carried our in large groups of hyperlipidenic patients. For example, the Lipid Research Clinics Coronary Primary Prevention Trial studied the effect of Cholestyramin treatment in 3806 patients and the Cholesterol Lowering Atherosclerosis Study (CIAS) followed up the combined effect of Colestipol and nicotinic acid in 163 patients for two years [2,3]. The CLAS trial reported a 43% decrease of plasma LDL, and a 37% elevation of plasma HDL. A significant reduction of preexisting lesions, along with the formation of new atheromas, was recorded; in 16.2% of the cases a regression of atherosclerosis was noted [5]

The purpose of this study was to:

- synthesize cationic dextran gels with aminic group wich contains an alkyl substituient with variable length (C2 -C12)
- to test in vitro the ability of these polymers to bind bile acids;
- perform biochemical investigation of the synthesized compounds regarding their hypolipidemic action in normolipemic rats.

MATHERIALS AND METHODS

The synthesis of aminated polymers wich contains an alkyl substituient with variable length (C2 - C12) was carried out in two stages: *crosslinking and amination.* The crosslinking of dextran was performed in an anorganic suspension, with diclorethane as a suspension medium and cellulose acetate butyrate as a suspension stabilizer. The crosslinking agent was 1-cloro-2,3-epoxypropane. The reaction mixture was agitated for 16-20 h at 50°C. The spherical particles were sieved and the fraction with a diameter of 30-50 µm was selected.

The crosslinking degree was estimated by measuring the water uptake capacity of the resulted hydrogels, which at same time is a measure of the swelling porosity of these gels.

The capacity *of binding bile acids for the synthetised polymers* was estimated by measuring ionisation constante ($k_{0)}$,cooperation constante (u) and general bond stability constante(k), according with the calculation from "Nearest Neighbour Interaction Model"

Biochemical al studies were carried out on adult male Wistar rats treated under similar laboratory conditions. The animals weighed 160-170 g; they received the test substance in the form of a suspension via an endogastric probe. Each animal received a single daily dose. Plasma concentrations of lipid fractions and enzymes were found by classic methods. To investigate the influence of aminated polymers on intestinal transit, a single daily dose of 0.6 g/kg in the form of normal saline suspension, not exceeding 1.5 mL per rat, was administered via an endogastric probe. The intestinal transit rate was compared with that recorded in normal rats receiving a similar diet and the same volume (1.5 mL) of normal saline solution.

RESULTS AND DISCUSIONS

Synthesis of Aminated Polymers

Dextran used as macromolecular matrice for synthesis of antihypercholesterolemic drugs is a nontoxic biocompatible substance. This polysaccharide is not absorbed in the gastrointestinal tract. Linear dextran is slowly biodegraded possibly due to the action of an enzyme specific to 1-6 links between glucopyranosidic cycles [27]. A recent study revealed that crosslinked and aminated dextran, orally administered in rats, is eliminated unchanged from the body [30]. Chemical structure of the aminated and crosslinked polysaccharides synthetised is shown in fig. 1.

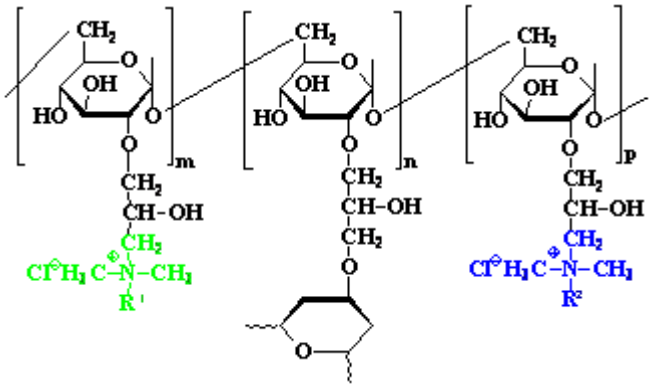

Fig 1 : Chemical Structure of the compounds tested as hypolipemic agents

The crosslinking degree is estimated by the water uptake capacity of the respective hydrogels, which at same time is a measure of the swelling porosity of these gels.(Table 1)

Table 1

Physico-chemical characteristics of the compounds tested as hypolipemic agents

Gel Type A: m = 25 %, p = 0 Type AB : m+p = 55 mol %	Aminic Groups Content, moli %				Swoling in water at equilibrium (optimum domain)
	R^1		R^2		g H_2O/g dry weight
A2	Etil	25	-		3.861
A4	Butil	25	-		3.692
A8	Octil	22	-		3.330
A12	Dodecil	23	-		3.550
ABO (A polymer)	**Etil**	**55**	**Dodecil**	**0**	**3.967**
AB5	Etil	50	Dodecil	5	3.085
AB25 (B polymer)	**Etil**	**35**	**Dodecil**	**25**	**3.087**

Binding of Bile Acid Anions

Binding capacity for bile acid anions was considered to be an indicator for the possible use of an aminated polymer as a hypolipidemic drug. Until now, investigations have been mainly concerned with cholestyramin [12,13] and cellulose derivatives [5,6]. For comparison, the bile acid binding capacities of a number of commercially available polymers with different chemical structures, such as Questran, Colestid and Secholex, were studied [27]. In these study, conjugated and unconjugated bile acids in aqueous solutions were prepared with concentrations close to these in physiologic fluids, without micelle formation [13]. In some cases, adsorption media contained electrolyte buffers (NaCl, NaHCC3, or anions of carboxylic acids) [5,12,13,15].

The capacity *of binding bile acids for the synthetised polymers* was estimated by evaluating the ionisation constante ($k_{0)}$, cooperation constante (u) and general bond stability constante(k), according with the calculation from "Nearest Neighbour Interaction Model" (fig 2)

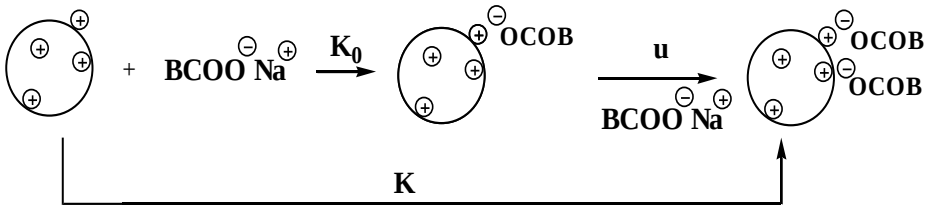

Fig 2 : Nearest Neighbour Interaction Model

Study of the values of the binding constants determined for sodium cholate (NaCA) showes that physico-chemical properties of these polymers can influence their binding capacity in the following manner: B > A >Cholestyramin. The polymers obtained have greater affinity for bile acids as the swelling porosity decreases. This may be explained by the role of the "inclusion" size of the anion in the polymeric network. Cholesteramine has the lowest bile acid binding capacity among the studied polymers studied. It requires a longer time to establish ecruilibrium in the adsorption process (24 h as compared to 2 h for polymers obtained from polysaccharides

Adsorption isothermes of sodium cholate on aminated polymers are sigmoide shape (fig. 3, 4)

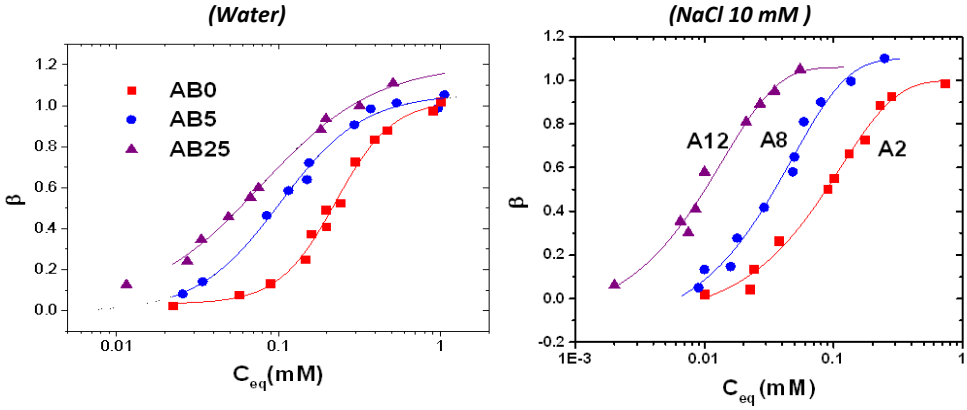

Fig 3:NaCA Binding Isothermes for AB gels in water and NaCl 10nM

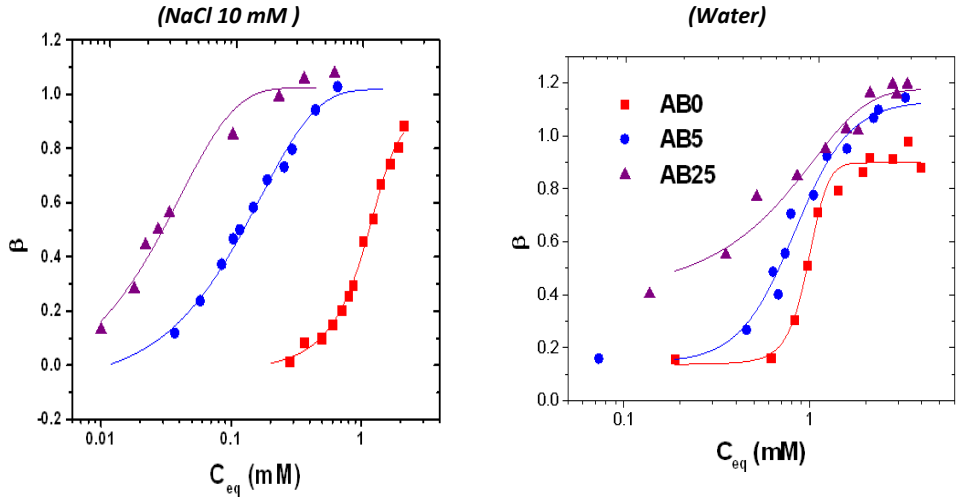

Fig 4 : NaCA Binding Isothermes for A gels in water and NaCl 10nM

The values of the main characteristic parameters for binding bile acid- ionisation constante ($k_{0)}$, cooperation constante (u) and general bond stability constante(k), are shown in fig. 5, 6 and 7.

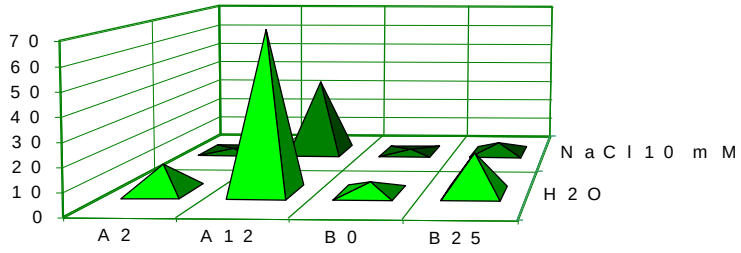

Fig 5: Stability constante (k) for binding NaCA

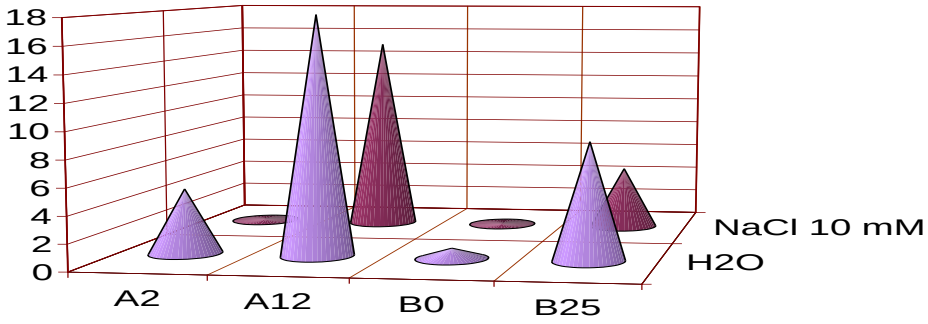

Fig 6 : Ionisation constante (k) for binding NaCA

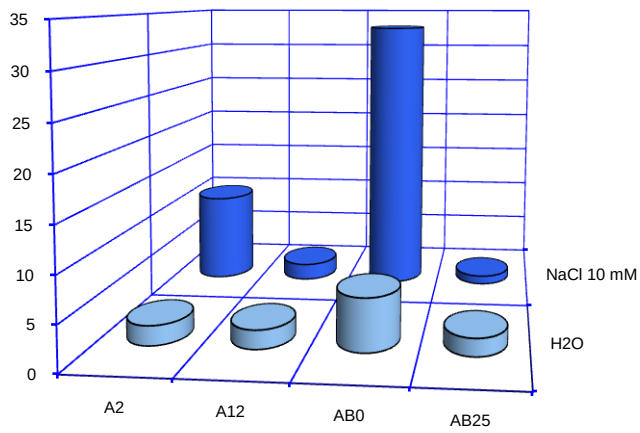

Fig 7: Cooperation constante (u) for binding NaCA

Analysis of the results presented in fig. 5, 6 and 7 shows that increasing of the length and the content of the R radical increase the values of ionisation constante($k_{0)}$ and general bond stability constante(k), but lower the cooperation constante (u), while increasing the ionic force lower the ionisation constante($k_{0)}$ and stability constante(k) but increase the cooperation constante (u) value for the considered polymers.

Table 2

Influence of Amynated Polymers on the Total Plasma Cholesterol, HDL, VLDL and Trygliceride Levels in Normolipemic Rats

Group	*Treatment length, days*	*Total Cholesterol*	*HDL*	*LDL+ VLDL*	*HDL / LDL+ VLDL*	*Triglycerides*
		mg/%	*mg/%*	*mg/%*		*mg/%*
Control	0	74.6	23.8	20.1	1.18	128
	21	73.5[a]	24,2 [a]	23.2 [a]	1.04	142 [a]
B	0	84.6	26.6	26.0	1.1	168
	21	62.5[b]	38.8[b]	16.2 [b]	2.39	102[b]
A	0	80.2	24.4	20.1	1.21	144
	21	76.5[a]	29.6 [a]	22.2 [a]	1.33	130 [c]
C	0	64.6	29.8	18.6	1.60	118
	21	52.5[c]	44,2 [b]	14.2 [c]	3.11	100[c]

Dose 1.2 g/kg /day

****Statistic significance vs. initial level: a not significant ;b <0.05 ; c<0.01***

Results for the in vivo study are shown in table 2.

In the case of compound B, the in vivo tests proved a good-efficiency of the product by lowering very statistically significant the levels of Ch-T, LDL + VLDL and TG and by increasing statisticaly significant HDL levels and HDL/ratio.

In the case of compound A, the in vivo tests shows a decreasing of the blood lipid fractions, but excepting TG, the variations were statistically not significant.

In the case of Cholestyramine, the in vivo tests proved a quite good-efficiency of the product by generating statistically very significant variations for the Ch-T and HDL while the levels of LDL + VLDL and TG varied statisticaly significant compared to the control group.

Comparing the in vitro bile acid binding results with the in vivo tests results for dextran based compounds, the efficiency order is similar (B > A > C); . Compound B with the highest bile acid binding capacity is also the most significant in reducing lipid levels. Taking both polymers into consideration, the in vivo results differ from the in vitro data, which demonstrate the following order of binding efficiencies: B > Cholestyramin >A. Never the less, both Cholestyramine and compound B produce a elevation of HDL/(LDL+VLDL): ratio. This ratio is more important in preventing the occurrence of atheromatous lesions than the level of each single lipid fraction.

The differences in the efficiency order for the binding of bile acids and the lowering of plasma lipid levels may be due to environmental variations in the digestive tract (pH, electrolytes, fatty acids).

In addition to the in vivo studies of plasma lipid levels, we also investigated the effect of aminated polymers on the function of other organs. The intestinal transit rates (Table 3) indicate that all administered products slowly decrease the transit time in the rats receiving a normal diet. No intestinal occlusions were observed.

Table 3

Influence of Polymeric Compounds Administration on the Speed of Intestinal Transit in Rats

Group	No. of rats	Single dose, g/kg per os	Speed of intestinal transit as compared to control	P
Control	10	0.6	100	p<0.01
A	10	0.6	63	p<0.01
B	10	0.6	48	p<0.05
Cholestyramin	10	0.6	41	p<0.05

The plasma levels of transminases and and alpha-amylases after administrating compounds B and A for 3 weeks in rats (Table 4) indicate that hepatic and pancreatic functions are not significantly affected by these substances.

Table 4

Influence of Aminated Polymers Administration on Plasma Levels of Some Enzymes in Rats

Group	Dose, g/kg/day/ per os	*Treatment period, days*	*TGP (I.U)*	*Alpha-amylase (Amylase units)*
Control	0	0	24.3±1.8	35672±1587
		21	39.4±2.8	20626±2862
A	0.6	0	24.3±1.8	35672±1587
		21	32.8±3.8	2662±1872
B	0.6	0	24.3±1.8	35672±1587
		21	30.0±6.6	30426±3862

CONCLUSIONS

The present paper reports on the derivatization of polysaccharide (dextran), along with the bile acid binding capacities (invitro) and plasma lipid level lowering capacities (invivo) of the products. These are compared to a commercially hypolipidemic drug, Cholestyramin, with the following results:

1. In vitro study of the values of the binding constants determined for sodium cholate (NaCA) showes that physico-chemical properties of these polymers can influence their binding capacity in the following manner: B > A >Cholestyramin.
2. In vivo results differ from the in vitro data, which demonstrate the following order of binding efficiencies: B > Cholestyramin >A. The differences in the efficiency order for the binding of bile acids and the lowering of plasma lipid levels may be due to environmental variations in the digestive tract (pH, electrolytes, fatty acids).
3. Aminated polysaccharides administered in rats do not affect the digestive system, liver or pancreas functions.

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Comparative aspects respecting the morphology of the oropharynx in *Cygnus cygnus* and *Cygnus olor*

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The study was about the main differences between the oropharynx in Cygnus cygnus and Cygnus olor. There are a lot of differences regarding external and internal morphology. The most of them are on the roof of oropharynx, on the dorsal face of the tongue, on the dorsal face of larynx.

Key Words: Cygnus Cygnus, Cygnus olor, oropharynx, anatomy

Matherials and methods

For this studies we used 3 head of C. olor and 4 head of C. cygnus, which was detached from different birds from Zoo Bucharest and from the Faculty of Veterinary Medicine, along a few years, died from different skeletical problem. The heads was prepared, photographed and scaned on PC.

The names of anatomical structures was corelated to N.A.A.- 1993.

Results

Both species, so closed phylogenetical, have a lot of morphologycal peculiarities, special to oropharyngeal cavities. So, we find that the superior valve at C. olor is widder that C. cygnus and the superior border is concave. In caudal segment has a proeminent caruncle.

On the lateral borders of superior valve in C. olor there are 52-55 transversal horny ridges. At C. cygnus there are only 39-40 transversal horny ridges.

In C. olor on the oropharyngeal roof there is a median ridge with tubercles who becomes bigger in the caudal half of it. This is lined by one row of tubercles each side.

In C. cygnus the median ridge is bigger in the middle third of the oropharyngeal roof. At caudal extremity of the medial ridge there are only 3-4 rounded tubercles. In C. cygnus the roof of oral cavity is demarcated by the pharyngeal roof from a transversal row of papillae who missing in C. olor.

In C. olor the tongue is widder, filliform papillae from the lateral parts at the rostral half are longer, and in the caudal extremity of it there is one row of large transversal lingual papillae and caudally another smaller row. In C. cygnus in this area there is three rows of smaller papillae.

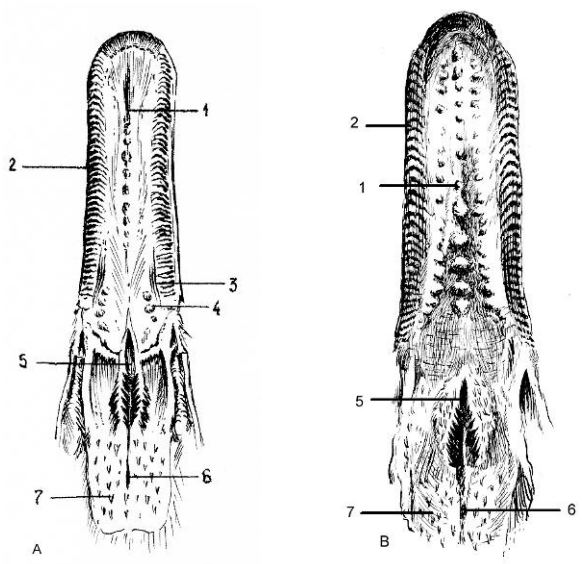

Fig. 1 The oropharzngeal roof in C. czgnus (A) and C. olor (B)
1-median ridge; 2- tranversal horny ridges; 3- lateral longitudinal rideges; 4- wide-based papillae; 5-palatine slip; 6- common opening of faringeal tube; 7- faringeal papillae

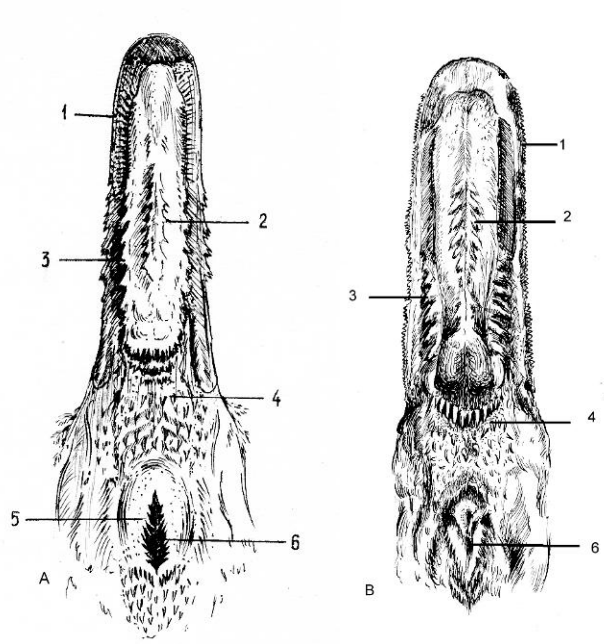

Fig. 2 The orofaryngeal floor of in C. czgnus (A) and C. olor (B)
dorsal lamellae; 2- papillae linguale; 3- large papillae; 4- pharyngeal papillae; 5- laryngeal mound; 7-inlet of the larinx

In the rostral part of laryngial inlet in C. olor there is a mucous fold who becomes thinner caudally and divide the laryingeal inlet in rostral part.

Conclusions

1. On the lateral borders of superior valve in C. olor there are 52-55 transversal horny ridges. At C. cygnus there are only 39-40 transversal horny ridges.
2. In C. cygnus the roof of oral cavity is demarcated by the pharyngeal roof from a transversal row of papillae who missing in C. olor.
3. In the rostral part of laryngial inlet in C. olor there is a mucous fold who becomes thinner caudally and divide the laryingeal inlet in rostral part.

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The influence of the administration of different concentrations of *Satureja Larstensis L* and *Anetlum graveolens L*. as botanical additives, on the hematological indicators in broilers

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Growth biostimulators are used in order to ensure a quick body mass growth cells specific action mechanism, it was proven that generally the bioactive components that act sinergically, penetrates the cell membrane of the pathogen bacteria, duo to their lipophilie properties, and determine the phospholipids cathalisation.

The suspicions concerning the possible remanence of antibiotics in animal products and the installation of the bioresistance in consumers lead to the search of alternatives to antibiotics usage.

In vitro studies with essential oils revealed a powerful antimicrobial action. Starting from these results, we tested the effect of essential oils on animals and we established that they have great antioxidant activity, stimulating enzymatic intestinal secretion, and powerful antimicrobian effect.

Key Words: biostimulators, bioresistance, fodder additives

Growth biostimulators are used in order to ensure a quick body mass growth cells specific action mechanism, it was proven that generally the bioactive components that act sinergically, penetrates the cell membrane of the pathogen bacteria, duo to their lipophilie properties, and determine the phospholipids cathalisation. Other ways of action consists in the lyses of the cellular wall, and the spilling of the cellular contents, the inhibition of the ionic transport mechanisms the inhibition of protein transcription and of certain enzymes.

We outlined the fact that the main antimicrobian activity of asomatic and spicy medicinal herbs, as well as that of the extracts and essential oils obtained from those, is regulated either by mixing two an several herbs, either by their administrative concomitant with different natural substances from the probiotic and prebiotic group (especially organic acids).

The suspicions concerning the possible remanence of antibiotics in animal products and the installation of the bioresistance in consumers lead to the search of alternatives to antibiotics usage.

The effect of certain active components can be influenced by the protein concentration and by the amount of energy of the food recipe used.

Material and methods

Our research was centered on some spicy and asomatic medical herbs, used as they came or as essential oils.

In vitro studies with essential oils revealed a powerful antimicrobial action. Starting from these results, we tested the effect of essential oils on animals and we established that they have great antioxidant activity, stimulating enzymatic intestinal secretion, and powerful antimicrobian effect.

The determinations were made on broilers Rass 308. We created three experimental groups: a whiteness group and experimental group (L_1) who was administrated 0,6% thyme, and a second experimental group (L_2) who was administrated 0,3% thyme and 2,3% dill. We studied a total of 45 broilers, 15 for each group.

The blood samples taken from the 42 days old broilers were the subject of biochemical determination in the Physiology Laboratory of the Zootechny Faculty.

Results and Dissensions

The experimental protocol established the testing of the biostimulator effect of the thyme *Satureja Hostensis L* administered in a concentration of 6%, and of a mix of thyme 0,3% and dill 0,3% *Anethum Graveolens L.*

We used complete combined fodder, specific for each growing period: starter (PB 22%, 3005 KCAL EM/Kg), growth (PB 20%, 3100Kcal/EM/Kg), growth-finissage (PB 18%, 3153Kcal/EM/Kg), to which we added the herbs supplements.

The chemical composition of thyme is represented by a certain quantity of volatile oils, which varies between 0,5-2,7%; monoterpenic Lidrocarbonates: α-β terpinen (25,8%), α and β pinen, p-cimen (7%), m-cimen, α-triem, camphsono, mircen, D_3 and D_4 caren, sabinen, α-felandren, limonem, terpenoidie alcohols and phends: 3-octanol, linalool, terpinen-4-al, trans-8-trianol, α-terpined, geraniol, myrthenol, borned, terpinde, carvacral, which can vary between 39-90%, thyme, engenol, carvacrol-methyl-ether.

Carvacrol and thymole are the main components of both the garden and culture thyme, and have the most important pharmacadynamic action.

The relative toxicity of the bioactive components is 2, considered to be a medium toxicity (0-nontoxit, 1-2-secure, 3-very toxic).

The active components in leaves, strain and fonits offer dill its therapeutically properties: volatile oils 50%, monoterpenic hidrocarbonates: α and β pinen (under 1%), sabinen, mircen, α-felandren (15-20%), β-felandren (2-3%), limonene (25-30%), mentatriena, p-cimen (1-3%), cis and trans acimen, 8-terpinen (1-2%), terpenoidic acids cis and trans-limonen epoxide, terpinen-4-ol, dillether (5%), monoterpenic cetones: cis and trans-dihydrocarvona (4%), (+)-carvona (25-40%), characteristic compound of the dill oil, fencona, cis and trans-carveols, dihydro-izocarveol, 1-p-menthen-ol, terpenoidic and non-terpenoidic esthers: dihydrocarvil acetate, transcarvil acetate and others; very low quantities of sesquiterpenoids: dillapiol, miristicina, β-cariephylene.

The bioactive chemical compounds of dill act sinergically, having the next main actions: carminative, digestive, antidiarrheaic, antispastic, major antiinfection (antibacterial, antiviral, antiparasitary and antimicotic), antalgic immunostimulatory, antivomitive, galactogen. The toxicity of the dill biocomponents is mainly due to cetones, which are neurotoxic.

There is a direct link between the alimentation and metabolism of animals, the homeostazia of the organism being dependent on nutritive substances and biological active compounds.

We determined the parameters of the proteic, energetic, mineral and enzymatic metabolism, to determine if there are any essential modifications after the administration of the mentioned above botanic additives compared to the whitness group.

Specification	L_w	L_1	L_2
Glucoses (g ‰)	2,31	2,34	2,30
Total proteins (g %)	4,35	4,4	4,31
Total lipids (mg %)	439	440	437
Cholesteride (mg %)	1,29	1,28	1,28
Phospholipids (mg %)	188	192	186
Natrium ions (mEg/l)	72	79	78
Potassium ions (mg/l)	159	161	160
Calcium ions (mg/l)	4,8	5,6	5,3
TGO	6,8	7,2	7,1
TGP	170,1	172	170,4
LDH	42,4	47,5	41,9
CPK	1850	1968	1860
Lipases	239	274	265
CLE	2580	2422	2480

Glucide are the main energy source, due to their abundance in nature and their availability for oxidative degradation.

The (blood glucoses) glicemia had medium values of 2,34 in L_1 and 2,30 in L_2, similar to those presented in medical literature. The energy deficiency in the nutriments produces growth retardation and an inefficient assimilation of nutritional compounds.

Age is one of the most important factors influencing the concentration of plasmatic proteins, proteic deficiencies of the fodder for 2-3 weeks leads to growth delays, as well as lipoproteinemia.

The total proteins had a concentration of 4,4 g% in L_1, and 4,31 g/% in L_2; both values are normal.

The total lipids had a concentration of 44, mg/% in L_1, and 437% in L_1.

The cholesterolemia in 42 days old broilers was 1,28 mg/% both in L_1 and L_2.

The phospholipids had concentrations of 192 mg% in L_1 and 186 mg% in L_2.

Triglicerides store energy and liberate the fat acids needed during the oxidation processes in tissues, their concentration was 92 mg% in L_1 and 78 mg% in L_2.

Sodium ions had values of 161 mEg/l in L_1 and 160 mEg/l in L_2. both the concentrations of the potassium and calcium ions are normal.

We also studied the main seric enzymes, knowing the fact that some herbs used as botanic additives have some components with toxic effect if administered in large quantities that can affect hepatic and muscular functions.

Glutamoxalacetic transaminase (TGO) had a concentration of 44,7 U/l in L_1 and 41,9 U/l in L_2.

Glutampiruvic transaminase (TGP) had a concentration of 47,5 U/l in L_1 and 41,9 U/l in L_2.

Lacticdehidrogenase (LDH)in 42 days old broilers plasma had a concentration of 1968 U/l in L_1 and 1860 U/l in L_2.

Creatinphosphokinasis (CPK) had a concentration of 274 U/l in L_1 and 265 U/l in L_2.

The broilers plasma had a lipase concentration of 1,5 U/l in L_1 and 1,60 U/l in L_2.

Cholinesterase (CHE) had a concentration of 2422 U/l in L_1 and 2480 U/l in L_2.

Conclusions

1. We consider that the fodder additives: garden thyme and dill, used in this experimental study, didn't have a significant influence on the parameters we studied.
2. The test made to evaluate the possibility of muscular remanence of some herbal substances, determined that these active components could not be identified. Most of these active components are rapidly metabolized in the liner, and excreted in less than 24 hours.
3. The positive physiological results obtained consequently the administration of spicy and asomatic plants recommend their use as growth promoters that can successfully replace antibiotics.

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The influence of the administration of various concentrations of *Satureja hortensis L* and *Anethum graveolens L*, used as growth biostimulators, on the qualities of the meat

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All over the world, some medical and spicy herbs were studied (botanical additives) in order to test their biostimulating potential when used supplement to animal fodder.

The herbs were administered as powder compounds of 4 medicinal herbs, wich can be biologic constituents as well as secondary metabolites (terpenoids, phenols glycosides, alkaloids, alcohols, cetones, acids, etc.), interact with each other and are in direct relation with other active secondary components.

The determinations were made on broilers Rass 308. Were chose 3 experimental groups: a witness group, an experimental group which we administered thyme 0,6% (L1), an experimental group which was administered thyme 0,6% and dill 0,3% (L3). We used combinated fodder, specific for each growing period: starter (PB 22%, 3005 Kcal/EM/kg) growth (PB 20%, 3100 Kcal/EM/kg), growth finishing (PB 18%, 3153 Kcal/EM/kg), which added the herb supplements.

Key Words: biostimulators, meat quality, medicinal plants

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Within the organism, these biostimulators play several soles: antibacterial, bacteriostatic, antiviral, antimicotic, stomahic carminative, antihelminthic, antioxidant, immunoregulatory with positive effects on animal growth and development and a very efficient use of nourishment.

Material and method

A somatic and spicy medicinal plants were taken in study as n alternative to antibiotics looking for the most efficient way of administration in minimal dozes.

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In order to appreciate meat quality, several determinations were made: organoleptic control, daily weigh gain index consumption, dressing percentage, chemical composition.

Results and discussions

After the administration of growth biostimulators the results concerning the body weight were 6,5% higher then these of the witness group.

Evolution of the medium weigh, medium daily weigh gain and at 42 days.

Specification	L_m	L_1	L_2
Body weigh of 1 day old broiler	40,1	40,0	40,0
14 days	380	420	410
28 days	1380	1410	1390
42 days	2400	2554	2550
Daily weigh gain	56,1	59,85	59,76
Total fodder (g/broiler)	4222	4349	4443
I.C. nc/kg spor	1,79	1,73	1,77

I.C. was smaller then in the witness group by 3,4% in L_1 and by 1,1% in L_2, demonstrating that 0,6 thyme powder used as biostimulator leads to an improvement on growing performance and to a superior conversion of fodder.

The concentration of 0,6% indicates the fact that thyme had the best results, due to it's capacity to stimulate digestive secretions directly implicated in fodder digestion.

Thyme determines a raise in the level of the main digestive enzymes, and, through his antibacterial properties, maintains an intestinal environment favorable to the envelopment of the benefic intestinal microorganisms. It's antioxidant properties greatly contributes to stress diminishing.

Organoleptic characteristic of poultry meat were not modified; all of them proved to be in standard (STAS 6997/74, STAS 7031/83) in all experimental groups.

The medium carcass weigh was determined by weighing the whole group.

Dressing percentage for each group were: 71,5% in the witness group, 71,8% in L_1 and 71,5% in L_2.

All the figures proved to be in concordance with the body weighs of the days old broilers, but the values in L_2 were close to those of the witness group. Analyzing the data in the table and comparing the results, we can easily notice that L_1 (thyme 0,6%) had better results than both L_2 and the witness group, but without significant differences cells for carcass quality, the evolution was made by studying the general aspect of the carcass as well as the organoleptic, physic and chemical properties of meat.

The carcasses were designed as first quality.

Medium carcass and body parts weight

Specification	L_m	L_1	L_2
Carcass	1716	1833	1730
Chest	429	457	432
Calf of the leg	549	585	553
Wing	205	219	208
Cover	533	569	537

Comparing the data about the weight of the main organs, we can notice that L_1 broilers had values with 2,4% up to 6,6% higher than the witness group, but without truly significant difference.

Medium weight of the main internal organs

Specification	L_m	L_1	L_2
Liver	46	47,2	46,7
Heart	10,5	11,2	10,9
Gizzard	34,1	34,8	34,4

All internal organs were studied from clinical point of view: adipose deposits on internal organs were bigger in the witness group; the colour of the adipose tissue was a light yellow in the other group, compared to an intense yellow in the other groups, the liver appeared normal, with modifications bile appeared normal the pancreas was normal as well as the spleen.

Because in birds the chest meat has good quality and represents a big percent of the carcass, chemical determinations were made in fragments taken at that level.

Chemical composition is one of the most important indicator to evaluate meet quality.

Chemical composition of broilers meat

Group	Water%	S.U.%	P.B.%	Lipids%	Minerals%	Others%
L_m	71,7	28,3	22,4	4,2	1,2	0,5
L_1	71,2	28,8	22,6	4,8	1,2	0,2
L_2	71,8	28,2	22,1	4,5	1,2	0,4

Analyzing the data in the table we can notice that the values of the 3 groups are close to those presented in medical literature, the additives used did not influence meat chemical composition.

Conclusions

1. The results concerning the medium weights at 42 days old broilers from L_1 and L_2 groups were 6% and 2,3% higher than in the witness group. The same aspect was noticed in all the other indicators: weight of carcass, weight of the main trench portions and of the internal organs.
2. Meat quality, expressed through organoleptic, physical and chemical properties, was superior in the groups fed with herbal additives, certifying that one of the botanical additives qualities is to improve organoleptic meat qualities.
3. These results prove the fact that some of the herbs administered in broiler's fodder can have biostimulating effect, determining a superior growth and development.

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Observations upon the evolution of infectious granuloma at peacock

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At peacock, as in any other bird, granulomatous inflamations are produced by different pathological agents. These granulomas are localized at all major organs of the body and have various aspects depending upon the pathological agent, the state of the disease and the reactivity of the organism. This particular paper work wants to show the histopathological aspects of the evolution of infectious granuloma at a few peacocks.

Key Words: infectious granulomas, peacock, histology

The giant cell inflammation is a mainly prolifferative inflamation with chronical evolution that is characterized by the participation of all inflammatory cells and differentiation of the epitelioid and giant cells.

Regarding the classification of this inflammation, this is made after a lot of criterias:

- after extention are classified in: granulomatous and diffuse with giant cells;
- after etiology are classified in: infectious, micotic, parasitic, of foreign bodies and allergic.

Morphogenesis of infectious granuloma

The introduction of infectious agents in the animal body is followed by the neutrophile granulocytes invasion at the entry door.These mycrophages die in a few hours because they are not capable to phagocyte the pathogenical agent. Bacterian poliyzaharides next to fibrine and mediators relesed by granulocytes attract a number of monocytes that transform in macrophagues.

These macrophags produce a micronodular lesion called pregranuloma or macrophagic granuloma.

Under ceramides, glicophosphatides and phtioic acide action, macrophages differentiate in epitelioid cells resulting the epitelioid or fresh granuloma. This forms in approximately 2 weeks.

At the peripheria of the granuloma can be observed also the umoral defence represented by the presence of lymphocytes and plasmocytes .

Epitelioid cells under the action of the fusion factor of macrophages MFF or IL-4 gather in multinucleate cells Langhans and is born the epitelioid-giant or young granuloma.

After 3 weeks, in relation to the species, ischemia, lysosomal enzymes released by granulocytes and dead macrophages and some bacterian components produce focal necrosis of some structures. With the first necrosis nuclei the granuloma becomes mature.

In tuberculosis at birds, giant cells are quickly formed, in a large number and groupes around the cazeification necrosis, and the other levels are precisely arranged with a clear concetrical stratification.The necrosis area is very reach in bacteria and doesn't calcificate. In chronical colibacilosis, the coligranuloma produced by roots of E.coli, present epitelioid and giant cells with numerous vacuoles in cytoplasma, aspect characteristic to this infection.

Material and method

The research was made on a number of 7 peacoks males and females (Fam. Phasianidae, sp. Pavo muticus) with ages between 6 months and 1 year, good health, came from a particular breeder.

The studies were made on organ fragments came from fresh bodies.

The fixing was made in formaldehide water solution 10% and for the colouring was used Haematoxiline-Eosine - Methil blue and Pas method.

Results and discussions

At the 7 peacock bodies studied, the most affected organs were the liver, spleen, intestine and heart. At these was noticed macroscopically the presence of nodules of different dimensions from a few mm to a few cm, white in colour, high consistence, diseminated in all organ both at the surface and on section.

It was studied from histological point of view the evolution of granulomatous inflammation and the secondary lesions that appeared. At the liver , was noticed the presence of macrophages gather called macrpphagic granuloma or pregranuloma.

At the same organ from the same peacock was noticed the differentiation of macrophages in epitelioid cells and the constitution of the epitelioid granuloma.

At another peacock, at the liver, was noticed epitelioid cells that gathered in giant cells Langhans, giving birth to the epitelioid-giant granuloma or young granuloma. (Fig. 1)

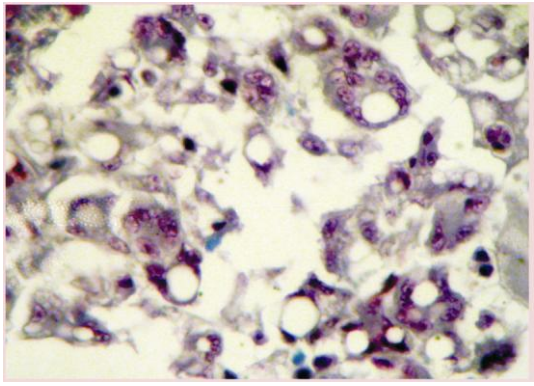
Another 2 cases presented at the liver and spleen were noticed mature granulomas with oxiphile necrosis center, giant cells are disposed around the necrosis foci and at the exterior fibrous hyperplasia.(Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 7, Fig.9,Fig.10).

In liver, spleen and heart was noticed giant cells that had vacuoles in the citoplasma, giving it a spongious aspect.(Fig. 6, Fig.8)

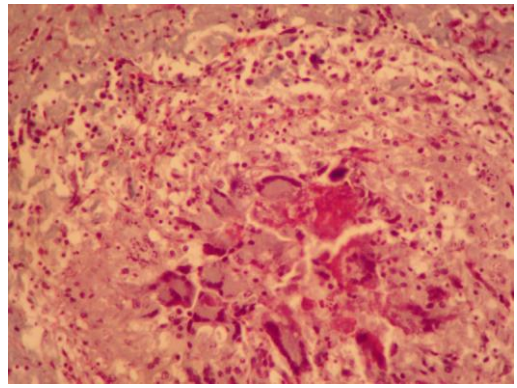
After this particular aspect of the giant cells the diagnosis could be coligranulomatosis, because the vacuolized aspect of the giant cells is characteristic to the chronical inflammation produced by E.coli at birds.

At the intestine, in another peacock, next the spleen and liver granulomas was noticed a big granuloma at which could be distinguished necrosis foci, epitelioid and giant cells, limphocytes and histiocytes.(Fig. 7)

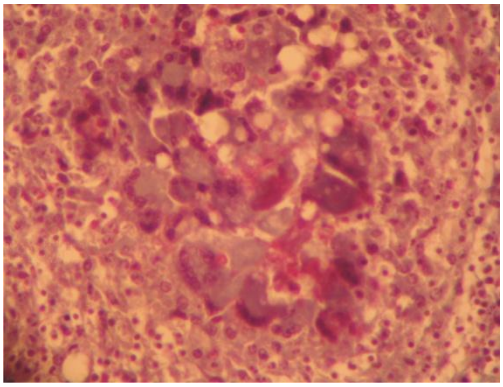
As an associated lesion was noticed liver amiloidosis, this also prooving the chronical evolution of the disease.(Fig.11)



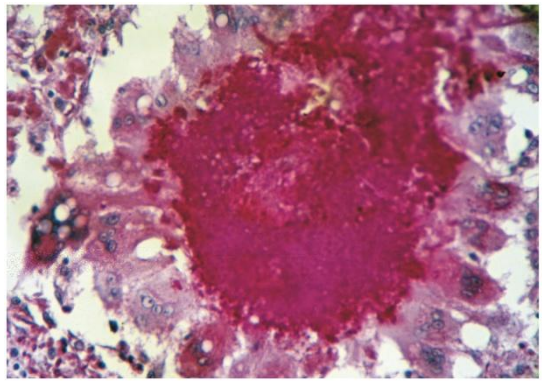
Epitelioid-giant granuloma.
Lung.
Col. HEAx400



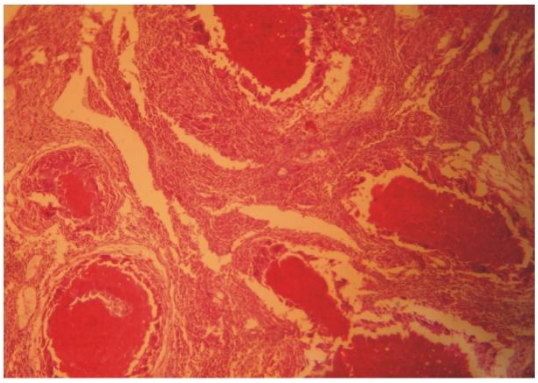
Constituition of the necrosis foci.
Liver.
Col.HEAx400



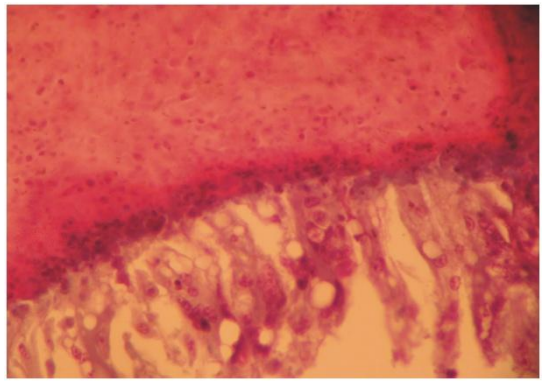
Constituition of the necrosis foci.
Spleen.
Col. HEAx400



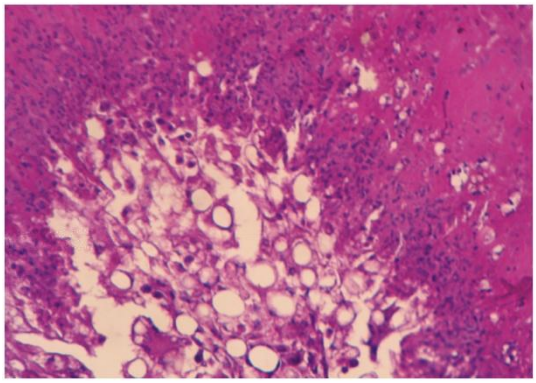
Mature granuloma.
Lung.
Col.PASx400



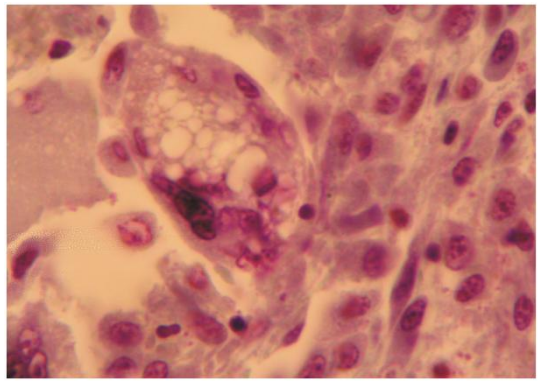
Mature granuloma.
Spleen.
Col. PASx100



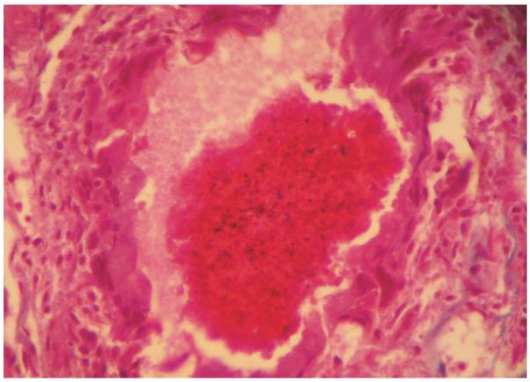
Giant cells with vacuolized citoplasma. Lung.
Col.PASx400



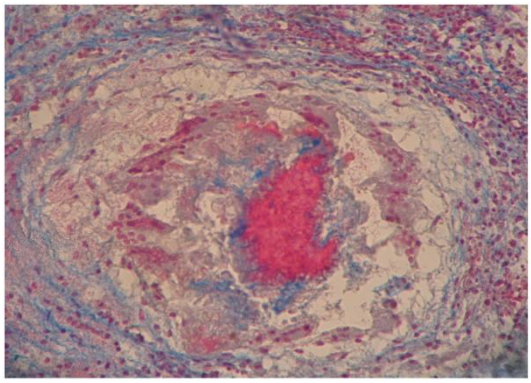
Giant cells with vacuolized citoplasma
Intestine.



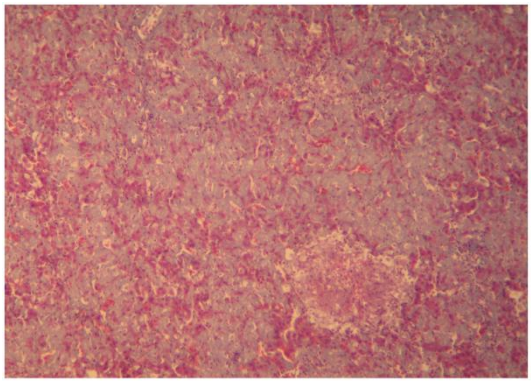
Giant cell with vacuolized citoplasma
Liver.



Unspecific cell proliferation and fibrous pheripheric
Liver. Col.PASx400



Pheripheric fibrous proliferation.
Intestine. Col.HEAx400



Amyloid hepatosis.
Liver. Col.HEAx400

Conclusions

1. At all the histological examined cases the infectious granulomas were noticed in different evolutive stages. Mature granulomas were very well structured presenting a central oxiphil necrosis, the epitelioid and giant cells disposed in a very well marked level as well as cell proliferations and peripheric fibrilar.
2. Liver amiloidosis observed at 3 cases shows the chronical evolution of the infectious process.
3. At all examined cases, the liver was constantly affected, granulomatous hepatitis being histologically evidenced through a multitude of granulomas in different stages of evolution, sublobular dimensions.
4. Vacuolized aspect of the giant cells from the structure of the observed granulomas is specific to colibacil infection.

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Acrylamide - toxic compound formed during the thermical process of aliments

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Acrylamide, a chemical compound obtained for the first time by Moureu in 1893 and industrially produced starting with 1954, is used in the production of polyacrylamide (in the treatment of wastewater and potable water), the production of plastics and food packaging materials, paper and pulp manufacture, in sugar refining, crude oil production. Starting with the first years of production and use numerous acrylamide intoxication cases appeared, due to the occupational exposure or general population exposure because of impurified potable water. The first signal regarding the presence of acrylamide in starchy foodstuffs exposed to thermical treatments has been given by the Swedish researchers in april 2002. Further OMS launched a reasearch and legislative programme regarding the optimal technological and culinary proceedings in order to obtain a minimum acrylamide level. In Romania, the acrylamide reasearches have been approached only from the analitical aspect and upon few aliments. The present project proposes an unitary approach of the acrylamide subject, starting with the indsustrial synthesis, continuing with the read-in of the mechanisms of formation, and ending with the negative effects upon animal and human organism and also possible ways of counteracting them.

Key Words: acrylamide, mechanism of formation, human exposure, carcinogenity

INTRODUCERE

Acrilamida este o substanţă chimică cunoscută încă din anul 1893, când a fost sintetizată pentru prima dată de către Moureu, în Germania, prin hidratarea catalitică a acrilonitrilului (1).

Acrilamida este un compus bifuncţional, care conţine o dublă legătură electrofilă şi o grupare amidică, grupări ce îi conferă atât caracter bazic cât şi acid (2) (fig. 1).

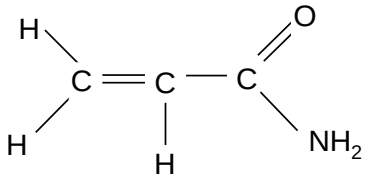

Figura 1. Formula structurală a acrilamidei

SINTEZA INDUSTRIALĂ ŞI FORMAREA ACRILAMIDEI ÎN ALIMENTE

Acrilamida este o substanţă chimică ce poate lua naştere pe două căi:

sinteza industrială, prin hidratarea acrilonitrilului (până în 2001, acrilamida era considerată o substanţă exclusiv sintetică) (3);

calea naturală de formare a acrilamidei în timpul procesului culinar de prăjire sau coacere (descoperire realizată în 2001, o dată cu observarea unor mari cantități de acrilamidă în cartofii prăjiți de către Departamentul de Chimie a Mediului de la Universitatea din Stockholm în colaborare cu cercetătorii de la AnalyCen) (1).

2.1Obținerea acrilamidei la nivel industrial

Producția la nivel industrial a debutat în 1954 în cadrul American Cyanamid Inc (3) (fig. 2).

Astăzi se cunosc trei procedee de obținere a acrilamidei la nivel industrial, pornind de la hidratarea acrilonitrilului: metoda cu acid sulfuric, metoda catalitică şi metoda de hidratare enzimatică cu ajutorul microorganismelor (4).

Metoda utilizată inițial a fost metoda de hidratare a acrilonitrilului cu acid sulfuric şi amoniac, urmată de îmbunătățirea procedeului prin utilizarea de catalizatori eterogeni bazați pe cupru şi oxizi de crom (4).

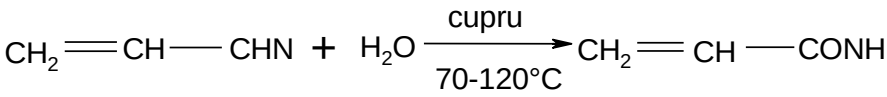

Figura 2. Obținerea acrilamidei la nivel industrial prin hidratarea catalitică a acrilonitrilului

Metoda de hidratare catalitică a acrilonitrilului este preferată şi este singurul proces utilizat în SUA începând cu 1981 (14). Prezintă multe avantaje față de metoda cu acid sulfuric în privința purității acrilamidei obținute (99.5 - 99.9% comparativ cu 98%), nu apar alți produşi de reacție nedoriți, randamentul este mai mare (97% comparativ cu 80%) şi un pas costisitor de purificare a acrilamidei este evitat (4). Metoda rămâne totuşi costisitoare prin necesitatea recirculării şi separării acrilonitrilului, precum şi a concentrării soluției de acrilamidă în vederea obținerii poliacrilamidei.

În anul 1980, în Japonia au început cercetările asupra unui procedeu de biotransformare pentru hidratarea acrilonitrilului. Nitto Chemical a realizat primul proces de biotransformare care folosea microorganisme imobilizate într-o matrice poliacrilamidică. Microorganismele conțineau enzima nitril hidrataza activă (5).

Provocarea consta acum în obținerea unor microorganisme care să dețină nu numai o mare capacitate de hidratare a grupării nitril ci şi o capacitate de hidroliză scăzută, astfel încât acrilamida formată să nu fie hidrolizată la acid acrilic şi amoniac. Răspunsul a fost găsit o dată cu obținerea microorganismului *Rhodococcus rhodochrous* J1, înlăturând etapele de recirculare, separare şi concentrare costisitoare (6).

Alți cercetători au folosit celulele provenite de la microorganismul *Brevibacterium* CH1, posedând o activitate crescută a nitril hidratazei asupra acrilonitrilului şi o activitate scăzută a amidazei asupra acrilamidei (6).

2.2 Formarea acrilamidei in alimente

În aprilie 2002, cercetătorii de la Swedish National Food Administration şi Stockholm University au anunțat prezența acrilamidei în alimentele bogate în amidon şi prelucrate termic prin prăjire sau coacere, întreaga lume devenind alarmată, dat fiind profilul toxicologic al acestui compus. Numeroase țări au luat inițiativă şi s-au implicat într-un program internațional care avea următoarele obiective: să dezvolte metode sensibile de analiză cantitativă a acrilamidei, să determine concentrația toxicului în alimente, să descopere mecanismul de formare, precum şi să determine posibilități de reducere a acrilamidei în alimente (7).

Ca mecanism de formare a acrilamidei în alimente au fost propuse multe ipoteze:

pornind de la aminoacizi (fie din alanină, prin aminare şi dezaminare, fie din asparagină sau glutamină prin scindare radicalică, fie din metionină şi dicarbonil) (8, 9, 10);

pornind de la acroleine (din glicerol prin deshidratare, din monogliceride prin oxidare, sau din fragmente lipidice prin recombinare, din amidon şi zaharuri prin scindare, fie din acetaldehidă şi formaldehidă prin recombinarea aldehidelor simple) (8, 9, 10);

pornind de la acidul acrilic (de la acroleină prin oxidare directă sau aminare, de la α-alanină şi β-alanină prin dezaminare, de la acid malic prin decarboxilare şi deshidratare, de la acid tartric, de la cisteină prin dezaminare, sau de la serină prin dezaminare şi deshidratare) (8, 9, 10);

pornind de la precursori Maillard: de la N-glicozide prin reacţii de descompunere termică (8, 9, 10); aminoacizi (asparagine) şi zaharuri reducătoare prin reacţia Maillard (11, 12) sau de la aminoacizi şi compuşi dicarbonilici prin degradarea Strecker (13).

Experimental s-a demonstrat că mecanismul principal de formare al acrilamidei îl constituie reacţia Maillard, având ca precursori asparagina şi zaharuri reducătoare (11, 12, 13). Formarea acrilamidei pornind de la intermediari ca trigliceridele, acroleina sau acidul acrilic nu este susţinută de suficiente date experimentale (14).

Luând în considerare caracteristicile reacţiei Maillard, precum şi rezultatele obţinute în diverse experimente, procesul de formare a acrilamidei este influenţat de numeroşi factori: timpul de prăjire/coacere (15, 16), **temperatura de coacere** (17) **conţinutul în apă** (17, 18), **conţinutul în asparagină** (9, 10, 11, 12, 13), **zaharuri reducătoare** (13, 20, 21), **raportul molar aminoacid/zaharuri reducătoare** (10), **lipidele (**8, 14, 19), **proteinele** (14), **carbonatul de amoniu** (14), **condiţiile de depozitare** (13, 17), **condiţiile agricole** (21).

3. EXPUNEREA LA ACRILAMIDA

Încă din primii ani de producţie şi utilizare s-au semnalat numeroase cazuri de intoxicaţie cu acrilamidă, datorate fie expunerii profesionale, fie expunerii generale a populaţiei prin impurificarea apei potabile (23).

Expunerea profesională la acrilamidă poate avea loc în toate stadiile de preparare şi utilizare ale acesteia (prepararea monomerilor şi polimerilor de acrilamidă, utilizările poliacrilamidei, prepararea gelurilor de poliacrilamidă) (3). Expunerea profesională poate avea loc prin inhalarea prafului, pudrei sau vaporilor de acrilamidă (substanţa solidă sublimează lent la temperatura camerei) sau prin contact cu forma solidă sau soluţiile apoase ale monomerului. Nivelul expunerii este maxim în timpul procesului de producţie a monomerului, în timp ce stadiile târzii ale producţiei de poliacrilamidă prezintă un risc minim, deoarece acrilamida este fixată în matricea polimerică (2).

Expunerea generală a populaţiei

Acrilamida poate ajunge în organismul uman prin ingestia de apă potabilă impurificată sau alimente ce conţin acest compus (23).

Prima semnalare referitoare la prezenţa acrilamidei în alimente bogate în amidon, supuse tratamentelor termice, a fost făcută de cercetătorii suedezi în aprilie 2005 (1). Comitetele de experţi FAO/OMS au estimat expunerea la acrilamidă prin ingestia de alimente, utilizând date privind consumul alimentar din Australia, Norvegia, Elveţia, Suedia şi Statele Unite. Estimările minime ale expunerii prin consum alimentar pentru un adult au fost de 0.3-0.8 μg/Kg/zi. Pentru copii, valorile au fost de 2-3 ori mai mari, raportate la suprafaţa corporală (23). Aceste estimări nu dau nici o indicaţie asupra limitei maxime a nivelului de ingestie, dar dacă comparăm cu limita stabilită de OMS pentru acrilamidă în apa potabilă (0.5 μg/L) (23), şi multiplicăm cu 2 l (consumul zilnic de apă indicat unui adult) şi raportăm la 70 kg (masa corporală medie a unui adult), obţinem un nivel maxim de 0.014 μg/Kg /zi, valoare aflată cu mult sub limita estimărilor făcute de FAO/OMS pentru consumul zilnic al alimentelor ce conţin acrilamidă.

Expunerea generală pe cale dermică poate avea loc în principal în urma utilizării produselor cosmetice şi mai puţin prin contact cu textile ce conţin poliacrilamidă (23).

Expunerea generală a populației mai poate avea loc prin inhalarea aerului poluat cu fum de țigară sau prin consumul direct de țigări. Conținutul în acrilamidă a fumului rezultat de la o țigară este de 1.1-2.34 μg. Presupunând că un adult de 70 de kg fumează 20 de țigări pe zi, doza medie inhalată este de 0.67 μg/Kg /zi (23).

TOXICOCINETICA

Date privind toxicocinetica acrilamidei la oameni sunt reduse. Oricum, simptomele observate în urma intoxicației cu acrilamidă indică faptul că aceasta este absorbită de organismul uman atât prin ingestie cât şi pe cale dermică şi inhalatorie (3, 24).

Datele privind absorbția, distribuția, metabolizarea şi eliminarea acrilamidei la rozătoare sunt mai numeroase. Experimentele pe şoareci şi şobolani arată că acrilamida se absoarbe atât pe cale orală, cât şi în urma aplicării dermice sau prin inhalare (23). Acrilamida este metabolizată pe două căi: prin conjugare cu glutation sau prin oxidare la glicidamidă, un epoxid toxic (25). Prin conjugarea directă a acrilamidei cu glutationul se formează N-acetil-S-(3-amino-3-oxopropil)cisteina şi S-(3-amino-3-oxopropil)-cisteina, care se excretă prin urină (24, 25, 26). Glicidamida se excretă ca atare în urină sau, mai departe, parcurge acelaşi traseu de conjugare cu glutationul, formând N-acetil-S-(3-amino-2-hidroxi-3-oxopropil)cisteina şi N-acetil-S-(1-carbamoil-2-hidroxietil)cisteina, de asemenea eliminate pe cale renală (25). Principala izoenzimă implicată în metabolismul acrilamidei este citocrom P450E1 (27), iar ceilalți citocromi nu metabolizează acrilamida în absența P450E1 (26). Atât acrilamida cât şi glicidamida formează cu restul de valină N-terminal al hemoglobinei aducți. Acrilamida formează N-(2-carbamoiletil)valina, în timp ce glicidamida formează N-(2-carbamoil-2-hidroxietil)valina şi N-(1-carbmoil-2-hidroxietil)valina (26).

TOXICOLOGIE

Primul efect observat în urma intoxicației acute sau subacute cu acrilamidă la oameni este neurotoxicitatea. Efectele asupra sistemului nervos central apar după câteva ore până la câteva zile de la intoxicație şi presupun: confuzie, probleme de memorie şi concentrare, oboseală, vorbire incoerentă, halucinații. Neuropatiile periferice se dezvoltă în urma unei perioade de latență care poate dura de la câteva zile până la câteva săptămâni: parestezii, amorțeală, slăbiciune musculară, reflexe scăzute ale tendonului. Tremorul şi tulburările de mers se pot dezvolta ca urmare a afecțiunilor la nivelul cerebelului şi creierului mijlociu (28, 29). Anorexia, pierderea în greutate, nistagmus, transpirația, vasodilatația periferică, dificultatea la urinare şi defecație au fost de asemenea observate. În general, simptomele neurologice continuă să scadă în intensitate în următoarele 4-5 săptămâni de la încetarea expunerii, după care se va observa o îmbunătățire graduală a stării generale în următoarele luni. Este posibilă recuperarea completă la majoritatea intoxicaților cu acrilamidă dacă expunerea nu a fost prea îndelungată (23, 29).

Cercetări experimentale pe animale de laborator (şoareci, şobolani) au demonstrat că acrilamida deține acțiune cancerigenă (2, 30, 31, 32, 33), genotoxică (2, 30, 32), precum şi acțiune toxică asupra dezvoltării şi reproducerii (2, 23, 24, 32, 33).

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Morphological aspects in pulmonary protostrongilosis in roe - deer (*Capreolus capreolus*)

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The paper presents lesional aspects in parasitic invasions with Protostringilus spp. in roe - deer (Capreolus capreolus), observed through morphologic macroscopic and histologic examination of 3 animals taken after shooting.

Necropsic examination revealed multilobular compactisation foci, alleatory dispersed in the diaphragmatic lobes. Histologic examination revealed tissular modifications consisting of lymphohistioplasmocitar hyperplasia, with many eosinocites and alveolar epithelial hyperplasia.

The apparition in underbronchiolar air spaces of eggs and larvae of the etiologic agent proves usefull for diagnostic. The eggs appear as oxyphile spheres with black dots, measuring 15/20 - 30 µm and the smallest of the larvae measures 5/15 µm.

Key Words: roe - deer (Capreolus capreolus), Protostringilus spp granuloma, lung

Morphological and cytochemical particularities of nictitating gland in large breed dogs

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This study was conducted to determine some morphological and cytochemical particularities of the nictitating glands in large breeds dogs. Fourteen dogs of various large breeds were used as the study materials.

To determine the morphological and cytochemical particularities of nictitating gland in large breed dogs, the periodic acid Schiff (PAS), Alcian blue, Novelli, Papanicolaou, Giemsa for tissue and Steedman-Mowry stains was used for the presence of mucosubstances. The glandular acini and nictitating gland ducts present PAS-positive secretion (proteoglicans) in their lumen. PAS-positive granules are to the apical pole of epithelial cells of glandular acini, the acini in different phase of secretion too.

In the anterior nictitating conjunctiva are the pigmented cells in lamina propria and among the cells of basal layer of epithelium. The palpebral nictitating conjunctiva and bulbar nictitating conjunctiva presents folds that magnified the surface and amount of secretion.

Off the lymphonoduls, the nictitating conjunctiva presents rare goblet cells. The part of nictitating gland in palpebral side of cartilage of third eyelid is less developed than the part of nictitating gland in bulbar side of cartilage of third eyelid.

Key Words: nictitating gland, morphology, cytology, large breed dogs

Third eyelid (*plica semilunaris conjunctivae; palpebra tertia; membrana tertia; membrana nictitans*) is a fold of conjunctival mucosa of inferior conjunctival sac what it looks like half-moon in medial angle of eye (5, 6, 7, 10, 12).

Third eyelid is constituted by next structures: conjunctiva, lymphoid tissue associated to the third eyelid, nictitans gland, blood vessels, nerves and smooth muscular fibres (11). The lymphoid tissue associated to the third eyelid belongs to *organized mucosa-associated lymphoid tissue*-O-MALT (4).

The nictitans gland (G.N.) is a tubulo-acinous gland and its secretion is eliminated by exocitosis to contribute on production of fluid precorneal tear film (3, 8, 9).

The epithelium of bulbar nictitating conjunctiva is simple cubical or columnar epithelium that sustain in evident basal lamina, and palpebral nictitating conjunctiva is formated by nonkeratinized stratified pavimentous epithelium with numerous cells charged with proteoglicans, among there are rare melanocytes (2).

In palpebral nictitating conjunctiva are difuse lympho-plasmocitar infiltrations in lamina propria, and in bulbar nictitating conjunctiva these lympho-plasmocitar infiltrations are more intensive.

The glandular acini of nictitans gland to the anterior side of third eyelid cartilage are composed by high columnar cells that contain more proteoglicans. The nictitating gland to the posterior side of third eyelid cartilage is much more developed in mean third, in some areas being structurated

by 3-4 glandular lobuls separated by thin connective tissue septum. As part of glandular lobuls, the secretive acini contain great amount of proteoglicans (1).

The nictitating gland to the posterior side of third eyelid cartilage is much more developed than the gland to the anterior side of cartilage, extending dorsal to the free margin of third eyelid with 1875-2025 µm, in regard to the anterior side of nictitating gland (2).

MATERIALS AND METHODS

The third eyelid with nictitans gland from both eyes were assayed from 14 dogs (3 German Pointer, 3 Rottweiler, 8 mongrel dogs,) 8 male and 6 female, between 3 to 14 years old and body weight 25 to 40 kilograms. The eyelid were assayed from the animals alive, after induction of anesthesia and from euthanasied animals.

After sampling, the nictitant membranes were prefixed in 10% formalin solution for five hours, then were cut and cut off medio-sagittal and transversal (3 transversal sections-by upper, mean and lower third).

The obtained fragments were fixed in Orth and included in paraffin and cut off to 5 µm. Used stain was PAS, Alcian blue, Novelli, Papanicolaou, Giemsa for tissue and Steedman-Mowry obtaining 524 permanent histological preparation that were interpretated to the microscope. The principal morfological and cytochemical aspects were photographed for illustration this paper.

RESULTS

Third eyelid is lined up by nictitating conjunctiva that is differentiated in palpebral or anterior nictitating conjunctiva and bulbar or posterior nictitating conjunctiva. The palpebral nictitating conjunctiva is following up with palpebral conjunctiva of inferior eyelid, making a palpebralo-nictitating conjunctival sac in internal angle of the eye. The bulbar nictitating conjunctiva is following up with bulbar conjunctiva of the eye, making a nictitanto-bulbar conjunctival sac in internal angle of the eye. Therefore, the inferior conjunctival sac in internal angle of the eye is separated by third eyelid resulting two inferior conjunctival sac: palpebralo-nictitant and nictitanto-bulbar.

The third eyelid is backed up by own „skeleton" represented by hialin cartilage in form „T", whose the horizontal section lines the free margin of eyelid (fig.1, 2, 3).

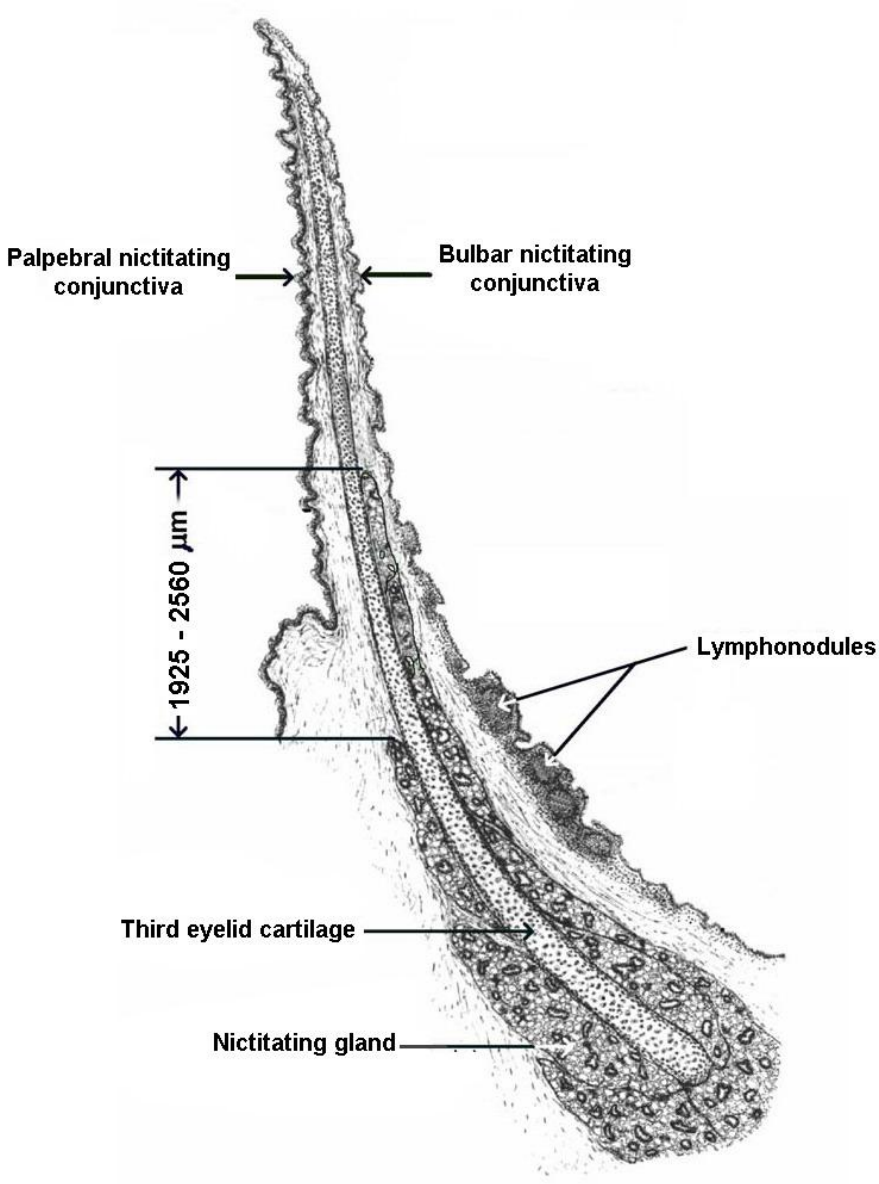
The palpebral nictitating conjunctiva is structurated by nonkeratinized stratified pavimentous epithelium, with 3-5 lines of polyhedral, round or oval cells whose PAS-positive secretion, demonstrates secretion of proteoglicans (fig.9, 10). In the anterior nictitating conjunctiv are the pigmented cells in *lamina propria* and among the cells of basal layer of epithelium (fig.9).The palpebral nictitating conjunctiva presents folds that magnified the surface and amount of secretion (fig.10). The glandular acini and nictitating gland ducts on the palpebral side of cartilage of eyelid present PAS-positive secretion (proteoglicans) in their lumen (fig.11, 12). PAS-positive granules are to the apical pole of epithelial cells of glandular acini and the acini in different phase of secretion too (fig. 14,15,16). The glandular lobuls are separated by connective tissue septum (fig.13,14).

The bulbar nictitating conjunctiva is thiner being constituted from nonkeratinized pavimentous stratified epithelium with 2-3 lines of round or oval cells.The bulbar nictitating conjunctiva forms the deep folds that magnify the surface and amount of secretion (fig.1, 2). In lamina propria are lymphonoduls and numerous capillary. Off the lymphonoduls, the nictitating conjunctiva presents rare caliciform cells. (fig. 3,4,5). The epithelial cells of glandular acini of nictitating gland in bulbar side of cartilage have PAS-positive contain (proteoglicans) (fig. 6,7,8)

The part of nictitating gland in palpebral side of cartilage of third eyelid is less developed than the part of nictitating gland in bulbar side of cartilage of third eyelid (fig. 17,18). On the basis of

gland are more interlobular ducts with PAS-positive secretion (proteoglicans) in lumen (fig. 19, 20).

The nictitating gland to the posterior side of third eyelid cartilage is much more developed than the gland to the anterior side of cartilage, extending dorsal to the free margin of third eyelid with 1925-2560 µm, in regard to the anterior side of nictitating gland (scheme 1).



Scheme 1. Morphology of the third eyelid and nictitating gland in large breeds dogs (medio-sagittal section).

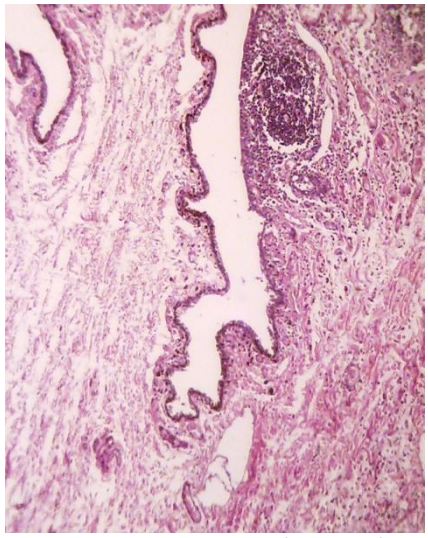


Figure 1. Nictitating membrane in dog: fold of bulbar nictitating conjunctiva. PAS; x 80.

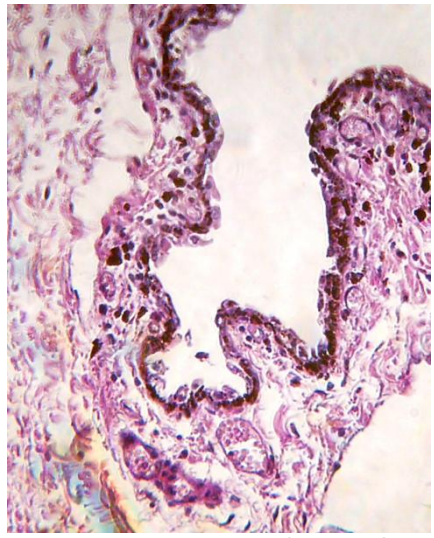


Figure 2. Nictitating membrane in dog: fold of bulbar nictitating conjunctiva. PAS; x 200.

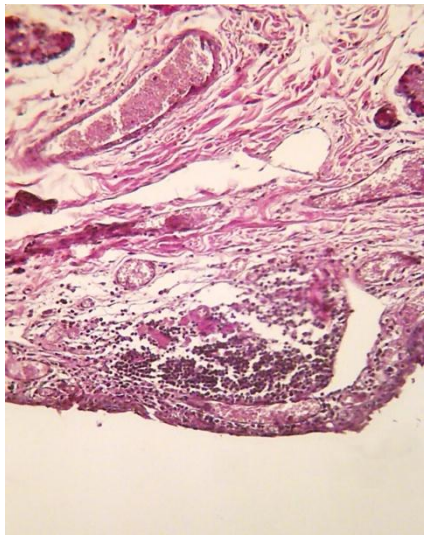


Figure 3. Nictitating membrane in dog: lymphoid follicle in bulbar nictitating conjunctiva. PAS; x 80.

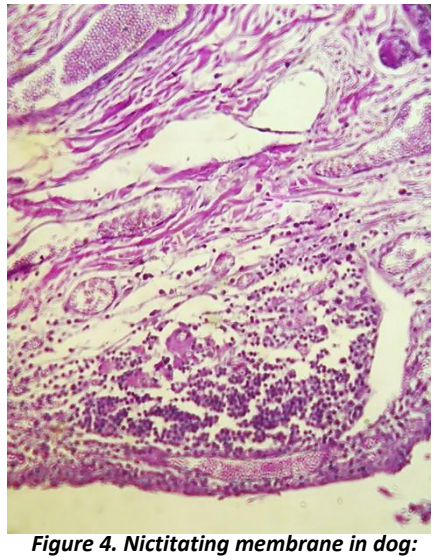


Figure 4. Nictitating membrane in dog: lymphoid follicle in bulbar nictitating conjunctiva. PAS; x 200.

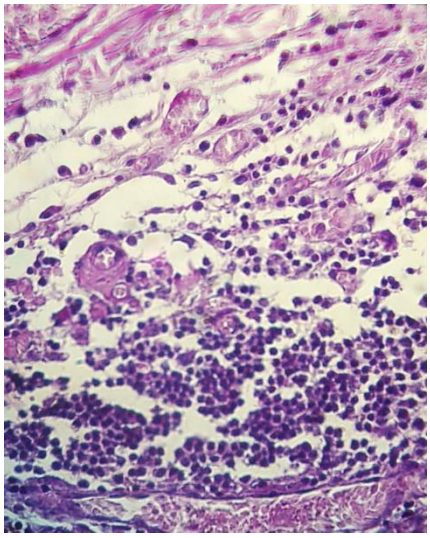


Figure 5. Nictitating membrane in dog: lymphoid follicle in bulbar nictitating conjunctiva. PAS; x 400.

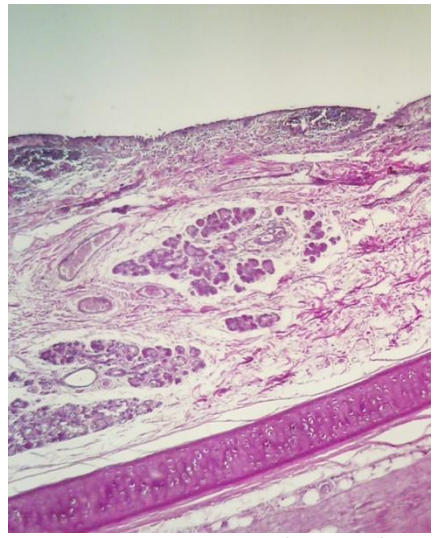


Figure 6. Nictitating membrane in dog: first glandular formations of gland peak in bulbar side of cartilage and lymphoid follicles.

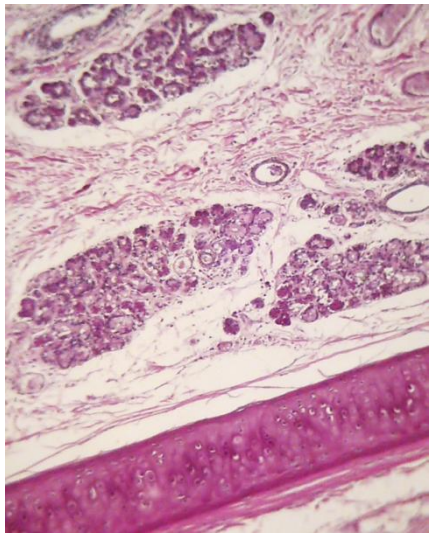


Figure 7. Nictitating membrane in dog: first glandular formations of gland peak in bulbar side of cartilage. PAS; x80.

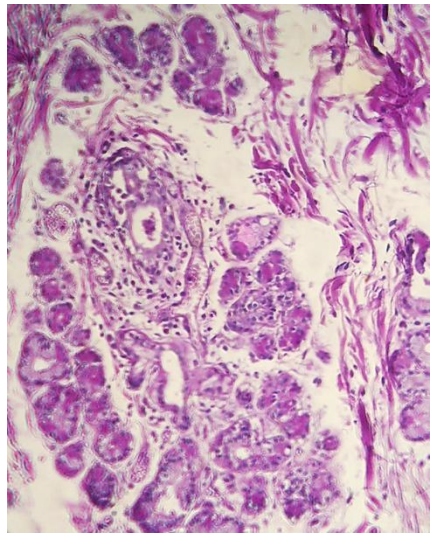


Figure 8. Nictitating membrane in dog: glandular formations of gland peak in bulbar side of cartilage. Glandular acini with PAS-positive secretion. PAS; x 200.

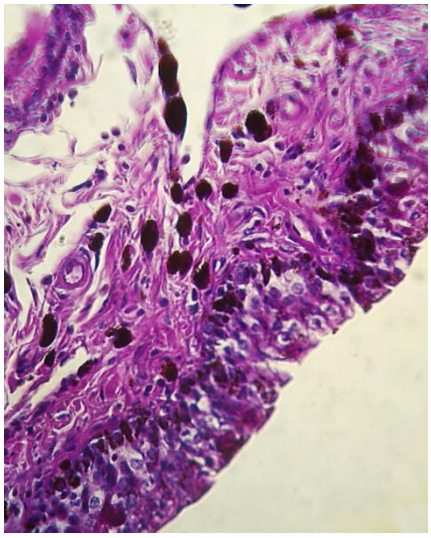


Figure 9. Nictitating membrane in dog: palpebral nictitating conjunctiva, numerous melanocytes in lamina propria and among cells of basal layer of

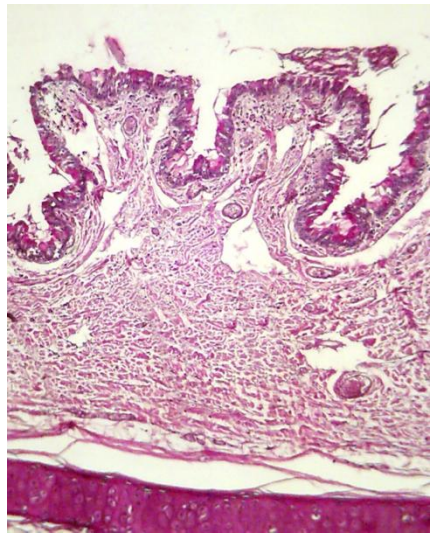


Figure 10. Nictitating membrane in dog: palpebral nictitating conjunctiva, folds of mucosa. PAS; x 80.

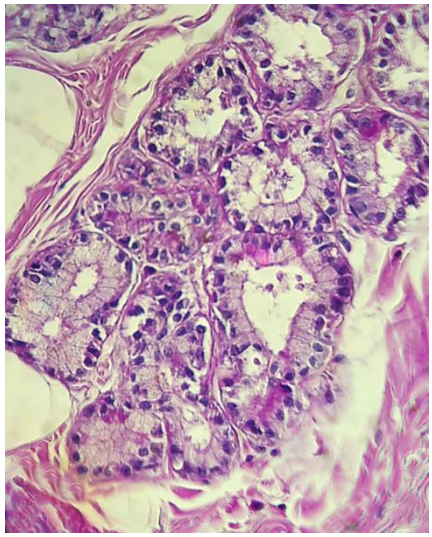


Figure 11. Nictitating membrane in dog: bulbar nictitating conjunctiva, glandular acini with secretion in lumen. PAS;x 400.

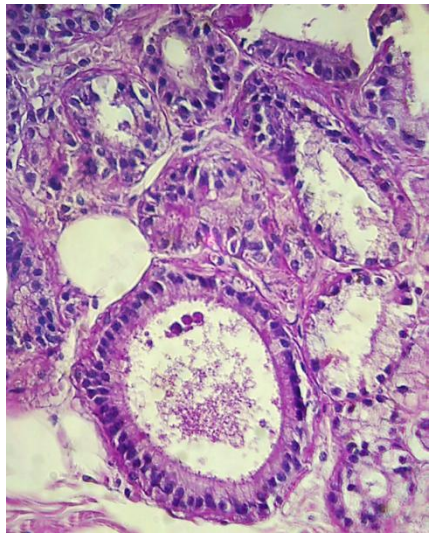


Figure 12. Nictitating membrane in dog: palpebral nictitating conjunctiva, duct with secretion in lumen. PAS;x 400.

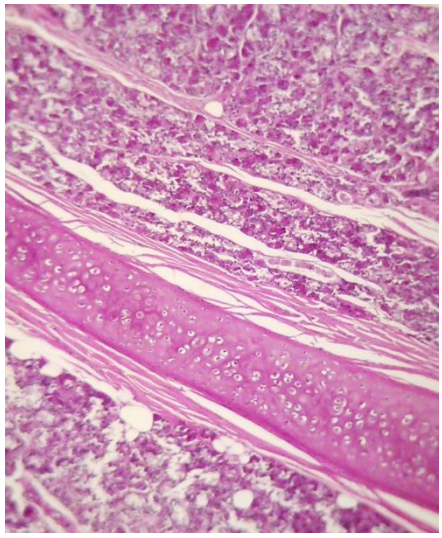


Figure 13. Nictitating membrane in dog: glandular lobuls separated by connective tissue septum. PAS; x 80.

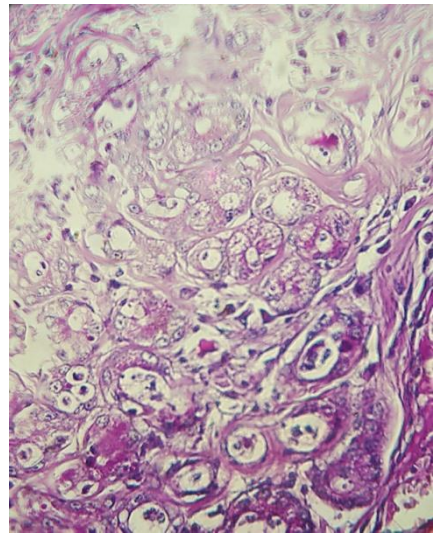


Figure 14. Nictitating membrane in dog: secretion granules to the apical pole of alveolar epithelial cells. PAS; x 400.

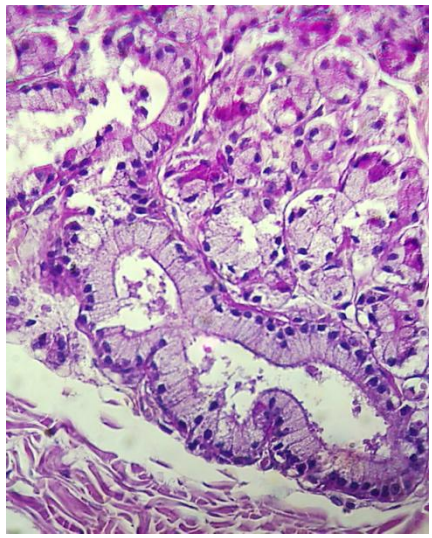


Figure 15. Nictitating membrane in dog: glandular lobuls separated by connective tissue septum. PAS; x 400.

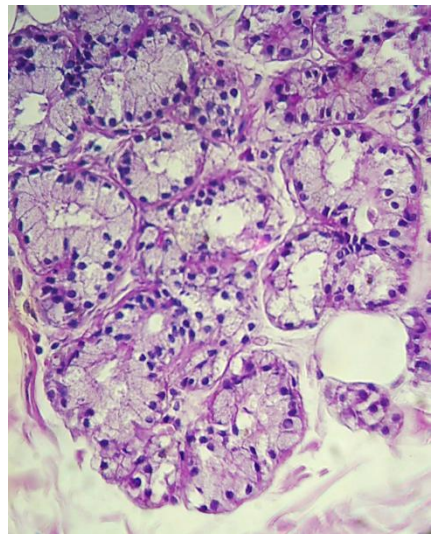


Figure 16. Nictitating membrane in dog: elaboration of secretion process in glandular acini. PAS; x 400.

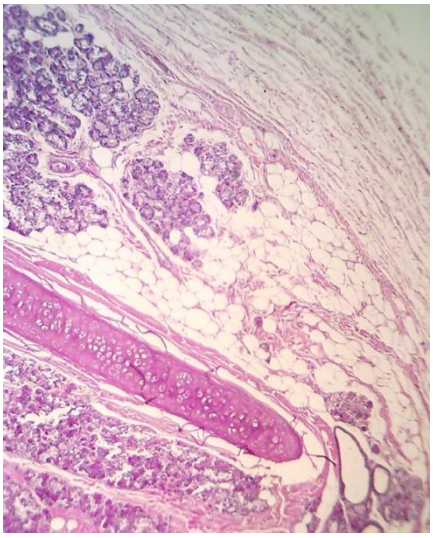


Figure 17. Nictitating membrane in dog: nictitating gland in palpebral side of cartilage is less developed. PAS; x 80.

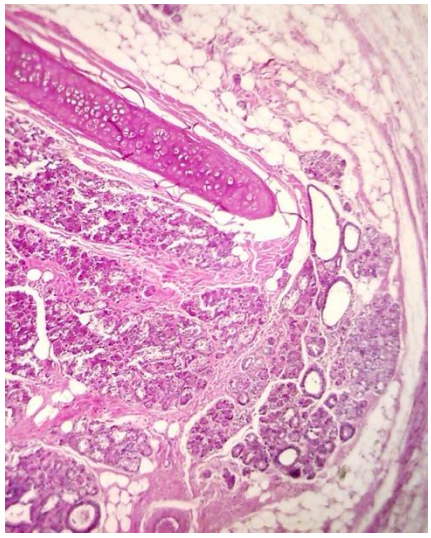


Figure 18. Nictitating membrane in dog: nictitating gland in bulbar side of cartilage is quite developed. PAS; x 80.

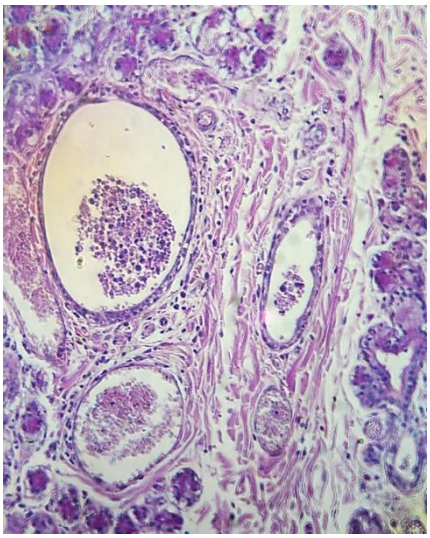


Figure 19. Nictitating membrane in dog: nictitating gland in palpebral side of cartilage of third eyelid, three interlobular ducts with secretion in lumen. PAS; x 200.

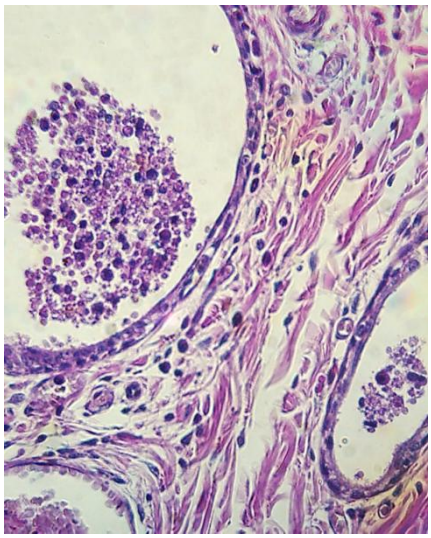


Figure 20. Nictitating membrane in dog: nictitating gland in palpebral side of cartilage of third eyelid, three interlobular ducts with secretion in lumen. PAS; x 400.

CONCLUSIONS

1. In the anterior nictitating conjunctiva are the pigmented cells in *lamina propria* and among the cells of basal layer of epithelium.
2. The palpebral nictitating conjunctiva and bulbar nictitating conjunctiva presents folds that magnified the surface and amount of secretion.
3. Off the lymphonoduls, the nictitating conjunctiva presents rare goblet cells.
4. The part of nictitating gland in palpebral side of cartilage of third eyelid is less developed than the part of nictitating gland in bulbar side of cartilage of third eyelid.
5. On the basis of gland are more interlobular ducts with PAS-positive secretion (proteoglicans) in lumen.

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Morphology of the external ear canal in dogs (*Canis familiaris*)

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This study was conducted to determine morphological properties of the external ear canal in dogs. Seven dogs of various breeds, sex and ages were used as the study materials. To determine the morphology and histochemistry of the external ear canal of dogs, the periodic acid Schiff (PAS) stain was used for mucosubstances. The epidermis of the external ear canal is represented by a keratinized stratified squamous epithelium. The dermis contains hair follicles, sebaceous and ceruminous glans, vessels and connective tissue fibers. The cartilage of external ear canal consist of elastic cartilage tissue. PAS-positive staining reveals secretions of mucosubstances in secretory epithelial cells of ceruminous glands.

Key Words: morphology, histochemistry, external ear canal, sebaceous and ceruminous glands, dog

The external ear canal extens from the base of pinna to the tympanic membrane (2-5, 7). The external one-third of this canal is composed of cartilage with the temporal bone making up the roof of the remaining part (7). The external ear canal is lined up with skin, what contain thin hair follicle, sebaceous and ceruminous glans. The ceruminous glans secrete the cerumen (1, 2, 4, 7). It is composed of a combination of desquamated cornified flat epithelial cells, fat and the fatty secretions of the ceruminous glands. Cerumen serves as an important barrier to infections by microorganisms and also protects the skin against injury. The cerumen has also been reported to facilitate the growth of the *Malassezia pachydermatis* fungus. Secretions of the ceruminous glands prevent the skin of the ear from drying and irritations, but accumulation of cerumen in the external ear canal predisposes the external ear to inflammation (6).

This study was conducted to fill in some morphological aspects of the external ear dog and histochemical properties of the ceruminous glands.

MATERIALS AND METHODS

The materials were obtained from seven dogs of various breeds, sex and ages. The dogs with no ear disease were euthanasied in a private veterinary clinic,

because they had suffered severe trauma or incurable disease. The specimens were taken from the external ear canal of both ears from each animal. For morphological and histochemical study, the tissue specimens were fixed,

dehydrated and blocked in paraffin, cut into five-micrometer thick and stained with Periodic Acid Schiff's (PAS).

RESULTS

The external ear canal in dog is lined up with keratinized stratified squamous epithelium histological like smooth skin. The epidermis contains 2-3 celular lines that belong to basal layer, spinous layer and rare cells of granular layer and the stratum corneum.

The dermis have not the dermal papillae being made by connective tissue fibres, among are the alveoli of sebaceous gland, the canals of ceruminous glands and hair follicles. The connective tissue of dermis is confused with the connective tissue of the outermost layer of the perichondrium of the cartilage of ear canal and the absence of adipose cells demonstrates the

absence of hypodermis from the skin that lies up the external ear canal. The cartilage of external ear canal is elastic.

The hair follicles from dermis have a less diameter in regard to pinna skin, being the secundary follicles.

The sebaceous glands are numerous and arranged round of hair follicles.

The ceruminous glands are modified sweat glands, coiled tubular glands, apocrine with large irregular lumen. The canals of ceruminous glands are more deep, under the alveoli of sebaceous glands. The columnar or cuboidal epithelial cells have PAS-positive secretion, to the apical pole. The PAS-positive secretion is being in the lumen of ceruminous glands, suggesting the secretion of mucosubstances. The epithelial cells of ceruminous glands canals go upon on basal layer being round by mioepithelial cells.

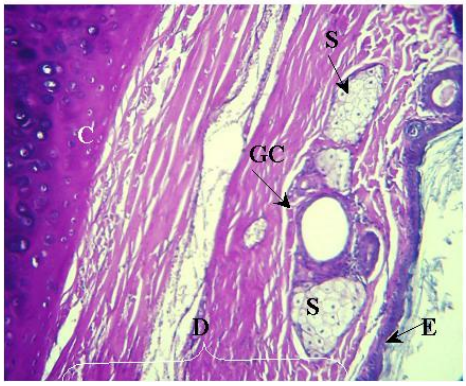

Figure 1. External ear canal in dog :. Epidermis (E), dermis (D) with sebaceous (S) and ceruminous glands (GC), elastic cartilage of external ear canal (C). PAS, x 100.

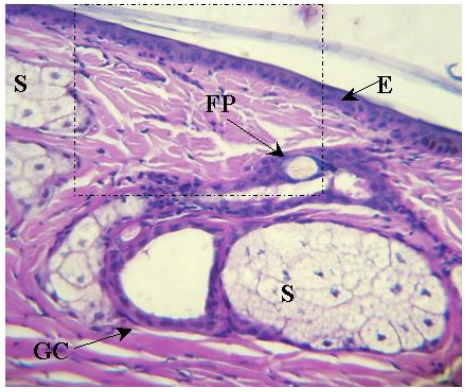

Figure 2. External ear canal in dog: Epidermis (E) ; sebaceous glands alveoli (S) satellited hair follicle (FP); cerominous glands canals (GC) between sebaceous glands alveoli. PAS, x 400.

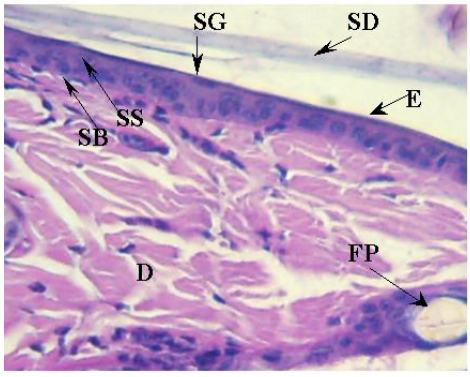

Figure 3. External ear canal in dog: Epidermis (E) with 2-3 lines of cells: basal layer (SB), spinous layer (SS), granular lamina (SG), stratum corneum (SD); dermis (D); hair follicle (FP). PAS (larged image from bordered zone in the figure 2).

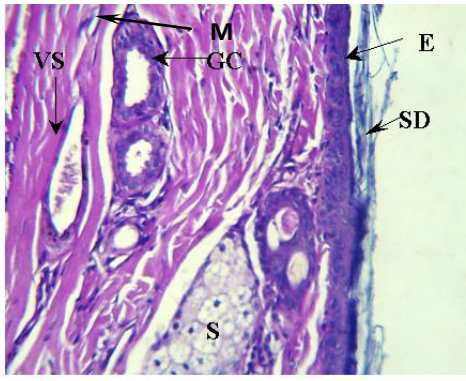

Figure 4. External ear canal in dog : Epidermis (E), stratum corneum of epidermis (SD), ceruminous glands canals (GC), sebaceous glands alveoli (S), mioepithelial cells (M),blood vessel (VS). PAS, x 400.

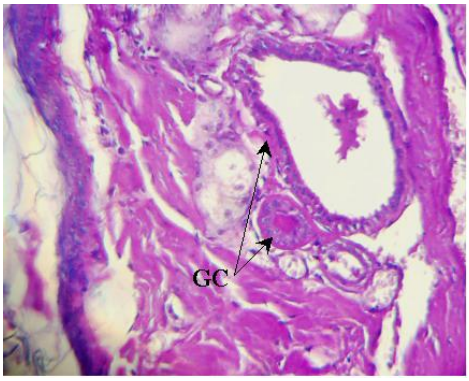

Figure 5. External ear canal in dog: epitelial cells of ceruminous glands alveoli (GC) with PAS-positive secretion to the apical pole. The ceruminous gland canals with PAS- positive secretion in lumen. PAS, x 400.

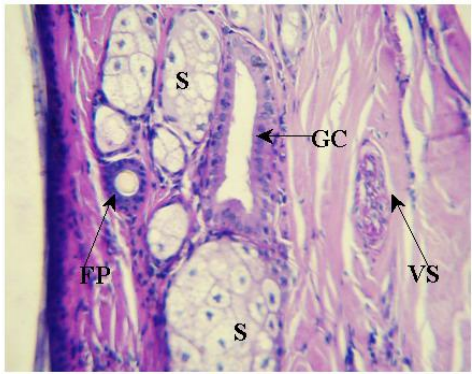

Figure 6. External ear canal in dog : hair follicle (FP), sebaceous glands alveoli (S), ceruminous glands canals (GC), blood vessel (VS). PAS, x 400.

CONCLUSIONS

1. The external ear canal in dog is lined up with keratinized stratified squamous epithelium histological like smooth skin.
2. The epidermis contains 2-3 celular lines that belong to basal layer, spinous layer and rare cells of granular layer and the stratum corneum.
3. The dermis have not the dermal papillae and the canals of ceruminous glands are more deep, under the alveoli of sebaceous glands.
4. The connective tissue of dermis is confused with the connective tissue of the outermost layer of the perichondrium of the cartilage of ear canal. The absence of adipose cells demonstrates the absence of hypodermis from the skin that lies up the external ear canal.
5. The columnar or cuboidal epithelial cells have PAS-positive secretion, to the apical pole, and in the lumen of ceruminous glands, suggesting the secretion of mucosubstances.

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The effect of certain differential fodder rations on the hematological and productive indicators in meat chickens

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To raise good, healthy Broiler chickens their diet must meet two main requirements: to provide the best energetic level, and to provide a high protein level for a quick growth, with minimum food.

Among other elements, the crude protein contains some esential aminoacids: lysine, argynine, isoleucine, metyonine, cystine, treonyne, and triptophane.

The mineral systems control the metabolism: the increasing or the decreasing of the proportion of one element in the body may cause a primary or a secondary deficiency at other elements, with negative effects concerning the health and the productive performances of the Broiler chickens (2, 3).

The purpose of our research is to demonstrate the effect of a high metyonine and selenium diet on the hematological and productive indicators in meat chickens.

Key Words: chicken, meat quality, fodder influence

Materials and methods

The experiment focused on 3 homogenous groups of RED JA chickens, 15 chickens/group (W – a witness group, and E_1, E_2 – two experimental groups).

The chickens have been differentially fed:

- group I (W) – with a commercial fodder;
- group II (E_1) – with a diet containing soy beans grist (as the main protein source), and a supplement of 5g Se/100kg fodder.;
- group III (E_2) – with a diet containing soy beans grist (as the main protein source), and a supplement of 5g Se/100kg fodder (like in E_1), plus a supplement of DL metyonine 0.2% from the fodder structure (during the first period of the experiment), and 0,1% (during the second part of the experiment).

The experiment went on a period of 42 days (6 weeks), in two phases:

- during the 1st phase (1-28 days), the chickens were fed with 21-1 fodder;
- during the 2nd phase (28-42 days), the chickens were fed with 21-2 fodder.

The fodder was „ad libitum", 23 hours out of 24, with constant running water. The fodder was weighed every day, and the groups of chickens were periodically rotated in order to minimize the effect of the external factors on the experiment. At the end of each weak, the chickens were weighted individually, to find out the medium weight (1).

At the end the experiment, we took fodder and blood samples for chemical and hematological tests. During tests, we observed the erythrocyte number, the hematocrite (through Wintrobe method), the hemoglobin (through Sahli method), the leukocyte number and the level of VEM, HEM, CHEM.

Results and discussions

Table 1

The Hematological Parameters at W chickens (n=5), in the 42nd day

n	Erythrocyte mil/mm³	Leukocyte thousand/mm³	Hematocrit %	Hemoglobin g/dl	VEM µ³	HEM pg	CHEM g/dl
1.	2.1	23.5	31.1	7.5	148	35.7	24.1
2.	2.8	20.5	35.2	9	125.7	32.1	25.5
3.	2.4	21.7	34.3	8.7	142.9	36.2	25.3
4.	2.3	21.9	32.8	7.9	142.6	34.3	24
5.	2.5	22.2	31.9	8.9	127.6	35.6	27.8
x	2.42	21.96	33.06	8.40	137.36	34.78	25.34
Sx	0.12	0.48	0.75	0.30	4.48	0.74	0.68
S	0.26	1.08	1.69	0.66	10.03	1.65	1.53
CV	10.70	4.90	5.10	7.90	7.30	4.76	6.05

We can see that the number eritrocites in the 42nd day reached 2.42±0.12 mil/mm³, still remaining inside the normal limits (2.35±0.25 mil/mm³).

The number of leucocytes was 21.96±0.48 thousand/mm³, also remaining inside the normal limits (26.0±4.0 thousand/mm³).

The hemoglobine was 8.40±0.30 g/dl, without passing the normal limits. We noticed a increasing in hematocrit's level (5).

Also, VEM reached 137.36±4.48µ³; HEM – 34.78±0.74 pg, and CHEM – 25.34±0.68 g/dl.

Table 2

The comparative evolution (W-E_1) of the hematological parameters (n=5) and the meanings of the differences

Hematological parameters	Group	x±Sx	CV%	Differences	Mann-Withney test	
				abs	%	
Erythrocyte mil/mm³	W	2.42±0.12	10.70	+ 0.32	13.22	0.1171
	E_1	2.74±0.12	9.86			
Leukocyte thousand/mm³	W	21.96±0.48	4.90	+1.14	5.1	0.3472
	E_1	23.10±0.79	7.62			
Hematocrit %	W	33.06±0.75	5.10	-1.46	4.4	0.3472
	E_1	31.60±1.20	8.51			
Hemoglobin g/dl	W	8.40±0.30	7.90	-1.08	12.8	0.0215
	E_1	7.32±0.15	4.67			
VEM µ³	W	137.36±4.48	7.30	-20.62	15.01	0.1745
	E_1	116.74±8.98	17.23			
HEM pg	W	34.78±0.74	4.76	-7.8	22.42	0.0162
	E_1	26.98±1.76	14.62			
CHEM g/dl	W	25.34±0.68	6.05	-2.08	8.20	0.0472
	E_1	23.26±0.89	8.57			

There are not significant differences regarding the number of the erythrocytes p<0,05 from one group to another: at W, the number of erythrocytes was 2,42±0,12 mil/mm³, and at E_1 the number of erythrocyte was 2,74±0,12 mil/mm³.

There is also no significant difference regarding the leukocyte number: 21,96±0,48 mii/mm³ at W, and 23,10±0,79 mii/mm³ E_1.

The hematocrite level reached similar values: 33,06±0,75 % (W), and 31,60±1,20 (E_1).

But the hemoglobin showed an increased level at W: 8,40±0,30 g/dl; E_1 reached only 7,32±0,15 g/dl for p<0,05.

There are also differences between HEM and CHEM at W chickens comparative with E_1 chickens.

Table 3

The comparative evolution (W-E_2) of the hematological parameters (n=5) and the meanings of the differences

Hematological parameters	group	x±Sx	CV%	Differences		Mann-Withney test
				abs	%	
Erythrocyte mil/mm³	W	2.42±0.12	10.70	-0.34	14.04	0.0367
	E_2	2.08±0.07	7.13			
Leukocyte thousand /mm³	W	21.96±0.48	4.90	+6.26	28.50	0.0090
	E_2	28.22±0.11	0.85			
Hematocrit %	W	33.06±0.75	5.10	-5.3	16.03	0.0090
	E_2	27.76±0.37	2.98			
Hemoglobin g/dl	W	8.40±0.30	7.90	-1.48	17.61	0.0121
	E_2	6.92±0.37	11.93			
VEM µ³	W	137.36±4.48	7.30	-3.18	2.13	0.7540
	E_2	134.18±5.82	9.71			
HEM Pg	W	34.78±0.74	4.76	-1.58	4.54	0.4674
	E_2	33.20±1.30	8.77			
CHEM g/dl	W	25.34±0.68	6.05	-0.42	1.65	0.6015
	E_2	24.92±1.52	13.62			

The number of erythrocytes at E_2 was 2,08±0,07 mil/mm³, lower than the one at W (2,42±0,12 mil/mm³).

But the number of leucocytes was bigger, p<0,01, at E_2: 28,22±0,11 mii/mm³, comparative with the number of leucocytes at W (21,96±0,48 mii/mm³).

The hematocrite and the hemoglobin reached lower levels at E_2 than those at W: p<0,01 at hematocrite, and p<0,05 at hemoglobin.

VEM, HEM, CHEM and the same levels at both groups.

STOICA *et all* (4) showed that replacing the animal protein with vegetal protein causes no significant in metabolic parameters; such modification in diet causes variations in the activity of some hepatic and mussel enzymes only. The supplementation of the metonine in chickens' fodder has the same effects, as presented above.

Table 4

The average weight at meat chickens

Day	Average weight (g)		
	W	E_1	E_2
1	18	18	18
7	117	119	118
14	278,57	275,13	301,02
21	488,21	477,13	401,03
28	674,81	674,06	608,92
35	930,08	960,76	1109.50
42	1195,83	1289,23	1259.50

E_1 chickens, fed with a supplement of selenium, reached an average weight of 1289,23 g, while W chickens reached only a 1195,83 g average weight.

E_2 chickens, fed with a supplement of metyonine, reached an average weight of 1259.50 g, while W chickens reached only a 1195,83 g average weight.

Table 5

The quantity of fodder used by W, E_1, and E_2 during the experiment, comparative with the needed quantity for a kg of meat

	W	E_1	E_2
Used fodder g/group	25650	26540	21990
Used fodder g/chicken	1832,14	1895,7	1466
Kg needed fodder/kg meat	1,53	1,47	1,32

It is obvious from the previous table that E_2 chickens had the lowest level of the needed fodder / kg meat (1,32kg fodder/kg meat), but reached the biggest weight.

We specify that the level of these productive parameters is under the level recommended for RED JA chickens.

CONCLUSIONS

- *Selenium and soy grots in the diet of meat chickens lead to the improvement of the productive parameters, but with a slight negative effect on the hemoglobin level with no repercussions on chickens' health or production.*
- *The supplementation of metyonine reduces the needed fodder/kg meat, increases the body weight, increases the leucocytes density, and decreases the values of hemoglobin and hematocrite.*
- *The supplementation of selenium, soy grots and metyonine determined positive effects on the bioproductive parameters, and had a slight suppressing effect on the hematological indicators.*

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The dynamics of γ – LH cell in the adenohypophysis of estrus cow

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As a research material we have used the hypophysisl of six estrus cows. The samples have been fixed in Orth, Carnoy and Helly, led up to paraffin and serially sectioned at 5 µm. We have finally obtained 250 serial sections stained through the methods: Novelli, PAS, Steedman-Mowry and MH_2. The adenohypophysis contains a high frequence of the γ – LH cells (29 %), with increased numbers of hightly granules, the precursors of the LH. Studying the dynamic aspect of LH – γ cells we can see the prevelance of the maximum function therefore their total degranulation with 48,3 – 55,2 % of the cells. The our researches demonstrate that in the estrus cow there is a making active of secretory hormones (LH-RH, LH and estrogen).

Key Words: cow, adenohypophysis, estrus, LH- γ cell

The dehiscence of the ovarian follicles in estrus cows is helped by the action of LH-RH and LH (6, 7, 8, 9, 10). The LH- γ cells in the adenohypophysis secrete LH. We have studied this problem in order to describe the cell dynamics in the adenohypophysis of the estrus Holstein cows at the age two-seven years.

MATERIAL AND METHODS

As a research material we have used the adenohypophysis of six estrus Holstein cows, at the age of two-seven years, which were taken just after the females has been killed. The fragments of adenohypophysis were fixed in Orth, Carnoy and Helly, led to paraffin and sectioned at 5 µm in thickness. The cytochemical stains were: Novelli, PAS, Steedman-Mowry and MH_2. In the chromophil population of the adenohypophysis we have identifed on the basis of morphological and tinctorial aspects, the main types of the cells. As for the LH-γ cells we have found the following aspects of the cell dynamics: maximum, medium and minimum functions, division (mitosis), interphase and cellular involution (pyknosis and cytolisis).

REZULTS AND DISCUSSIONS

In the chromophil population of the estrus cows at the ages of two-seven years we have investigated, the LH-γ cells are predominant (29 %) (table 1). In the high frequencies we have also noticed FSH-β cells (19 %), which through FSH they secreted, preparated the maturation of the ovarian follicles. The high percentage of LH- γ cells accounts for the elaboration of the increased LH quantity which is necessary in the dehiscence of the ovarian follicles at the estrus phase (6, 7, 8, 9, 10) (fig. 1). The other cells, TSH-delta, STH-alpha, LTH-eta and ACTH-epsilon, don't suffer greet changes in the estrus of the cows. Mention should be made of the presence of the degranulated chromophobe cells (5 %), which are interpreted as LH- γ cells that have secreted the hormone and thus they appear with a clear cytoplasm.

Studying the dynamic aspects of the LH- γ chromophil cell (table 2), we have noticed the predominance of the maximum function (48,3-55,2 %) at all ages. This may be explained by their

complete degranulation for the achievement of a high level of LH necessary in the in the estrus of the cows.

The presence of divisions (3,3 – 6,5 %) is explaned by the fact that the renewal the LH- γ cells takes place by mitosis in the estrus stage of the cows.

The cytolisis aspects (3,2-6,9 %) we found for the cell of two-seven years (8,5 %) can be interpreted by virtue of the holocrine way of secretion which secures an increased level of LH during the estrus stage of cows.

Table 1

The percentage of the cells in the adenohypophysis of the estrus Holstein cows, at the age two-seven years (%)

The phase of LH- γ cell	The age (years)	Number females	Cell types (in %)							
			Chromofobes x)		Cromofile					
					PAS-pozitive			PAS-negative		
			D	P	β – FSH	γ – LH	δ - TSH	α - STH	η – LTH	ε - ACTH
Proestrus	2 - 7	5	7	-	27	20	12	11	10	13
Estrus	2 - 7	6	5	-	19	29	11	11	12	13
Metestrus	3 - 8	5	5	10	10	22	11	10	21	11
Diestrus	3 - 7	5	11	16	13	14	10	11	13	12

x) D – degranulated; P – primordial; We have studied 100 cells for each case

The dynamics of γ – LH cell in the adenohypophysis of estrus Holstein cows, at the age two-seven years (%)

The phase of LH- γ cell x)	The age of females in years					
	2	***3***	***4***	***5***	***6***	***7***
Maximum function	48,3	55,2	53,4	51,6	50,0	51,9
Medium function	20,7	24,0	26,7	25,8	26,7	24,2
Minimum function	10,4	6,9	10,0	9,7	13,4	13,8
Mitosis	3,5	3,5	3,3	6,5	3,3	3,4
Interphase	-	-	-	-	-	-
Pyknosis	-	3,5	3,3	3,2	3,3	3,4
Cytolisis	6,9	6,9	3,3	3,2	3,3	3,4

x) The phase of LH- γ cell: maximum function (total degranulation)
medium function (partial degranulation)
minimum function (minimum degranulation)

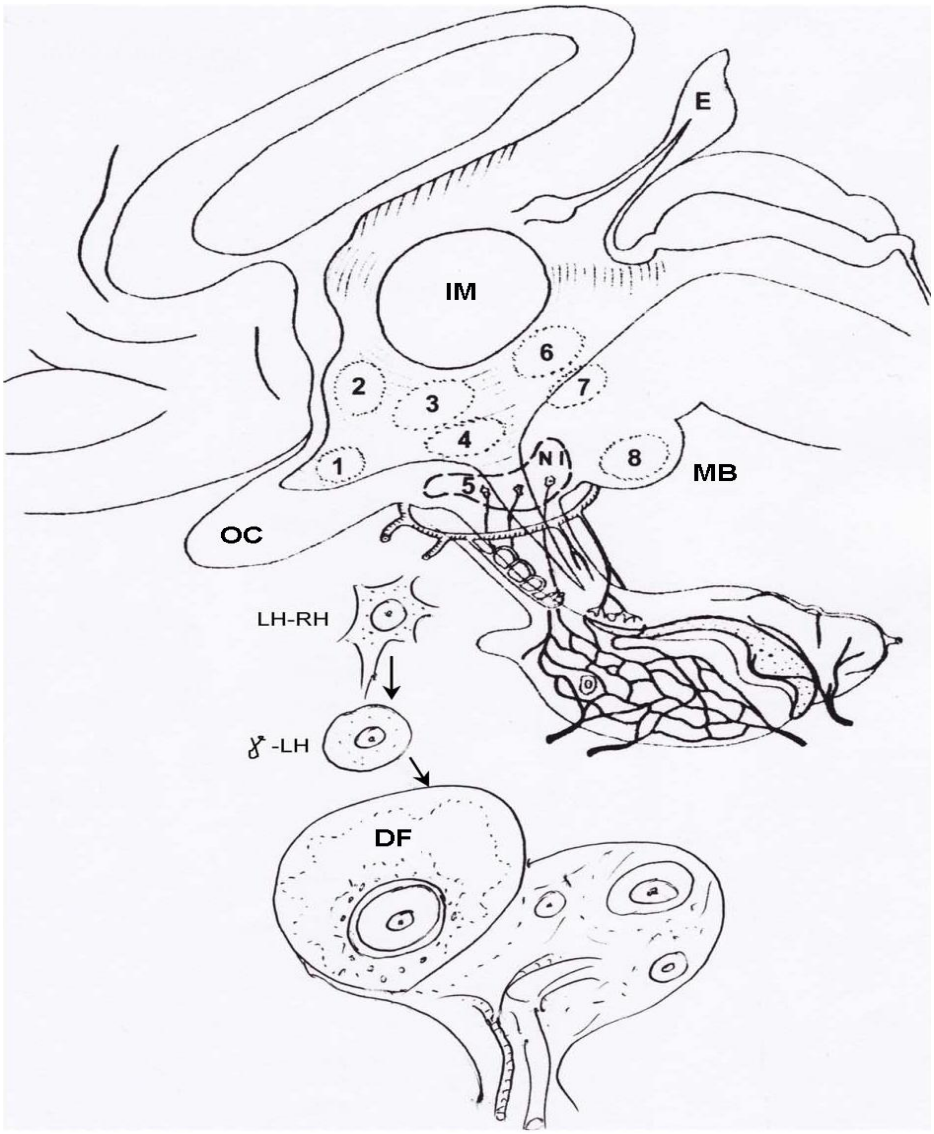

Plate I. The hypothlamo-hypophyseal and epithalamo-epyphyseal trats:
- Anterior groups (1. nuc. supraopticus; 2. nuc. paraventricularis);
- Lateral groups (3. nuc. hypotalamicus dorsomedialis; 4. nuc. hypotalamicus ventromedialis);
- Midlle groups (5. nuc. infundibularis);
- Posterior groups (6. nuc. periventricularis caudalis; 7. nuc. premamilaris;
8. nuc. corporis mamilaris)
E – epiphysis; IM – intermediate mass; OC – optical chyasma; MB – mamilaris body
DF – dehiscence follicle
Plate I
The estrus cow adenohipophysis

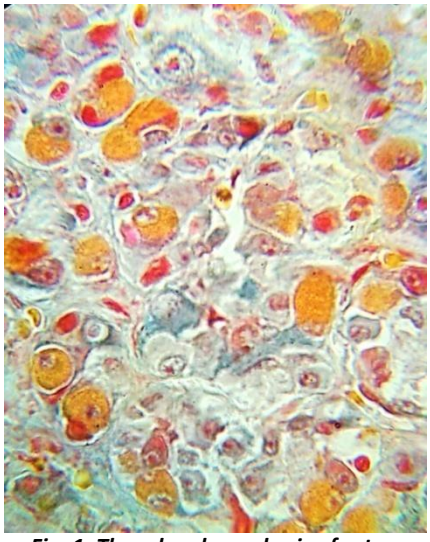

Fig. 1. The adenohypophysis of estrus Holstein cow at the age of two years. The total degranulation of the γ-LH cells (48,3 %). MH_2 stain; x 400

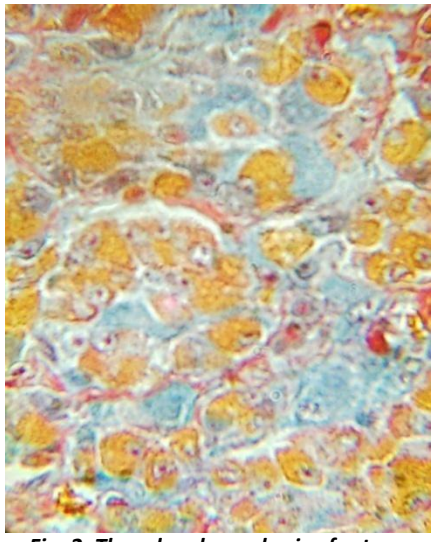

Fig. 2. The adenohypophysis of estrus Holstein cow at the age of four years. The maximum function of the γ-LH cells (53,4 %). MH_2 stain ;

Col. MH_2: x 400

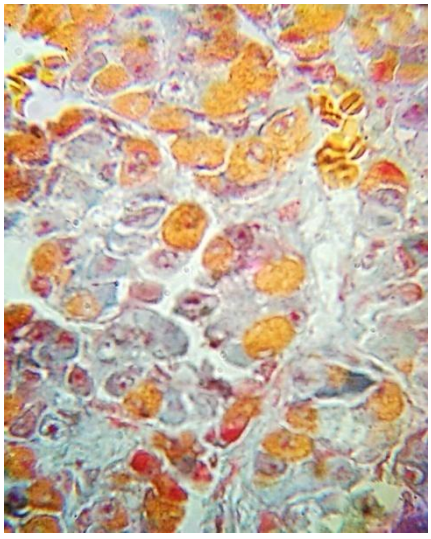

Fig. 3. The adenohypophysis of estrus Holstein cow at the age of five years. The maximum function of the γ-LH cells (51,6 %). MH_2 stain; x 400

(51,6 %). MH_2 stains ; x 400

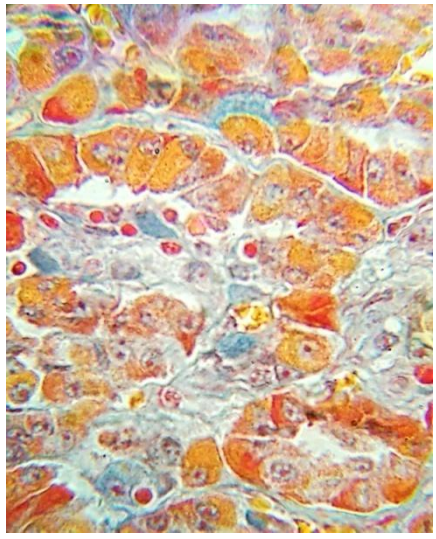

Fig. 4. The adenohypophysis of estrus Holstein cow at the age of seven years. The total degranulation of the γ-LH cells (51,9 %). MH_2 stain; x 400

CONCLUSIONS

1. In the estrus stage of Holstein cows at the age of two-seven years , in adenohypophysis LH- γ cells are predominant (29 %).
2. Studying the dynamic aspect of LH- γ cells, we can see the preeminence of the maximum function therefore their total degranulation of 48,3-53,2 % of the cells.
3. In estrus stage of Holstein cows at the age two-seven years we noticed a intensive secretions of specifical hormones (LH-RH, LH and estrogen) at the those three levls of the hypothalamo-hypophiso-ovarian axis.

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Quantitative and qualitative collagen changes in some tissular parasitoses of cattle and pigs

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Quantitative and qualitative determinations of collagen in the Longissimus dorsi muscle were made in healthy adult cattle (Holstein crossbreds) and in cattle with echinococcosis-hydatidosis, fascioliasis and dicrocoeliasis, as well as in young pigs experimentally infested with Trichinella spiralis.

In the Longissimus dorsi muscle of poorly managed cattle with echinococcosis-hydatidosis, the total collagen rose significantly compared with the healthy animals ($P < 0.001$), the same rising tendency being found also with the insoluble collagen in ¼ Ringer solution ($P < 0.01$), or the soluble collagen ($P < 0.001$).

In the cattle with fascioliasis and dicrocoeliasis, the total collagen alterations showed a significant rise ($P < 0.05$), while qualitatively the insoluble collagen remained within the same range ($P > 0.05$), and the soluble collagen rose distinctly significantly ($P < 0.01$).

Four months after the experimental infestation of swine with Trichinella spiralis there was found a very significant rise in the total collagen ($P < 0.001$) compared with the noninfested congeners raised under identical conditions. The insoluble collagen rose distinctly significantly ($P < 0.01$) compared with the clinically healthy piglets, while the soluble Ringer solution showed a tendency of nonsignificant statistical rise ($P > 0.05$).

In tissular parasitoses of cattle and pigs there was a rise in the amount of total collagen, as well as changes in the degree of collagen polymerization, notably in cattle with fascioliasis and dicrocoeliasis and to a lesser extent in the experimental trichinellosis of pigs.

Key Words: collagen, tissular parasitoses, catle, pigs

INTRODUCTION

Collagen is a scleroproteid similar to the two components of the conjunctive tissue: elastine and reticuline. Collagen represents 15-50% from the total conjunctive tissue and 30-35% from the total protein of the body (5, 8).

Quantitative and qualitative modifications are highly important in the understanding of different biologic processes such as growth and development or pathologic processes with the implication of this protein, which is spread very much in the body (1,2, 9, 15, 18).

Tissular parasitoses in animal are the cause of functional alteration of organs and tissues. The pathological processes arosen are predominantly proliferative inflammations, with an initial vasculo-exudative reaction, followed by the cystic parasitic granuloma constitution (in echinococcosis) or predominantly fibrous with tendency to collagen deposition in nemathodes invasion. The older the lesion is, the greater extent of collagen deposition takes place and the peripheric reaction is reduced. The amount of collagen formed per unity of tissue weight is a directly proportional to the number of the existing granuloma as well as to fibroblasts activity.

Collagen is the most abundant and important scleroproteide, generally constant in the adult organisms, under the control of certain physiological and mainly physiopathological states which indice both quantitative and qualitative changes in collagen levels (3, 4, 10, 20).

In tissues, collagen may exist in the following forms:

- acid neutral insoluble collagen which quantitatively predominates, can't be extracted without 90ºC heating in tricloracetic acid and has a 50 – 100 days turn – over;
- acid soluble collagen, extractable with diluted acids or citrate buffer;
- neutral soluble collagen, witch can be extracted both with neutral saline solution or weak alkaline buffers; its turn over varies from 2 – 3 hours to 2 days.

The special functions of collagen in the animal organisms, as well as the multitude of factors influencing its biosynthesis and molecular stability, determined us to investigate the quantitative and qualitative changes of collagen in the Longissimus dorsi muscle from cattle with echinococcosis-hydatidosis, fascioliasis and dicrocoeliasis as well as from pigs experimentally infested with Trichinella spiralis. All the obtained results were compared with those of healthy animals.

MATERIALS AND METHODS

The experimental groups investigated were:

- 5 healthy cattle
- 5 cattle with echinococcosis-hydatidosis
- 5 cattle with fascioliasis and dicrocoeliasis.

The animals were sacrificed in a slaughter house. The diagnosis and the infestation degree were determined on the basis of organs and liver laboratory examination.

The experimental infestation with trichinella spiralis was performed at a number of 6 two month old piglets by administration of 10 g meat containing 1500 encapsulated lawae. Four months after the infestation they were sacrificed, concomitantly with a control group of 6 noninfested congeners, raised in identical conditions.

The quantitative and qualitative determination of collagen were performed on Longissimus muscle samples prelevated from all the experimental animals and controls. Collagen was assayed by an indirect biochemical method, consisting in evaluating of hydroxylproline content (16). The hydroxyproline content conversion to collagen was calculated by multiplying the results with 7.25 (11). The quality of collagen was estimated on the basis of determination of its solubility in ¼ Ringer solution (13).

Results were statistically processed and interpretated, by calculation of X – arithmetical mean, s – standard deviation, s_x – mean standard error, V% – variation coefficient. Difference significance – t, was determined by comparing the experimental and control results (19).

RESULTS AND DISCUSSIONS

The amount of total collagen, soluble and insoluble, in the Longissimus dorsi from healthy and sick cattle are presented in table 1.

In the echinococcosis-hydatidosis cattle, the content of total collagen increases statistically significantly ($P < 0.001$). The mean value of the control group was 338.94 ± 25.57 mg/100 g fresh muscle, and increased to 521.64 ± 25.57 mg/100 g fresh muscle from the infested animals.

The collagen increase was realized in the charge of muscular tissue diminution, in the conditions of a tendency to amiotrophy provoked by the quantitative and qualitative muscular fibres diminishing (6, 7, 17).

A similar behaviour emphasized the insoluble collagen. The mean value for the control was 261.72 ± 27.12 mg/100 g fresh tissue and 358.15 ± 12.17 mg/100g tissue of parasitated animals.

The soluble collagen mean content was of 77.2 ± 9.84 mg/100g tissue in the control group and increased to 163.48 ± 13.69 mg/100 g tissue in the infested group. The difference was very significant statistically ($P < 0.001$).

The echinococci affect the parasitated organism not only trough their toxicity, but in a mechanical mode too. Their size and number disturb the hepatical circulation and finally cause hepatic cirrhosis. The irritation produced by E. granulosus embryos development in organs and tissues determines strong reactions materialized in development of connective tissue.

The hepatic insufficiency, consequence of mechanic and toxic actions of hidatic cyst, results in a decrease of free aminoacids in plasma, and at the level of striated muscle in synthesis of increased amounts of collagen. The chronic evolution of echinococcosis lead to the synthesis of a stable collagen, with a high degree of polymerization, as well as an insoluble one, characterized by a low degree of polymerization and a rapid turn-over (4, 10, 12).

A significant increase of the total collagen ($P < 0.05$), from 338.94 ± 25.57 mg/100 g fresh muscle to 462.12 ± 26.42mg/100 g muscle, has been emphasized in the Longissmus dorsi from cattle with dicrocoeliasis and fascioliasis. The results reflect an intensive activity of fibroblast and an increased collagen synthesis.

The insoluble collagen presents higher values, but without statistical significance ($P > 0.05$), while the soluble collagen increases distinctly significantly. The establish changes in collagen content reflect a reduced level of the polymerization degree associated with an intensification of the biosynthesis of the rapid turn-over collagen and with no effect on slow turn over collagen.

The piglets experimentally infested with Trichinella spiralis emphasized, after 4 months, a verry significant increase in total collagen ($P < 0.001$). The collagen ranges were 270.94 ± 16.45 mg/100g muscle for the control and 374.85 ±14.51 mg/100 g muscle for the infested animals (table 2).

The total collagen increase was due to the insoluble form which reached a mean value of 245.00 ± 20. 76 mg/100 g muscle confronted by 160.98 ± 12.98 mg/100g muscle in the control group. The soluble collagen presented a tendency of nonsignificant rise ($P > 0.05$) showing that the biosynthesis process in osteoblast and fibroblast develops normally, rising the capacity of slow turn over collagen synthesis, comparative to the rapid turn over collagen.

The collagens are structural proteins composed of units of three polypeptide chains arranged in triple helical configuration. There are now more than 14 recognized types of collagens grouped into a least two types by Irwin Leav (14), his classification of collagens: fibrillar (I, II, III, V, XI) and non – fibrillar (IV, VI, VII, VIII, IX, X, XII, XIII, XIV).

The fibrillar collagens assemble into cross – linked insoluble multimers (fibrils) of high tensile strength and are of most relevance in the wound healing process.

This is the structure of the procollagen type I molecule, the intracellular precursor form of collagen, which is now secreted into the extracellular space. There the terminal peptides are cleaved off and the triple helical segments are cross – linked covalently by formation of highly reactive aldehydes at selected epsilon amino ends of lysine and hydroxylysine, a reaction catalyzed by the enzyme lysyloxidase and essential for the polymerization of collagen fibers and stabilization of the extracellular matrix. This has been verified experimentally by feeding rats with sweet peas (*Lathyrus odoratus*) which contain ß – amino – propanetriol, and inhibitor of lysyloxidase. These animals develops marked connective tissue fragility, a condition that has been called lathyrism.

COLLAGEN FIBRILLAR

Type	***Distribution***
I.	Skin, bone, tendon
II.	Hyalin cartilage, vitreous body
III.	Skin, vessels
V.	Skin, bone, synovium, placenta
XI.	Hyalin cartilage

COLLAGEN NON – FIBRILLAR

Type	***Distribution***
IV.	Basement membranes
VI.	Skin, vessels, intervertebral disk
VII.	Dermo – epidermal junction
VIII.	Endothelial cells, descemet's membrane
IX.	Hyalin cartilage, vitreous body
X.	Growth plate (cartilage)
XII.	Embryonic tendon and skin
XIII.	Endothelial cells
XIV.	Fetal skin and tendon

These results emphasize that tissular parasitoses in cattle and pigs are associated with qualitative and quantitative changes of collagen. The affected the amount of total collagen as well as the degree of collagen polymerization. The noticed changes reflect the structural destabilization of the collagen helix, which depends on cooperative interactions established both intra and intermolecular, within the collagen fibrils.

CONCLUSIONS

1. In the longissmus dorsi muscle of cattle with echinococcosis-hydatidosis the total collagen increased significantly, the same rising tendency was found for both ¼ Ringer solution insoluble and soluble collagen.
2. In the cattle with fascioliasis and dicrocoeliasis, the total collagen showed a significant rise but qualitative changes of the insoluble and soluble collagen were noticed. The insoluble collagen remain within the same range while the soluble form rose distinctly significantly.
3. Four month after the experimental infestation of piglets with Trichinella spiralis there was found a very significant rise in the total collagen, while the insoluble collagen also increased and the soluble in Ringer solution showed only a tendency of nonsignificant statistical rise.

Table 1

THE COLLAGEN, INSOLUBLE AND SOLUBLE, CONTENT IN THE LONGISSIMUS DORSI MUSCLE OF CATTLE WITH ECHINOCOCCOSIS, FASCIOLIASIS AND DICROCOELIASIS, COMPARED TO HEALTHY ANIMALS (mg/100 g FRESH TISSUE)

Statistical parameters	Control lot			Echinococcosis			Fascioliasis and dicrocoeliasis		
	total	insoluble	soluble	total	insoluble	soluble	total	insoluble	soluble
X	338.94	261.72	77.21	521.64	358.15	163.48	462.19	338.20	123.97
Range	290.00 - 425. 93	203.00 - 319.00	47.12 - 106.94	462.18 - 592.69	335.31 - 402.37	126.87 - 199.37	407.81 - 543.75	271.87 - 404.19	94.25 - 148.62
s	57.17	49.46	21.99	53.76	27.20	30.61	59.07	58.98	21.54
s_x	25.57	22.12	9.84	24.04	12.17	13.69	26.42	26.38	9.63
V %	16.87	18.90	28.48	10.31	7.59	18.72	12.78	17.44	17.38
t				/ ***	/ **	/ ***	/ *	/ n. s.	/ **

Key **** = significant difference***
***** = distinctive significant difference***
****** = highly significant difference***
n. s = nonsignificant difference

Table 2.

THE TOTAL COLLAGEN, INSOLUBLE AND SOLUBLE, CONTENT IN THE LONGISSIMUS DORSI MUSCLE OF PIGS EXPERIMENTALLY INFESTED WITH TRICHINELLA SPIRALIS, COMPARED TO HEALTHY ANIMALS (mg/100 g FRESH TSISSUE)

Statistical parameters	Control lot			Experimentally infested pigs		
	total	insoluble	soluble	total	insoluble	soluble
X	270.94	160.98	109.96	374.85	245.00	129.85
Range	214.24 - 320.81	135.94 - 216.41	78.30 - 145.55	332.05 - 410.35	185.97 - 282.75	107.30 - 146.08
s	40.31	31.80	24.01	32.46	41.53	14.77
s_x	16.45	12.98	9.80	14.51	20.76	6.60
V %	14.88	19.75	21.83	8.66	16.95	11.37
t				/ ***	/ **	/ n. s.

Key ** = distinctive significant difference

****** = highly significant difference***

n. s = nonsignificant difference

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Sex hormones and environment involved in sex determination and growth in eels, *Anguilla Anguilla* - basic and applied aspects

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In animal growth, many biological processes are involved, including internal and external parameters, some apparently more important than others. Over the twenty years, during which the growth of the European eel, Anguilla Anguilla, has been researched, many aspects influencing eel growth have been documented. In the present paper, we propose a model, which describes the relationship between growth, sex hormones, environment and sex determination. Much of the model's supporting data has been collected in our laboratory.

Key Words: sex hormones, Anguilla

INTRODUCTION

Increased pollution and over-fishing of rivers in Europe are bringing about a decrease in the natural eel population, and an increase in the value of the European eel (*Anguilla anguilla*). The European eel is a catadromic teleost species with a complex life cycle, which takes place both in sea and freshwater environments (Tesch 1977). Significant differences between growth rate and size have been observed between the two sexes, with females growing faster and larger than males (Degani et al., 2003b). The environment has been found to affect the growth and sex determination in eels; females continue to grow, while the males cease. Some males are between 15-200 g. Many theoretical and applied aspects of the European eel have been investigated (Degani and Gallagher 1995). In this paper, a model is presented, which suggests that the relationship between the environment, hormones and sex determination affects growth. The relevance of such a model, not only in providing basic information, but also with respect to aquaculture, is described.

RESULTS and DISCUSSION – THE MODEL

A high variation in growth was discovered in European eels grown under aquacultural conditions (Degani and Levanon 1983). Under natural conditions, the sex ratio in silver eels varies considerably between localities, ranging between 0 to100% females (Tesch 1977). Under aquaculture conditions, starting from the glass eel stage, the majority of eels will develop male gonads (Tzchori et al., 2004a), which possibly results from the high density in which eels are reared in captivity. Indeed, it has been shown that low densities of captive eels favor female differentiation, while high densities support male differentiation, implicating that social factors play an important role in sex determination (Degani and Kushnirov 1992). These results support the environmental effect on sex determination in the model (Fig.1 - A to C). It was postulated that sex determination in the eel, as in other fish, is regulated by the ratio of androgen to estrogen levels at the critical time of sex determination, and steroid intervention at this period alters this balance. About 95% feminization was obtained by

oral treatments with estrogen (Colombo and Grandi 1990). These results, confirmed by Degani and Kushnirov (1992) and Tzchori et al. (2004a) in our laboratory, indicate that the density of the

eels, during their growth, affects their steroid production. High density favors androgen secretion and low density encourages estrogen secretion, as suggested in the model (Fig.1 - A to C). However, more direct studies are necessary to support this hypothesis.

The steroid, 17β – estradiol (E_2), which is produced by the female gonad (Fig.1- F), and which controls vitellogenesis, augments the growth rate (Degani 1986) (Fig.1 - G) and affects oogenesis (Fig.1- H). When E_2 was provided in the diet, there was an increase in the growth rate of the eels. Aromatase (P450) is the key enzyme that transforms testosterone into estradiol in vertebrates. The cloned complementary deoxyribonucleic acid (cDNA) of P450 from the European eel contains an open reading frame of 1539 bp, encoding a deduced protein of 513 residues (Tzchori et al., 2004b). Its mRNA expression during sex determination was significantly higher in females than in males. All of these results support the model (Fig.1- A to C). Treatment of the eels with a P450 inhibitor reduced the female percentage during sex determination. These data support the E_2 contribution to growth, which is significantly higher in female than in male European eels. Follicle stimulation hormone (FSH), which controls vitellogenesis by induction of E_2 secretion, seems to have an indirect effect on growth, as described in the model (Fig.1- L, M, N, H). The FSH β subunit cDNA, which consists of 1068 bp, encodes a 108 amino acid peptide (Degani et al., 2003a). The expression of FSH and luteinizing hormone (LH) mRNA were examined in males and females after sex determination, and the mRNA levels were found to be significantly higher in females than in males. In conclusion, these results suggest that FSH secretion from the pituitary has an effect on E_2 secretion, which in turn has an effect on the increase in the growth rate, as presented in the model. In the male, 11-ketotestosterone (11KT) is the main androgen, and apparently this hormone does not affect growth, but rather influences spermatogenesis (Sbaihi et al., 2001) (Fig.1.C)

Growth hormone (GH) is secreted from the pituitary of the eel, as supported by a study of Degani et al. (2003b), in which the GH gene was cloned and its transcription level determined in males and females (Fig. 1- D), as well as its influence on eel growth demonstrated (Degani and Gallagher 1985). According to the mRNA expression of the cloned and sequenced cDNA of the European eel GH, females exhibited a significantly higher GH transcription than males. Insulin administration to the eel diet significantly increased the growth rate of eels (Degani and Abraham 1992) (Fig.1 - K). These results support the pathway which is described in the model (Fig.1 - I to K) that GH is affected directly by the insulin like growth factor (IGF), although more detailed research is required. Montero et al. (1998) have determined the localization of cyclase-activating polypeptide (PACAP)-immunoreactive neurons in the central nervous system of the European eel, using an antiserum raised against PACAP27. The data of this study revealed that pituitary PACAP stimulates GH secretion from cultured eel pituitary cells (Montero et al., 1998) (Fig.1- I). The gonadotropin-releasing hormone (GnRH), did not alter GH release in the European eel (Rousseau et al., 1999).

In conclusion, the proposed model, regarding the relationship between the complex phenomena of eel growth and the hormones involved in its reproduction, may clarify the interaction between the two complex systems (growth and reproduction) and the environment.

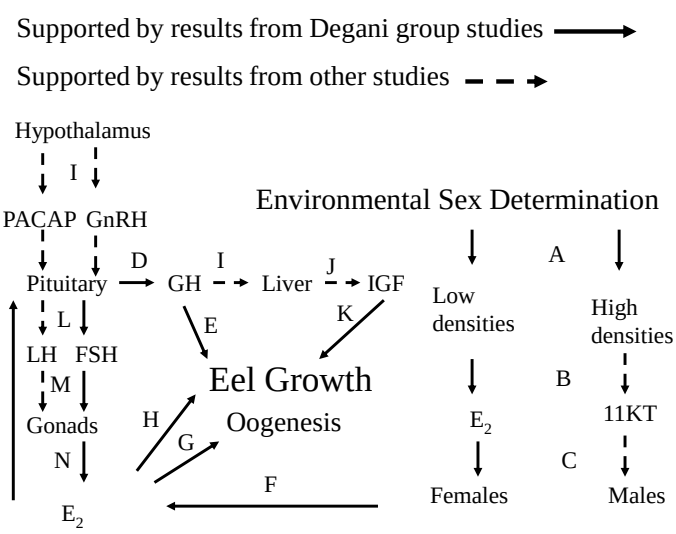

Figure 1. Model proposing hormonal involvement in sex determination, growth, gonad development and oogenesis in the European eel. 17β – estradiol (E_2), follicle stimulation hormone (FSH), luteinizing hormone (LH), growth hormone (GH), cyclase-activating polypeptide (PACAP), gonadotropin-releasing hormone (GnRH), 11-ketotestosterone (11KT), insulin like growth factor (IGF).

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Sanguine biochemical profile of Haflinger and Lipizzan horses

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Current pathology orientation towards the insight of the metabolic health aims at the discovery, as early as possible, of the critical metabolic area between normal and abnormal, at the prevention of the clinical diseases and at the establishment of the limits of physical oscillations caused by different internal metabolites; such variations represent undoubtedly a useful step in the achievement of data with regards to the future prevention of dis-metabolies (1, 2). The quantitative change of the sanguine constituents represents a diagnosis revealer and it is the consequence of some functional disorders – either organic lesions, or caused by the self-defense reaction as a results of some aggressions (1, 2). The objective of this paper work was to present reference data concerning the sanguine biochemical profile in two horse breeds: Haflinger and Lipizzan, used as recreation animals.

Key Words: horse, Haflinger, Lipizzan breed, blood biochemical profile

Materials and methods

The clinical and paraclinical investigations were performed in 14 Haflinger and Lipizzan horses, used as recreation animals in a private farm from the centre of the country. These horses have benefited by the following forage intake:

- natural hay 15-20 kg/day; its chemical analysis has confirmed the following amounts: crude protein 9.33%; humidity 9.23%; cellulose 30.63%;
- concentrated feed 4 kg/day (2 kg oat and 2 kg maize); concentrates` chemical analysis has confirmed: crude protein 9.32%, humidity 10.62%;
- vitamin-mineral premix 100 g.

Blood taking was carried out form the jugular vein; in laboratory, we have determined: plasmatic protein (refractometric), calcium (Elliot method), phosphorus (the micro-method without deproteinization), magnesium (the method with titan yellow), uremia, alkaline phosphatase, AST and ALT (kits).

Data achieved was biostatistically processed, using the software Excel for calculations. In order to test the difference significance, we have used the test Mann-Whitney.

Results and discussions

From a functional point of view, the plasmatic proteins are involved in nutrition constituting a part of the common protein fund; they may be sued as reserve of proteins necessary for tissue repairing and growth. With regards to this function, we may remark that the plasmatic proteins are in a dynamic equilibrium with the hepatic proteins and other tissues` proteins. A part of the plasmatic proteins passes into the digestive tract, where they are submitted to a proteolysis process, and the resulting amino acids are adsorbed, being used within the synthesis of other proteins.

Table 1 shows that proteinemia had a mean value of 6.09±0.13 g/dl in Haflinger horses, a significantly higher value than in the Lipizzan horses (5.48±0.15) and with a lower coefficient of variability (CV = 5.45%).

Table 1

The comparative evolution of biochemical sanguine parameters in horses and significance of the differences

Specification		X ± Sx	CV%	Differences		Significances (Mann-Withney)
				abs	%	
Total protein (g/dl)	Lipzzan	5,48 ± 0,15	7,24	+0,61	11,13	0,0127
	Haflinger	6,09 ± 0,13	5,45			
Albumine (g/dl)	Lipzzan	2,90 ± 0,10	8,94	-0,18	6,20	0,2774
	Haflinger	2,72 ± 0,14	13,15			
Globuline (g/dl)	Lipzzan	2,57 ± 0,19	19,97	+0,82	31,90	0,0151
	Haflinger	3,36 ± 0,22	17,13			
Ca (mg/dl)	Lipzzan	15,68 ± 0,68	11,58	-4,14	26,40	0,0026
	Haflinger	11,54 ± 0,62	14,32			
P (mg/dl)	Lipzzan	2,88 ± 0,45	41,17	+0,16	5,55	0,6547
	Haflinger	3,04 ± 0,33	28,50			
Mg (mg/dl)	Lipzzan	2,43 ± 0,12	13,34	0,08	3,29	0,1417
	Haflinger	2,51 ± 0,08	8,56			
Uree (mg/dl)	Lipzzan	21,99 ± 1,99	23,92	0,23	1,04	0,4822
	Haflinger	22,22 ± 0,80	9,59			
Alkaline phosphatase UI	Lipzzan	96,50 ± 8,92	24,48	-21,85	22,64	0,1102
	Haflinger	74,65 ± 11,18	39,70			
AST UI	Lipzzan	57,56 ± 4,98	22,93	-1066	18,51	0,0350
	Haflinger	46,90 ± 1,36	7,68			
ALT UI	Lipzzan	8,30 ± 1,45	46,28	+0,62	7,46	0,5229
	Haflinger	8,92 ± 1,14	33,81			

Concerning the albuminemia, there are no significant differences between the two breeds, the values being similar (272±0.14% g/dl in Haflinger horses, respectively 2.90±0.10 g/dl in Lipizzan horses).

Popescu et al. (5), successive to some researches concerning the sanguine constants in sport horses and their variations according to breed, sex, age, physiological status and environmental factors, has noticed that some constants have higher values during summer (no. of erythrocytes, hemoglobin and total protein) and lower values during winter (calcemia and potassiemia).

Globulinemia is significantly higher in Haflinger horses, compared to the Lipizzan ones, the values being 3.36±0.22 g/dl, respectively 2.57±0.19 g/dl.

Calcemia is significantly higher in Lipizzan horses (15.68±0.68 mg/dl), compared to the Haflinger breed (11.54±0.62 mg/dl).

Vallete (6) has observed that, in the English thoroughbred horses, successive to a 1500 m effort, calcemia and magnesiemia levels have decreased and the level of phosphoremia has increased. On the contrary, in the American trotters, after a 2400 m-distance effort, he has not observed any increase in the calcemia, magnesiemia and phosphoremia levels.

Phosphoremia had similar values in both breeds: in Haflinger, it was 3.04±0.33 mg/dl, and in Lipizzan it was 2.88±0.45 mg/dl. Similarly, there are not any significant differences between the two breeds concerning magnesiemia and uremia. On the other hand, the alkaline phosphatase activity was higher in Lipizzan horses, 96.50±8.92 U/l, than 74.65±11.18 U/l in Haflinger breed.

The aspartataminotranspherase activity (AST) was higher in Lipizzan horses (57.6±4.98 U/l), compared to the Haflinger breed (46.90±1.36 U/l), and the alaninaminotranspherase activity (ALT) had similar values in both horse breeds.

The researches performed by Suzana Milinkovic-Tur et al. (3) concerning the activity of some enzymes (AST, ALT and GGT), during gestation and within the first period of lactation in Haflinger mares show that the aspartataminotranspherase (AST) seric activity was higher (146.8±5.6 U/l) than the activity of the other two enzymes. The researcher has also observed that the enzymatic activity in horses used for work is higher with 16% compared to the horses maintained within dismissal for a few days.

Noel de Burlin et al. (4) considers that in the sport horse, during its training period, some sanguine parameters have higher values (AST, ALT, CK, bilirubin) and only after 3-4 days they stabilize, becoming concordant to the physiological limits. This fact proves that animals are overwrought and it should be submitted to a dismissal period. Within this context, the authors recommend the clinical and paraclinical examination for each animal, a few days before the horserace (gallop) and 3-4 days after the horserace.

Conclusions

Successive to our comparative study concerning the evolution of some sanguine biochemical parameters in Lipizzan and Haflinger horse breeds, we may draw the following conclusions:

- proteinemia in Haflinger horses is significantly higher ($p<0.05$) compared to the Lipizzan ones;
- globulinemia, calcemia and aspartataminotranspherase (AST) activity are higher ($p<0.05$) in Lipizzan horses than in Haflinger horses;
- there are no significant differences between the two horse breeds concerning the other parameters studied.

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Researches about the metabolic profile of milk cows raised in small farms

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Periodical testing of chemical and haematological values in healthy cows allows the veterinarian to detect the shortcomings of the nutrition, and to observe the capacity of the body to adjust to the conditions of an intensive exploitation.

Periodical testing is an effective way of evaluating the specific and general defensive capacity of an individual in the herd.

In order to detect the causes of the pathological syndromes and in order to understand the aetiology of many production and reproduction disorders, more and more veterinarians check the metabolic profile of the animal and adjust the nutrition (1, 3).

The aim of our research is to add more specific data to the problem of the physical and enzymatic metabolic profile of blood in three cow breeds, and to observe if any differences occur regarding certain parameters.

Key Words: cow, metabolic profile

Materials and methods

In the experiment, we used 21 milk cows, during the stabulation season, in a private farm from Timis County.

The results obtained after having analysed the fodder components are presented in Tables 1, 2, 3, 4.

The fodder ration used in the present experiment differs from the standard diet, namely:

- a deficit in the dray substance (DS): only 81.02 %;
- a slight deficit of UFL (0.56UFL), but remaining between the normal limits, ± 10% (11.91 UFL);
- a supplementation of PDI with 67.36 g, but still remaining between the normal limits, ± 10% (namely 1490).

This disequilibrium, if of long term, may have negative effects on animals:

- the animal does not feel satiety;
- the ration does not cover the energo-protein needs.

Blood samples were taken from the jugular vein, and were stored in vacutainers, with sodium citrate 3.8%.

The following parameters were determined: the viscous aspect (Ostwald), the density (Sicnometer), the superficial tension (Traube stalagnometer), the globular resistance (Perkin-Elmer photocolormeter).

Table 1

With the help of VET-SCREN 8008 we determined the ASAT, ALAT, GGT, PA, and LDH. The obtained data were statistically analysed, using the EXCEL program.

Computing the rations

	Rations for maintaining	Rations for milk production	Total
UFL	1.4 + 0.6x6 = 5	19.1x0.43 = 8.21	13.21
PDI	100 + 0.5x600 = 400	19.1x50 = 9.56	1355
SAD	0.6x600 = 360	19.1x60 = 1146	1506
Ca	4.5 x 6 = 27	19.1x4.15 = 79.26	106.26
P	6x6 = 36	19.1x1.75 = 33.42	69.42
NaCl	5x6 = 30	19.1x2 = 38.2	68.2

LS = 20(0.4+ 0.15x3.7) = 19.1 l

SU = 0.02 x 600 + 0.33 x 30 = 18.6 kt

Table 2

Ration for a cow of 600 kg, with 20 l daily milk production of 3.7 % milk fat during winter season

	kg	SU	UFL	PDI	SAD	Ca	P	NaCl
Ration	-	18.6	13.21	1355	1506	106.2	69.4	68.2
Mountain hay	2	1.7	1.29	130.9	127.5	11.9	5.95	-
Lucerne hay	3	2.59	1.78	300.9	-	40.8	7.65	-
Barley straw	2	1.76	0.79	38.72	-	5.88	1.76	-
Corn silo	20	5.6	4.70	296.8	252	19.6	14	-
Total of hay	-	11.61	8.56	767.5	678	78.36	19.36	-
Difference	-	6.99	4.65	587.5	828	27.86	50.04	68.2
Kg fodder	5.6	4.87	4.76	582.45	674.24	26.88	56	56
Total	-	16.48	13.32	134.99	1352.8	105.2	76.36	56*

**** The difference of NaCl was provided by NaCl balls.***

Table 3

Add ration

Specificare	%	SU	UFL	PDI	SAD	Ca	P	NaCl
Corn	56	48.44	53.28	3342.3	3148	14	133.4	
Grain bran	20	17.42	13.58	1654.9	1933	17	186.35	
Soy groats	20	17.66	18.18	5403.9	6958	58	109.44	
Ca carbonate	1	0.99				390		
Monosodium phosphate	1	0.99					198	
NaCl	1	1						100
Zoofort	1	1						
Total	100	87.5	85.03	10401.2	12040	480	627.32	100
for 1 kg	1	0.87	0.85	104.01	120.4	4.8	6.27	10

**** The difference of NaCl was provided by NaCl balls.***

Table 4

Ration for farm cows

	kg	SU	UFL	PDI
Ration	-	1.6	13.21	1355
Lucerne hay	5	4.25	2.95	501.5
Corn silo	20	5.6	4.6	296.8
Total hay	25	9.85	7.55	798.3
Difference	-	8.75	5.66	556.7
kg fodder	6	5.22	5.1	624.06 .
Total ration	-	15.07	12.65	1422.36

Results and discussions

In Table 5 we presented the values of the investigated physical and blood parameters: the viscous aspect, the density, the superficial tension, the globular resistance.

Red Holstein

It is obvious from the chart that the average value of the relative density in Red Holstein cows is of 1051.71 ± 0.57, with a very low variability coefficient (VC = 0.14).

The viscous aspect reached the level of 5.10 ± 0.004, with a very low VC (= 0.22).

The superficial tension had an average level of 67.20 ± 0.21, with a reduced VC (= 0.82).

The globular resistance, detected in NaCl hypotonic solution, was of 0.300 in Red Holstein cows, and of 0.333 in Holstein Friza cows, and of 0.300 in Baltată romaneasca cows. The minimum globular resistance reached a value of 0.700 % NaCl in all studied cows. This value is lower than the known data obtain by other researchers.

Holstein Friza

The relative density in Holstein Friza cows had an average value of 1053.57 ± 0.53, slightly higher than in other cow breeds, but with a very low VC (= 0,13).

The viscous aspect reached a level of 5.25 ± 0.01, higher than it was in Red Holstein or in Baltata Romaneasca.

The superficial tension had an average value of 66.17 ± 1.02, with a VC of 4. 09.

Baltata Romaneasca

The relative density in Holstein Friza cows had an average value of 1051.29 ± 0.61, close to the one obtained for Red Holstein.

The viscous aspect was lower that in other breeds: 4.79 ± 0.06

The superficial tension had an average value of 67.47 ± 0.23, not far from the values obtained in the other breeds.

Table 5

The levels of the relative density (ρ), of the viscous aspect (CP), of the superficial tension (τ), and of the globular resistance in Red Holstein cows

	Red Holstein breed	Holstein Friza breed	Baltata Romaneasca breed
Relative density $\overline{X}$	1051.71	1053.57	1051.29
Sx	0.57	0.53	0.61
s	1.50	1.40	1.60
CV	0.14	0.13	0.15
Viscous aspect $\overline{X}$	5.10	5.25	4.79
Sx	0.004	0.01	0.06
s	0.01	0.02	0.15
CV	0.22	0.33	3.17
Superficial tension $\overline{X}$	67.2	66.17	67.47
Sx	0.21	1.02	0.23
s	0.55	2.71	0.61
CV	0.82	4.09	0.90
Globular resistance R.O. maxim % NaCl	0.300	0.333	0.300
R.O. minim % NaCl	0.700	0.700	0.700

Red Holstein

The results in Table 6 show that enzyme activity (GOT, ASAT) in Red Holstein cows reached an average level of 95.14 ± 3.81 U.I., and the VC was of 10.61. The enzyme's activity ranged between the normal limits.

The GPT, ALAT was 23.71 ± 1.21, within the physiological limits (14 – 38 U.I.), with a VC of 13.49.

The GGT presented a medium level of 14.14 ± 0.80 U.I., with a VC of 14.96.

After having studied the metabolic profile in cows with ovarian disorders, DRUGOCIU (2) concluded that the GGT's had reached very low values, close to the inferior limits: 19 – 33 U/I (compared with 220 U/I).

The value of the alkaline phosphate in cows with follicular cysts and persistent lutein formations had tended to the superior limit: 78- 95 U/I (compared with 196 U/I).

The enzyme activity (PA) reached a level of 130.71 ± 10.19 mV/ml, with a high VC (= 20.63).

The average value of LDH was of 1554.14 ± 30.38, with a very low VC (= 5.17). Increasing of serum activity may be caused by hepatic disorders. This does not always implies cell damages. It can be connected with modifications in the membrane's permeability (5).

Holstein Friza

The GOT activity in Holstein Friza cows was lower in comparison with Red Holstein and Baltata Romaneasca: 85.57 ± 3.09.

The GPT) reached an average value of 27.71 ± 1.38 U/I, with a VC of 13.13.

Stojenich et all, apud PECHOVA (4) observed the enzyme activity in milk cows with different physiological conditions, and declared that the highest level of ASAT had been registered during the first period of lactation, and the highest level of ALAT had been registered between the 46th day of lactation till the time of mammary repose.

The GGT was 22.29 ± 1.11 U/I, with VC = 13.14.

The activity of the alkaline phosphate was bigger (160.43 ± 4.06), in comparison with the alkaline phosphate level in Red Holstein (130.71 ± 0.19).

The LDH increased, 1769.71 ± 79.16 U/l, in comparison with Red Holstein and Baltata Romaneasca.

Baltata Romaneasca

The GOT, ASAT was significantly higher, 121.29 ± 4.45 U/l, in comparison with Red Holstein and Holstein Friza.

The GPT and ALAT was also more intense: 37.86 ± 2.86 U/l.

The GGT reached an average level of 16.57 ± 1.09, lower than in Holstein Friza cows.

The alkaline phosphate had an average value significantly lower (110.86 ± 7.81), in comparison with the one obtained in Holstein Friza cows.

The LDH was 1393.71 ± 79.76 U.I., with VC = 15.14.

Table 6

Enzyme activity in milk cows

	GOT, ASAT U/l	GPT, ALAT U/l	GGT U/l	PA mU/ml	LDH U/l
Red Holstein breed $\overline{X}$	95.14	23.71	14.14	130.71	1554.14
Sx	3.81	1.21	0.80	10.19	30.38
s	10.09	3.20	2.12	26.97	80.37
CV	10.61	13.49	14.96	20.63	5.17
Holstein Friza breed $\overline{X}$	85.57	27.71	22.29	160.43	1769.71
Sx	3.09	1.38	1.11	4.06	79.16
s	8.16	3.64	2.93	10.74	209.43
CV	9.54	13.13	13.14	6.69	11.83
Baltata Romaneasca breed $\overline{X}$	121.29	37.86	16.57	110.86	1393.71
Sx	4.45	2.86	1.09	7.81	79.76
s	11.77	7.56	2.88	19.33	211.01
CV	9.71	19.97	17.37	17.44	15.14

Conclusions

- Para clinical investigations revealed that there are significant differences between Red Holstein and Holstein Friza, concerning *the density* $p < 0.04$, *the viscous ascpect* $p < 0.001$, *the GPT* $p < 0.04$, *the GGT* $p < 0.01$, *the alkaline phosphate* $p < 0.01$, *the LDH* $p < 0.01$, and insignificant differences concerning *the superficial tension* $p > 0.06$, *the globular resistance* $p > 0.01$, and *the ASAT* $p > 0.06$.
- There are significant differences between Holstein Friza and Baltata Roamneasca breeds concerning *the density* $p < 0.02$, *the viscous aspect* $p < 0.001$, *the ASAT* $p < 0.001$, *the ALAT* $p < 0.01$, *the GGT* $p < 0.008$, *the alkaline phosphate* $p < 0.001$, and *the LDH* $p < 0.0$; and there are insignificant differences concerning *the superficial tension* $p > 0.06$.
- There are also significant differences between Red Holstein and Baltata Romaneasca breeds concerning *the viscous aspect* $p < 0.001$, *the ASAT* $p < 0.002$, *the ALAT* $p < 0.03$; and there are some insignificant differences as well: *density* $p > 0.56$, *superficial tension* $p > 0.14$, *GGT* $p > 0.09$, *alkaline phosphate* $p > 0.12$, and *LDH* $p > 0.08$.
- The differences are significant between Red Holstein and Holstein Friza, and between Holstein Friza and Baltata Romaneasca; the differences are mostly insignificant between Red Holstein and Baltata Romaneasca, due to the fact that these two breeds are closer related.

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Evaluation of the open market milk quality by measurement of some physico-chemical parameters

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Milk as one of the main ingredients of the human diet must meet certain quality standards that are basicaly common to most countries they are strictly regulated and monitored on the market.

The purpose of this work was to undertake as complex as possible an analysis of the milk quality on the open market in Iasi, on the basis of measurement of some physico-chemical parameters like density, the fat content, the dry fatless substance, the protein content and the crioscopic point.

Research was done on 20 milk samples taken from each of the three open markets of Iasi: Market A,B and C.

The chemical parameters of the investigated milk samples had significant variations: these of the Market B and C were found under the legal limites and suspected of being adulterated by skimming and for water addition. Those from the Market A were suspected of adulteration at a relatively low rate (5%), while those from the Market B and C were found in the same state at higher rates:25% and 30%, respectively.

Key Words: milk quality, physhico-chemical parameters, milk adulterations

MATERIAL ŞI METODE

Cercetările au fost efectuate pe probe de lapte recoltate din locurile de desfacere special amenajate în cadrul pieţelor agro-alimentare din Judeţul Iaşi.

Probele de lapte au fost recoltate şi pregătite în aşa fel încât să exprime cât mai exact caracteristicile sale, să fie suficiente pentru executarea analizelor şi eventual pentru repetarea acestora. Pentru realizarea acestor deziderate s-au avut în vedere:

- ✓ Omogenizarea manuală cât mai eficientă a laptelui,înainte de recoltare;
- ✓ Recoltarea unui volum adecvat de probă – 500 ml;
- ✓ Prelevarea probelor din recipiente cu ajutorul unei pipete din sticlă de 25 ml, spălată şi uscată în prealabil;
- ✓ Transvazarea probelor în eprubete de 40 ml, cu dop, curate, uscate şi etichetate corespunzător;
- ✓ Transportarea rapidă a probelor şi conservarea adecvată în timpul transportului (temperaturi de $+4^0$C).

Au fost analizate 60 de probe, pentru următorii parametri: densitate, grăsime, substanţă uscată, proteine, punct crioscopic. Determinările au fost realizate folosind analizatorul de lapte EKOMILK, special conceput pentru analiza rapidă şi cu costuri minime a principalilor parametri de calitate ai laptelui. Acest dispozitiv prezintă unele avantaje în folosire, deoarece permite

efectuarea unui număr mare de măsurători, pentru analiza tuturor parametrilor este utilizată o cantitate relativ mică de lapte şi nu sunt necesare substanțe chimice pentru efectuarea analizelor. Limitele de detectare pentru parametri măsurați sunt următoarele: densitate 1,016 – 1,039 g/cm^3, grăsime 0,5 – 9%, substanță uscată degresată 6-12%, proteine 2 – 6%.

REZULTATE ŞI DISCUȚII

În vederea stabilirii unor statistici cu privire la calitatea laptelui comercializat, s-a procedat la recoltarea a câte 20 de probe din cele mai reprezentative trei piețe agroalimentare din Județul Iaşi: Piața A, B şi C. Rezultatele obținute au fost următoarele:

Tabel 1.

Variația indicatorilor fizico-chimici măsurați

	Grăsime %	**Proteine %**	**Substanță uscată degresată %**	**Densitate g/cm^3**	**Punct crioscopic**
Piața A	4,57 ±1,13	3,07±0,16	8,51±0,18	1,027±0,00086	(-)0,55±0,01
Piața B	3,88±1,16	3,1±0,75	8,57±0,62	1,027±0,0025	(-)0,54±0,03
Piața C	3,08±0,62	2,98±0,16	8,26±0,43	1,027±0,003	(-)0,53±0,04
Valori normale	**3,5**	**3,3-3,5**	**8,5-9,5**	**1,026-1,034**	**(-)0,58-(-)0,53**

Din analiza datelor cuprinse în tabelul nr. 1 se pot realiza următoarele interpretări cu privire la variația parametrilor fizico-chimici luați în discuție:

Variația grăsimii laptelui. Determinarea grăsimii din lapte este un examen important pentru aprecierea integrității laptelui, putându-se decela astfel falsificările prin adaos de apă sau smântânire, situații când acest parametru scade sub valorile normale prevăzute ca standard.

Față de valoarea considerată normală pentru acest indicator, au fost întâlnite valori necorespunzătoare la probele recoltate din cadrul piețelor B şi C, în sensul scăderii cu mult sub limita admisă. Au fost depistate probe cu un conținut neadmis de mic de grăsime (pentru această categorie de lapte de consum) de până la 2,72% în cadrul Pieței B şi 2,46% în cadrul Pieței C. În cadrul Pieței A, valorile minime înregistrate pentru acest parametru se încadrează în limitele normale, însă valorile maxime sunt cu mult peste media întâlnită, sugerând un adaos de smântână în laptele supus comercializării.

Variația proteinelor laptelui. Determinarea proteinelor din lapte, în strânsă corelație cu determinarea densității, grăsimii şi substanței uscate negrase, constituie un real ajutor în aprecierea falsificărilor laptelui prin adaos de apă. Proteinele laptelui înregistrează scăderi ale valorilor în urma adaosului de apă, dar şi în afecțiuni ale glandei mamare.

Variația titrului proteic s-a încadrat în limite largi pentru probele recoltate din Piața B. În toate cele trei piețe au fost depistate probe de lapte cu un conținut în proteine sub limita admisă prevăzută ca standard pentru laptele integral, muls individual, valorile minime întâlnite fiind de 2,91% (Piața A), 2,35%(Piața B) şi 2,82%(Piața C). În cadrul piețelor A şi C, limitele maxime înregistrate nu se încadrează în intervalul considerat normal pentru acest parametru.

Variația extractului uscat degresat. Determinarea extractului uscat degresat are o însemnătate deosebită, întrucât valoarea sa scade sub limitele admise în cazul falsificărilor prin adaos de apă. Valori minime pentru acest parametru au fost înregistrate pentru probele de lapte recoltate din cadrul piețelor C şi B(7,83%, respectiv7,95%); valori sub limita admisă au fost înregistrate şi în cazul probelor recoltate de pe Piața A, însă diferența față de valoarea considerată normală nu este semnificativă.

Variația densității laptelui. Determinarea densității laptelui permite obținerea unor informații asupra integrității laptelui, condițiilor de recoltare, infecțiilor ugerului, precum şi identificarea

falsificărilor laptelui (diluare, smântânire, adăugare de lapte smântânit). Densitatea laptelui creşte când alimentaţia cuprinde nutreţuri sărace în apă sau în cazul falsificărilor prin smântânire si scade atunci când hrănirea se realizează cu nutreţuri bogate în apă, în inflamaţiile glandei mamare sau în cazul falsificărilor prin adaos de apă.

Din totalul probelor luate în analiză pentru determinarea acestui indicator, abateri semnificative de la limitele admise (prevăzute ca standard pentru laptele integral, muls individual) au prezentat cele prelevate din cadrul pieţelor B şi C care au inregistrat valori necorespunzătoare de 1,024g/cm^3, faţă de valoarea minimă admisă de 1,026g/cm^3,. Rezultatele obţinute la determinarea acestui parametru pentru probele recoltate din cadrul Pieţei A s-au încadrat în limitele considerate normale.

Variaţia punctului crioscopic. Temperatura de îngheţare a laptelui integral variază între (-0,58) şi (-0,53). O valoare mai mică de (-0,53) dă dreptul la o suspicionare a unui adaos de apă.

Variaţiile valorilor punctului crioscopic pentru probele de lapte recoltate din cele trei pieţe agroalimentare, în sensul scăderii sub limita minimă admisă, au fost semnificative în cazul Pieţei C [(-)0,49] şi B [(-)0,51]. Valoarea punctului crioscopic pentru probele de lapte recoltate de pe Piaţa A se încadrează în limitele normale prevăzute ca standard.

CONCLUZII

1. Laptele comercializat pe Piaţa A prezintă scăderi minime ale conţinutului de grăsime şi substanţă uscată degresată; conţinutul în proteine nu se situează la nivel normal pentru nici o probă de lapte analizată. Aceste rezultate permit aprecierea unui adaos modest de apă în unele probe supuse comercializării în cadrul pieţei A.
2. Rezultatele analizelor pentru probele recoltate de pe Piaţa B, permit suspectarea unor falsificări ale laptelui supus comercializării prin adaos de apă, existând o corelare în acest sens, cu scăderea valorii densităţii, grăsimii, substanţei uscate degresate, proteinelor şi punctului crioscopic. Asocierea dintre scăderea conţinutului în grăsime şi creşterea valorii densităţii peste media rezultatelor obţinute, permite suspectarea unor falsificări şi prin smântânire.
3. Scăderea semnificativă a conţinutului de grăsime asociată cu scăderea ponderii extractului uscat degresat a permis depistarea şi a unor falsificări prin adaos de apă şi smântânire pentru aceeaşi probă, în cazul laptelui comercializat în cadrul Pieţei C.
4. Prin raportarea datelor obţinute la valorile densităţii, grăsimii, proteinelor, substanţei uscate degresate, se poate aprecia prezenţa apei în probele depistate pozitiv pentru acest tip de falsificare, în raport de 5 % pentru laptele recoltat de pe Piaţa A, 25% pentru cel recoltat de pe Piaţa B, şi 30% pentru Piaţa C. Se poate ca în aceste condiţii, falsificarea probelor de lapte recoltate de pe Piaţa A să nu fi avut la bază reaua-credinţă, ci unele neglijenţe ale mulgătorului, precum apa rămasă după curăţire în vasele de muls şi de depozitare a laptelui.
5. Analiza complexă cu ajutorul determinatorului EKOMILK este o modalitate rapidă şi economică de evaluare a calităţii laptelui pe pieţele agroalimentare de desfacere.

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Efficiency of some antibiotic formulae for the treatment of the subclinical bovine mastitis

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The maintenace of a healthy udder is a major task of the farmer as its damage is one of the main causes of the economic losses in the breeding sector (1,4). The early diagnosis and treatment of subclinical mastitis is mandatory, to avoid the occurrence of a harsher process, sometimes with a very severe evolution (6,8).

The purpose of the present work, performed in a group of small private dairy farms in North Moldova, was to evaluate the efficiency of some market antibiotic formula in the subclinical mastitis treatment.

Key Words: subclinical mastitis, treatment, antibiotics, efficiency, R-Mastitest

Glanda mamară se deosebeşte de alte glande ale organismului animal prin faptul că, produsul său de secreţie – laptele – este folosit cu succes atât în alimentaţia omului, cât şi a tineretului animal. Menţinerea integră a sănătăţii ugerului constituie o problemă de actualitate, îmbolnăvirea sa fiind cauza unor pagube economice semnificative în sectorul creşterii animalelor (1,4).

Deşi în ultima vreme s-au înregistrat progrese semnificative în controlul şi combaterea mastitelor subclinice, folosirea unor preparate medicamentoase pe bază de antibiotice în mod abuziv, aplicate fără respectarea celor mai elementare norme de conduită terapeutică, conduc, de cele mai multe ori, la insuccese terapeutice, cu grefarea unor germeni cu potenţial microbian crescut, rezistenţi la tratamentele medicamentoase şi responsabili de cele mai grave forme clinice de mastită(2,3,5,7).În acest context, diagnosticarea şi tratarea formelor subclinice de boală se impun ca o necesitate, cu atât mai mult cu cât nerealizarea la timp a acestor obiective poate conduce la apariţia unor procese acute, uneori cu caracter evolutiv foarte grav (6,8).

Scopul lucrării este de a efectua o analiză cât mai complexă privind eficacitatea unor preparate comerciale pe bază de antibiotice în tratamentul formelor de mastită subclinică, rezultatele obţinute fiind prelucrate statistic.

MATERIAL ŞI METODE

Cercetările au fost efectuate în cadrul unui grup de microferme particulare din zona de Nord a Moldovei.

Materialul de studiu a fost reprezentat de vaci, rasele Bălţată cu Negru Românească, Brună Austriacă şi Bălţată Austriacă, cu un efectiv total matcă de 235 capete.

Depistarea formelor subclinice de boală s-a efectuat prin utilizarea testului rapid la grajd R-Mastitest. Interpretarea rezultatelor s-a realizat după aspectul amestecului lapte – reactiv (6,8):

- reacţie negativă (-) = amestecul rămâne fluid, omogen şi subţire ;
- reacţie dubioasă (±) = în amestec apar filamente şi flocoane;
- reacţie pozitivă (+) = amestec uşor consistent, cu flocoane şi fluiditate scăzută;

- reactie intens pozitivă (+ +) = amestec foarte consistent şi vâscos. Dacă se înclină placa amestecul rămâne prins de placă sau se scurge ca un lichid filant.

Tratamentul instituit a avut inițial caracter local, pentru cazurile nevindecate făcându-se, ulterior, asocierea cu terapia pe cale generală. Timpii de intervenție adoptați în aplicarea locală a preparatelor medicamentoase, au urmărit toaletarea riguroasă a sfertului afectat şi mulgerea cât mai completă a secreției patologice.

Preparatele medicamentoase pe bază de antibiotice folosite în terapia locală, au fost:

- Cobactan LC, suspensie intramamară: o seringă de 8g conține 75 mg cefquinomă sulfat.
- Mastijet Forte, suspensie intramamară: o seringă conține 200 mg tetraciclină, 250 mg neomicină, 2000 U.I. bacitracină, 10 mg prednisolon.
- Synulox LC, suspensie intramamară: o seringă conține 50 mg acid clavulanic-sare potasică, 200 mg amoxycilină trihidrat, 10 mg prednisolon.

Pentru a se testa eficacitatea fiecărui produs comercial folosit în terapia locală, s-a procedat la administrarea lor intramamară timp de trei zile consecutiv. După acest interval de timp,s-a reluat diagnosticul rapid la grajd, prin metoda R-Mastitest. Pentru cazurile reacționate în continuare pozitiv, s-a procedat la asocierea terapiei locale cu cea pe cale generală timp de două zile consecutiv (Enrofloxacină 10%, 2,5 ml/100 Kc, i.m.,timp de două zile).

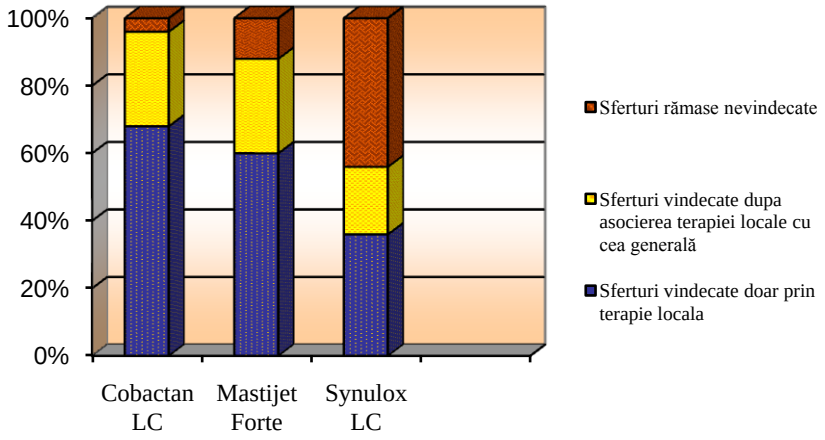
REZULTATE ŞI DISCUŢII

În vederea stabilirii eficienței terapeutice pentru produsele comerciale utilizate, s-a procedat la constituirea a trei loturi de animale, fiecare preparat medicamentos fiind folosit în tratarea a câte 25 sferturi reacționate pozitiv la reacția R-Mastitest. Rezultatele obținute au fost următoarele:

Tabel 1

Eficacitatea unor preparate comerciale în tratamentul mastitelor subclinice

	Cobactan LC		Mastijet Forte		Synulox LC	
	Nr.	%	Nr.	%	Nr.	%
Sferturi tratate	25	100	25	100	25	100
Sferturi vindecate doar prin terapie locală	17	68	15	60	9	36
Sferturi vindecate după asocierea terapiei locale cu cea generală	7	28	7	28	5	20



Grafic 1
Eficacitatea unor preparate comerciale în tratamentul mastitelor subclinice

Din analiza datelor cuprinse în tabelul 1 şi graficul 1, reiese faptul că, după administrarea locală a celor trei preparate comerciale, rezultatele cele mai bune s-au obţinut prin folosirea preparatului comercial Cobactan L.C.,utilizarea acestuia ducând la vindecarea unui număr de 17 sferturi din totalul de 25, ceea ce este echivalent, în exprimări procentuale, cu 68 din total. Rezultatele obţinute după administrarea intramamară a produsului Mastijet Forte au fost apropiate de cele rezultate după administrare preparatului Cobactan L.C.: 15 sferturi vindecate dintr-un total de 25, ceea ce este echivalent cu 60% din totalul sferturilor luate în calcul.

În urma administrării preparatului comercial Synulox L.C. s-a obţinut vindecarea unui număr de 9 sferturi afectate de mastită subclinică, ceea ce este echivalent cu un procent de 36 din total.

Asocierea terapiei locale cu cea pe cale generală a permis vindecarea unui număr de 7 sferturi (28%) în cazul în care terapia locală a fost cea cu Cobactan L.C., 7 sferturi (28%) în cazul administrării locale a preparatului Mastijet Forte şi 5 sferturi (20%) în cazul administrării locale a preparatului Synulox L.C.

CONCLUZII

În urma cercetărilor efectuate cu privire la stabilirea eficacității unor preparate comerciale în tratamentul formelor subclinice de mastită, se pot desprinde următoarele concluzii:

1. depistarea şi tratarea la timp a formelor subclinice de mastită devine o necesitate în contextul în care aceste procese inflamatorii pot trece în forme acute, cu evoluție clinică deosebit de gravă;
2. conduita terapiei mastitelor subclinice trebuie să respecte conduita generală de tratament a inflamațiilor glandei mamare, fiind urmărite, în general, următoarele deziderate: toaletarea cât mai riguroasă a sfertului afectat şi mulgerea cât mai completă a secreției patologice, în acest fel fiind favorizate difuzarea şi acțiunea preparatelor medicamentoase asupra structurilor mamare inflamate;
3. preparatele comerciale Cobactan L.C. şi Mastijet Forte au avut acțiune net superioară în tratamentul local al mastitelor subclinice, comparativ cu preparatul comercial Synulox L.C. S-a obținut vindecarea a 17 sferturi reacționate pozitiv la reacția R-Mastitest doar prin folosirea locală a preparatului comercial Cobactan L.C, ceea ce este echivalent cu 68% din totalul cazurilor. Prin folosirea locală a produsului Mastijet Forte s-a obținut vindecarea unui număr de 15 sferturi (60% din cazuri), iar prin folosirea locală a Synulox L.C. a 9 dintre sferturile afectate, ceea ce reprezintă 36 % din sferturile afectate.
4. în cazul nevindecării mastitelor subclinice doar prin folosirea tratamentelor intramamare, asocierea terapiei locale cu cea pe cale generală asigură succesul terapeutic într-un procent ridicat, de până la 96 % din sferturile afectate în cazul în care terapia locală s-a efectuat cu produsul comercial Cobactan L.C., 92% din total în cazul în care în terapia locală s-a folosit preparatul Mastijet Forte şi 84% din sferturile reacționate pozitiv în cazul folosirii locale a produsului Synulox L.C..

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Tissular reactions of the main organs in Equine Infectious Anemia (EIA)

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During 1994 - 2006 in Bacău region Equine Infectious Anemia (EIA) was diagnosed in 138 foci; 158 horses were extracted out of this region.

Organ fragments (encephal, lung, liver, splin, kidney) were prelevated from 7 of the euthanised animals, and used for morphological investigations.

The histopathological examination accomplished by parafine inclusion and Haematoxilin - Eosin (HE) and Perls coloration reveal autoimmune - determined modifications, consisting of vascular amiloidosis, hidroprotidic dystrophies, acute mesenchimal reactions; additionally, the splin is marked by a severe hemosiderosis.

Key Words: Equine Infectious Anemia (EIA), encephal, lung, liver, spleen, kidney, histology

Dinamics of umoral immunologic constants in cows

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Our research was made on milk cows from Holstein half-breed, between 2 and 8 years old, grouped according to physiologic and general condition and clinical healthy.

The usual laboratory methods allowed the determination of seric proteins and their dynamics during the 24 hours and during the whole year.

The results reveal variations of seric albumins and seric globulins during the day time and according to the season (winter, spring, summer).

According to season rhythm, seric albumins have bigger values in summer and lower in winter time. The lowest values in winter were recorded at 600 PM and the highest in spring and summer, at 1200 PM and 1200 AM.

Variations of α1-globulins were recorded with high values in spring and low ones in winter. As for hourly variations, the highest values was recorded at 600 AM in spring and at 1200 PM in summer. Minimal parameters were determined at 600 AM in winter.

Season dynamics of α2-globulins prove minimal values at 1200 AM and 1200 PM in summer and maximum ones at 1200 AM and 600 PM in winter and spring.

The quantity of β-globulins was high in winter and low in summer, with a peak in winter at 1200 AM and in summer at 600 PM.

γ-globulins evolution shows high values in winter and lower ones in spring; the highest ones were recorded at 600 AM and 1200 PM in summer, spring and winter, and the lowest at 1200 AM and 600 PM in summer, spring and winter.

Key Words: cow, immunity, rhythm

MATERIALS AND METHOD

Scientific research was performed on milk cows, Holstein half-breed, 2-8 years of age, brought up in identical conditions, healthy and grouped according to their physiological status.

Serumproteins were determined by electrophoresis.

Researchers everywhere (1,2,5) proved that the biologic process is a cascade of chemical reactions, that permanently requires simple and complex products that participate effectively in the process, as well as chemical structures that will ensure their development within the limits of the living organism. The mechanism ensuring biochemical reactions is accomplished by non-specific and mainly specific reactivity mechanism of living organism (3).

Every normal chemical reaction needs during it's circadian and season evolution, a variable amount of compounds.

Knowing these, our research tried to determine the circadian and sesonal dinamics of plasmatic proteins implicated in specific reactivity gamma globulins in cows. It's obvious that cows were and still are used everywhere in the world in human activities and survival, argument implying a carefull watch an maintaining the constancy of biochimic reactions (4,6,7).

The cuatification of results is shown in the table below.

The medium values of serumglobulins and serumalbumins at cows

No. crt.	Constant	Time	Season		
			Winter	Spring	Summer
1	Albumins (%)	6^{00}	39,18 ± 0,81	39,96 ± 1,20	44,26 ± 0,24
2	α_1 (%)		0,62 ± 0,12	12,04 ± 0,75	8,18 ± 1,10
3	α_2 (%)		10,18 ± 0,30	8,74 ± 0,50	3,74 ± 0,45
4	β (%)		10,20±0.90	10,80 ±0,65	6,76 ± 0,50
5	**γ (%)**		**39,87±1.10**	**28,74 ± 0,65**	**37,02 ± 0,55**
6	Raport A/G		0,64±0.20	0,63 ± 0,15	0,8 ± 0,10
1	Albumins (%)	12^{00}	39,22±0.56	44, 08 ± 0,50	47,52 ± 0,34
2	α_1 (%)		0,68±0,34	9,90 ± 0,65	8,42 ± 0,30
3	α_2 (%)		10,54±0.40	5,94 ± 0,35	3,66 ± 0,45
4	β (%)		10,26±0.26	9,12 ± 0,45	6,90 ± 0,33
5	**γ (%)**		**39,30±0.78**	**30,96 ± 0,75**	**33,50 ± 0,45**
6	Raport A/G		0,66±0,23	0,79 ± 0,55	0,91 ± 0,36
1	Albumins (%)	18^{00}	38,74±0,45	44,22 ± 0,90	46,00 ± 0,40
2	α_1 (%)		0,74±0.80	9,80 ± 0,75	7,98 ± 0,72
3	α_2 (%)		10,10±0,46	8,84 ± 0,15	3,98 ± 0,23
4	β (%)		10,82±0,63	9,44 ± 0,35	6,94 ± 0,10
5	**γ (%)**		**39,60±0,12**	**27,78 ± 0,14**	**35,10 ± 0,12**
6	Raport A/G		0,64±0,31	0,81 ± 0,30	0,85 ± 0,21
1	Albumins (%)	24^{00}	39,94±0,44	46,14 ± 0,40	47,24 ± 0,22
2	α_1 (%)		0,94±0,90	9,34 ± 0,30	7,80 ± 0,11
3	α_2 (%)		10,66±1,20	6,42 ± 0,55	3,66 ± 0,42
4	β (%)		11,84±1,11	8,40 ± 0,50	2,76 ± 0,74
5	**γ (%)**		**39,62±0,96**	**29,56 ± 0,25**	**34,54 ± 0,68**
6	Raport A/G		0,59±0,54	0,86 ± 0,15	0,89 ± 0,34

Following the evolution of gamma-globulins, the proteic component of plasma, playing a role in specific immunity *_immunoglobulines_* we could notice quantitative variations during day time and along the year.

Biologic rhythm is manifested through *high* values at 6^{00} in summer (37,02%) and winter (39,82%); *low* values at 12^{00} in summer (33,50%) and winter (39,30%) and at 18^{00} in spring (27,78%).

Seasonal dinamics of gamma-globulins revealed *high* values in winter (39,30-39,82%), *lower* in spring (27,78-30,96%) and *intermediar* values in summer (33,50-37,02%).

The results concerning alpha and beta globulins (components playing a mediator's part in immune processes) prove variations according hour and season.

The level of α_1-globulins registered a *peak* in spring (9,34-12,04%); *low* values in winter (0,62-0,91%); *intermediar* values in summer (7,80-8,42%). In 24 hours we noticed *high* values at 6^{00} in spring (12,04%) and at 12^{00} in summer (8,42%); the *lowest* values were recorded at 6^{00} in winter (0,62%).

Serumglobulins α_2 *raised* in winter (10,10-10,66%) and *diminished* in summer (3,66-3,98%) and *intermediar* values (5,94-8,84%) in spring. *Highest* values were recorded in winter at 24^{00}

(10,66%), spring 18^{00} (8,84%); *low* values were recorded at 12^{00} and 24^{00} (3,66%), in winter at 6^{00} (10,18%) and spring at 12^{00} (5,94%).

The dinamics of β-globulins prove *high* values in winter (10,20-11,84%); *low* values in summer (2,76-6,94%); *intermediar* values in spring (8,40-10,80%). In winter, the *highest* values were recorded at 24^{00} (11,84%) and *lowest* at 6^{00} (10,18%). In summer, the *lowest* values values were recorded at 24^{00} (2,76%) and the *highest* at 18^{00} (6,94%). In spring at 6^{00} *superior* values (10,80%) and *low* values at 24^{00} (8,40%).

The quantity of serumglobulins was *high* in summer (44,26 – 47,52%), *low* in winter (38,74-39,94%), with *intermediar* values in spring (39,96-46,14%).

The *lowest* values were recorded in spring and summer at 6^{00} (39,96%, respectively 44,26%) and at 18^{00} in winter (38,74%). The *highest* values were recorded in winter and spring at 24^{00} (39,94 - 46,14%) and at 12^{00} in summer (47,52%).

The general analysis of the results prooves that serumproteins levels are comparable to those obtained by researchers and stated in all the manuals and treaties.

CONCLUSIONS

1. Variations of gamma-globulins were noticed in 24 hours (circadian variation) as well as according to the season.
2. Circadian and seasonal variations were noticed in α and β-serumglobulins, as well as in serumalbumins.

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Morphologic and epidemiologic aspects in lerneosis at farm ciprinides

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Common carp (Cyprinus carpio) and Asiatic cyprinid's raised in fish farms in Romania can be affected by the exoparasitic invasion with the barnacle Lernaea cyprinacea.

The parasite has a cylindrical body and he is brace with the anterior extremity on the skin of the fish; in higher infestation degree, important lesions are produced, which can be complicated leading to the depreciation of commercial aspects.

The studies were made on common carp (Cyprinus carpio), silver carp (Hypophtalmichthys molitrix) and grass carp (Ctenopharyngodon idella).

The results of the studies showed proliferate lesions with a lymphohistioplasmocitary and even hemorrhagic-necrotic character, depending on the intensity of parasitic invasion.

Key Words: morphologic, epidemiologic, lerneosis, ciprinides

MATERIAL AND METHOD

The research was made in the fish nursery S.C. Piscicola S.A Pudu Iloaie on the following sweet water fish species: *Cyprinus carpio*, *Hypophtalmichthys molitrix* and *Ctenopharyngodon idell*.

The examination was made through inspection, palpation and section of the lesions, on 45 selected fish which had macroscopical changes immediately after fishing.

Fragments with lesions were taken 5 mm thick. These samples were fixed in formaldehyde 10% water solution and moved afterwards in Bouin mixture, included in paraffin, sectioned and coloured using the Haematoxyline-Eosine-Metil blue (HEA) method.

RESULTS AND DISCUSSIONS

After the macroscopical, microscopical, epidemiological investigations on the cases that came from the same farm were observed that inside it, evolve with important quality depreciation, one of the most important ectoparasitosis at nursery cyprinids and that is lerneosis. This disease evolves especially sweetwater fish but is sporadically met at wold fish.

The disease is produced by the females of some barnacle species belonging to the genre Lernea, more common in Europe being *L. cyprinacea*, but existing in other forms as well such as *L. ctenophryngodonis, L. elegans*. (1,3).

The etiological diagnosis can be established at the beginning of the necropsic examination; the parasite is relatively is to identify, having a cylindrical body, fixed through the proximal extremity in the skin (Fig. 1, Fig. 2).

The parasite body is wormlike, in the anterior parte is found the fixing apparatus which is made of 2 pairs of excrescences with auchor aspects and in the posterior thired the mature female pas 2 prolonged oviger sacks (Fig. 3, Fig. 4).

Macroscopically, through close inspection, at the implanting place is observed a haemorrhagical exsudate, visible also aromed the parasite (resembling to ring, red coloured).

After the forced take off the parasite in the fixing area is observed a haemorrhagic infiltration foci, intensively coloured in red-cherry in the central area and progressively less noticeable at peripheral areas (2, 4, 5).

Histologically can be observed changes both at skin and at the superficial muscles. So, in the debut stages congestions are found in the suscutaneous muscles, intraepidermical melanosis is observed (Fig. 5, Fig. 8, Fig. 9, Fig. 10).

I the late stages of parasitic invasions are observed next to a limphohistioplasmocitary proliferation a muscular fibres necrosis (Fig. 6, Fig. 7).

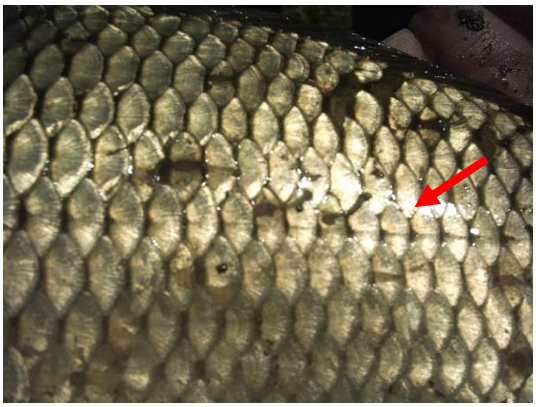

Fig. 1 Carp. Lernaea cyprinacea fixed at skin.

Fig. 2.. Lernaea cyprinacea – macroscopical aspects immediatly after the skin extraction.

Fig. 3 Lernaea cyprinacea – scanning electronical microscope aspect

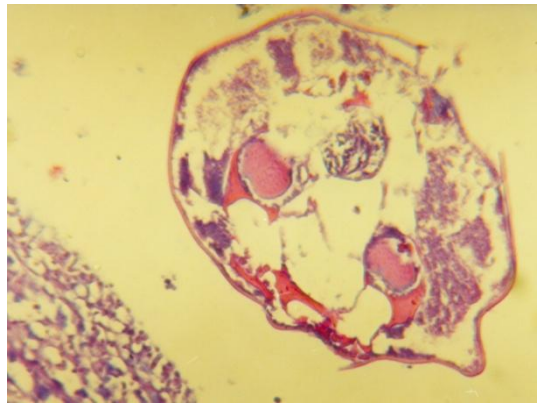

Fig. 4.. Lernaea cyprinacea. Female. Histopathological exam.. Col. HEA, x 200

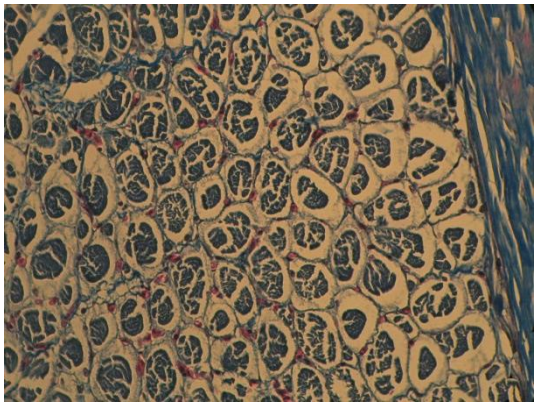

Fig. 5 Subcutaneous muscle. Congestion. Interfibrilar serous infiltration.. Col. HEA, x 100

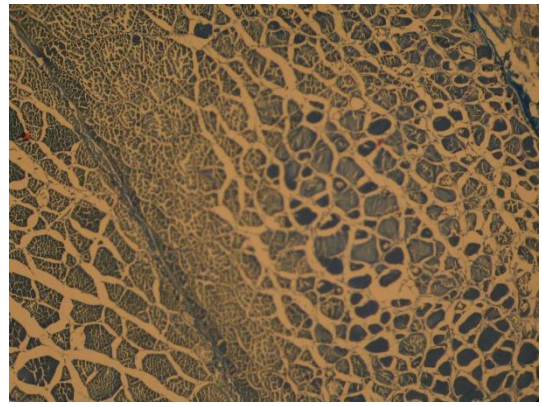

Fig. 6.Subcutaneous muscle. Fibrilar necrosis.. Col. HEA, x 100

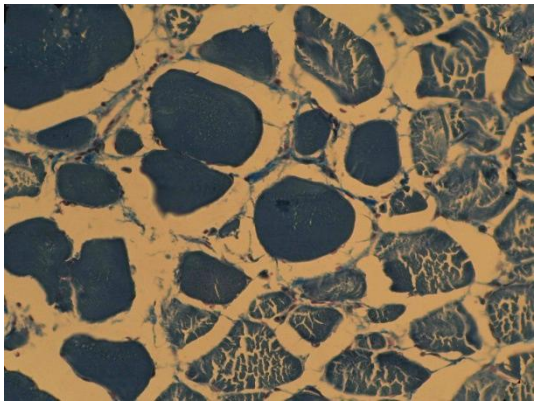

Fig. 7 – Necrotic muscle fibres.. Col. HEA, x 400

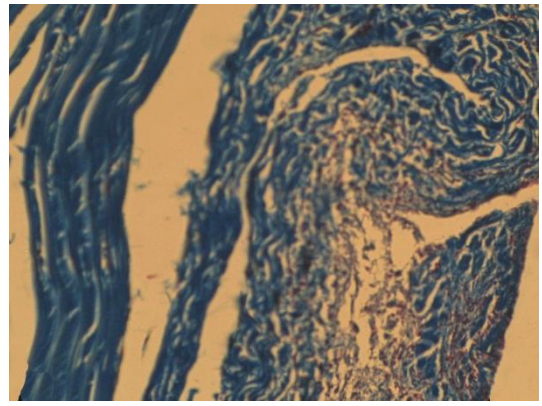

Fig. 8 – Scar conjunctive hyperplasia at the fixing place. Col. HEA, x 400

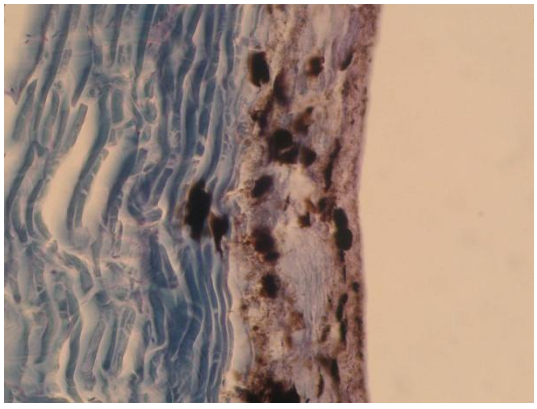

Fig. 9 – Epiderma. After scar melanosis. Col. HEA, x 400

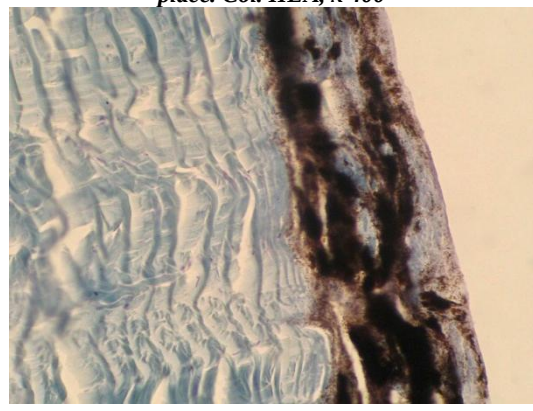

Fig. 10 – Intraepidermical focused melanosis. Col. HEA, x 400

CONCLUSIONS

The macroscopical examination of the fish immediately after fishing put in evience the presence of Lernaea *cyprinaceae* parasites on the surface of the skin.

Histological exams show next to the presence of the parasite in derma other lesions consecutive to the implantation as well.

In the acute phase of the parasitic invasion are noticed interstitial congestions and celular necrosis on small tissular areas.

The chronical stage of the disease is marked by respiratory changes: lymphohistiocitar hyperplasias, fibrosis, and focused melanosis after scars.

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Aspects regarding the rabbit embryonic development through echographycal analysis

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Sexual manifestations of male and female rabbit depend on the anatomic and functional integrity of their reproductive system, on their general heath, on alimentation and environment characteristics.

The diagnostic of pregnancy is necessary in order to take appropriate measures for repeating the mount of the female rabbits, to protect the pregnant ones, or to remove from the lot the sterile ones.

Key Words: blood vessels, fetal development, intrauterine crowding, placenta, uterine horn

Female rabbit's reproductive system counts two ovaries, two oviducts, two uterus, the vagina and the vulva and clitoris. The ovaries are situated in the posterior abdominal cavity (pelvic) with grey-pick colour and variable size, up to one and a half centimeters. Their surface has numerous follicles, with ovules in different developing stages. Then ovaries have a funnel shaped cover, with the ovules "fall" at the moment of the Graaf follicles opening, and are lead to the oviducts.

The oviducts one for each ovary are about 10 cm long each and lock like filliforme conducts who allow ovules passage toward the uterus, with ends as a very well developed pavilion, with an important role in fecundation.

The uterus in double, one for each ovary being a continuation of the oviduct, and has the role to receive feed and shelter the fecundated ovule until the end of pregnancy (30 day).

The two uterus resemble the uterus hours of the other mammals, yet they each have a separate uterine gorge to communicate with the vagina. The other mammals have an uterine body with an unique uterine gorge, while in female rabbits the uterine body is absent.

This characteristic conformation of the reproductive system of female rabbits allows the superfetation, which is the fecundation on different data and the successive development of the cubs of the same pregnancy.

The uterine gorge, one for each uterus, is closed during pregnancy and opens only during mating (copulation) and fecundation.

The vagina is about 6 cm long and communicates with the vulva. The mucous membrane of the vaginal vestibulum is folded and forms two pairs of vulvar labias.

Material and method

The diagnostic of pregnancy is necessary in order to take appropriate measures for repeating the mount of the female rabbits, to protect the pregnant ones, or to remove from the lot the sterile ones.

Pregnancy diagnostic can be made 14-16 day after mating through palpation of the abdomen.

Radiologic and echographic exams are alternative diagnostic methods.

We used the echograph of the veterinary Faculty Aquila Vet, and a 5 MHz probe.

Rezults and discussion

Most echographic studies one female rabbits were performed in order to confirm the pregnancy diagnostic.

We tried to trace the apparition of congenital anomalies, body and organ development.

Pregnancy diagnostic through echographic exam is difficult during the first days. The normal uterus can be mistaken with the intestine. During pregnancy until the 10-th day, the embryonic vesicle is easily mistaken with feces.

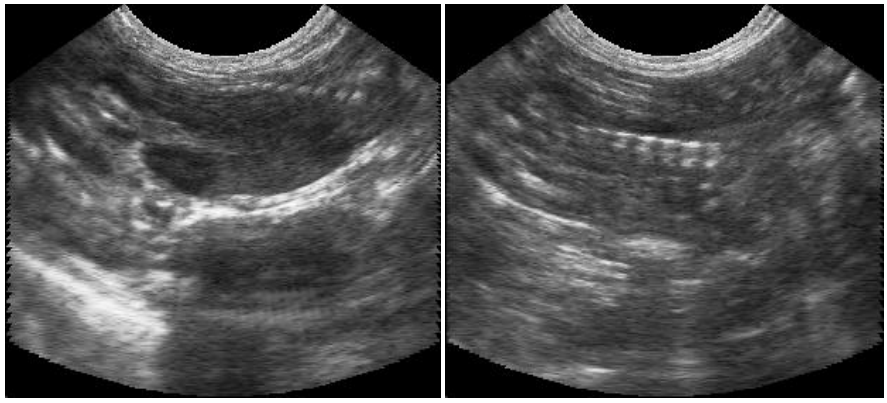
In day 8 one can notice anechogen images, and the number of fetuses can be established.

In day 17 the spine and limbs can be identified.

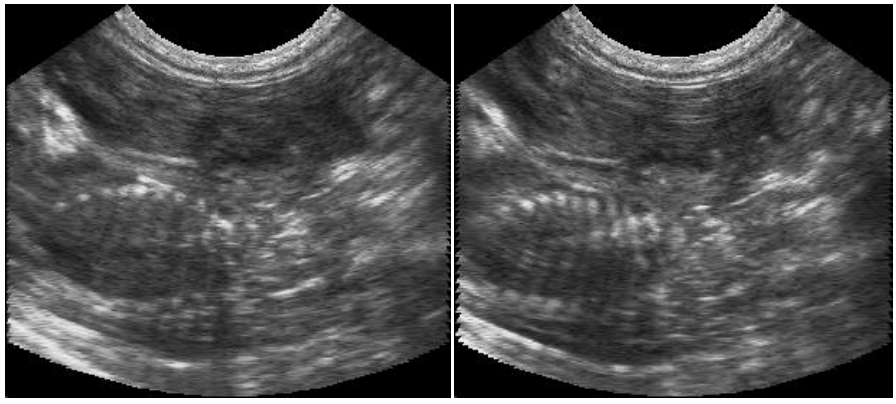
The end of organogenesis takes place in the 19^{th} day moment when the limbs are totally developed, the muzzle is long, the neck is visible.

The embryo becomes a fetus.

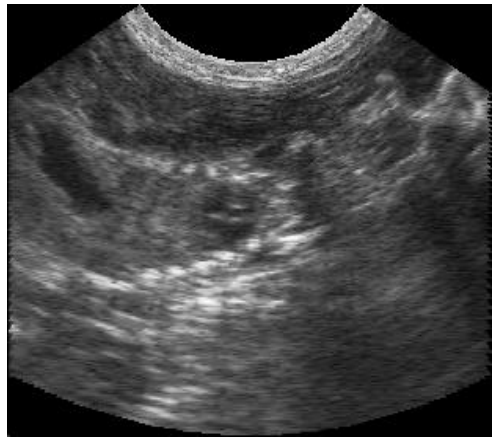
In day 27 we could measure the biparietal diameter, and in day 28 the whole skeleton was visible.



Ecographic images of rabbits fetus spine in day 29.



Thoracic cavity of rabbit fetus in day 29.



Heart and thoracic cavity of rabbit fetus in day 29.

Conclusions

1. Echographic images allows a close survey of fetus development during the whole pregnancy
2. Echographic exam helps notice morphologic anomalies and analyze in which pregnancy phrase certain embriotoxic products act.

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Observations on intrauterine growth and development at rabbit

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Embryology is a phase organism's individual development as an embryo, which begins immediately after fertilization and progresses though thousands transformations to an organism of a well defined form.

Scientific research is based on mother-fetuses relations, development status and embryo survival.

Key Words: blood vessels, fetal development, intrauterine crowding, placenta, uterine horn

Embryology is a phase organism's individual development as an embryo, which begins immediately after fertilization and progresses though thousands transformations to an organism of a well defined form.

During his development, the product of conception goes through several phrases: zygote (from the moment of fecundation to the moment of the implantation), embryo (from the moment of the implantation to the moment when placenta is formed) and fetus (which begins once with placentar circulation).

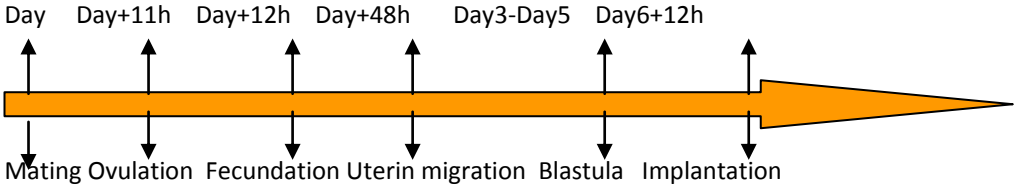
Immediately after the fecundation, the segmentation begins. The process is represented by several mitotic segmentations of the zygote, the dimensions of the entire structure remaining unchanged. The new cells(the blastromeres) get smaller with each division, resulting in a pluricelular construction.

During this whole process, the zygote migrates towards the uterus.

In rabbits, zygote segmentation is total, slightly unequal and asinchronic. The segmentation begins 8 hours after fecundation, when the zygote has reached half way through the oviduct.

Morula (M) reached the uterus in about 3 day after fecundation and is covered with a thick albumin caver, secreted by ovocites.

Blastula (B) appears in 4 day after fecundation. In 24 hour it's volume is double, pellucid zone gets very thin and disappears towards the end of the 4th day cell the beginning of the 6th day the blastocit is bilaminar and the albumin cover disappears.



The process of blastocit attachment on the uterine epithelium is called implantation. Blastocit's protrusion, in the epithelium and his inclusion in end metes compact layer begins in day 8.

Material and method

Scientific research is based on mother-fetuses relations, development status and embryo survival.

The studies were made on 6 females, common breed and weighing of fetuses at the end of pregnancy.

The classic preoperatory preparations were made preparation of the surgical field, hair clipping and repeated use of antiseptic on the region.

The contention was made in dorsal decubit anesthesia narco-neurolephanalgezic.

The instrumentar used was the one necessary for incision, hemostasis and suture.

The ensure haemostatis and abdominal musculature suture, we used chromic cadgut Braun 2-0,and for skin suture we used neresorbabil monofilament Premilene 6-0.

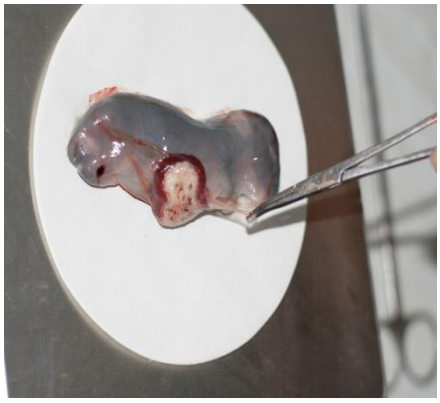
Surgical times:

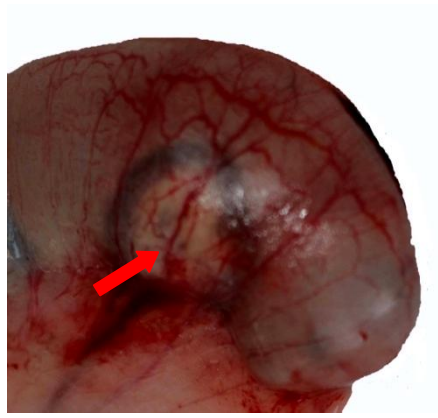
1. Skin and musculare incision
2. Pregnant uterus exteriorization
3. Laemostatis though uterine and ovary ligatures
4. Uterine ligatures previous to Lysterectomy
5. Ovario-Lysterectomy
6. Abdominal wall suture
7. Suture of the abdomen in continuous dual-leveled wire
   - musculo-peritoneal
   - subdermic suture
8. Intradermic suture

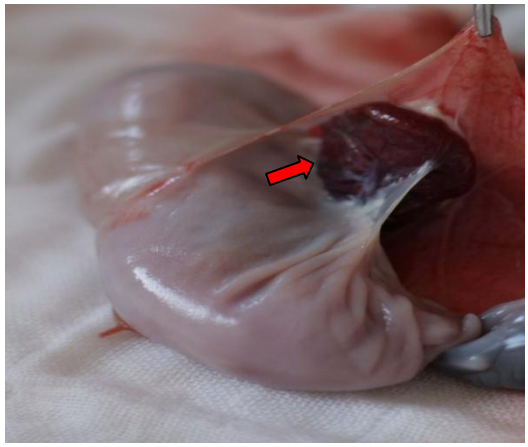
Rezults and discussion

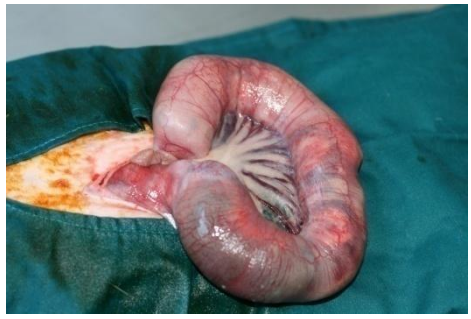
During surgery we recorded the number of fetus for each preagnancy, the degree of embrionar development, fetal irrigation.

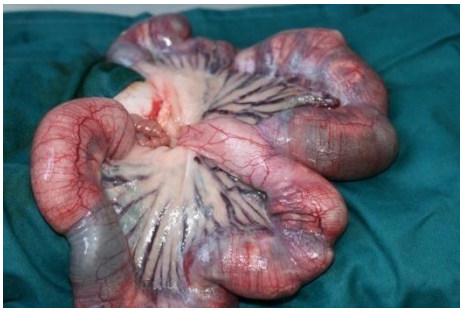
In rabbits, the place rata is hemocorial, with a discoidal form.

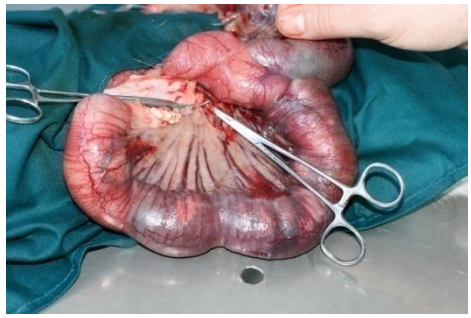


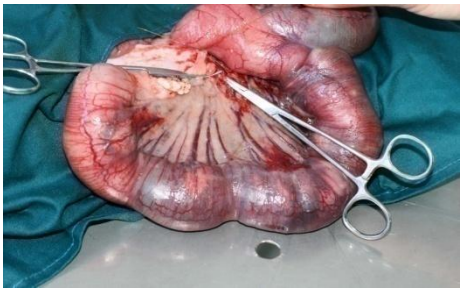




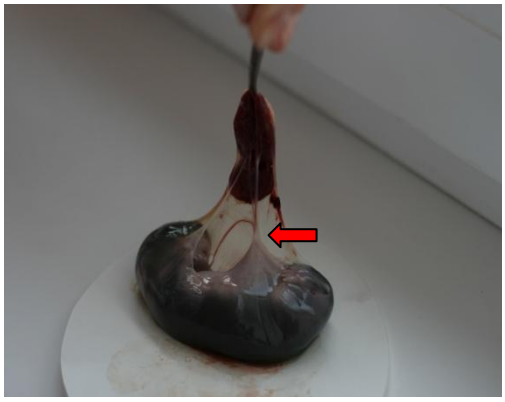








There are two umbilical arteries that flu near one of the fetus and a umbilical vein that leads to the liver. The umbilical card has 2 cm length.



After the 25th day of pregnancy takes place a strong cell lyses in the maternal portion of the placenta, fact that prepares the beginning of parturition. Rabbit pregnancy last 28 to 33 days.

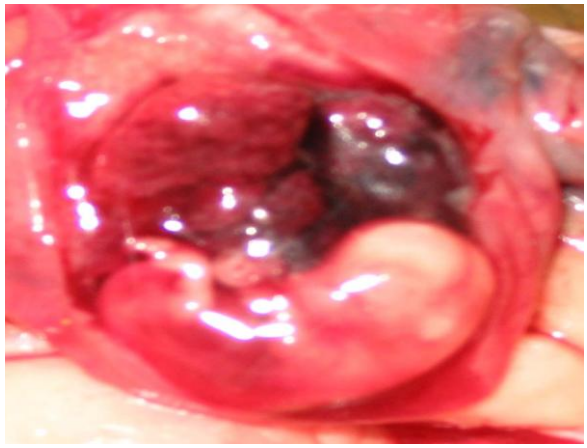
The beginning of parturition is the result of the decrease of progestin of axitocyn. Parturition lasts 30 minutes.

The embryos obtained at 20 days of pregnancy had the weight between 10.8-11.9 grams.

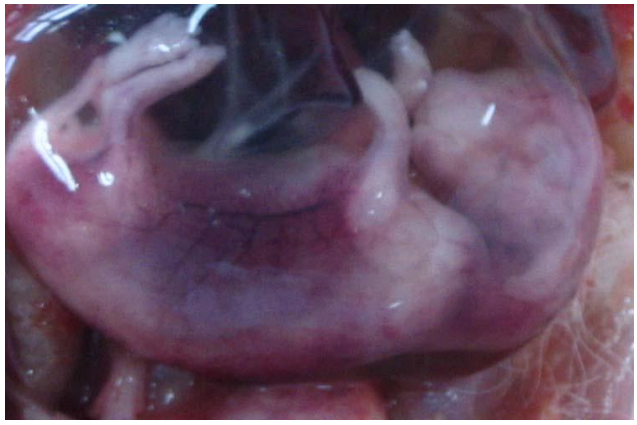
The embryos during it's development begins to gram in length from the 8th day of pregnancy, in the 11th day the body behind.

Beginning with the 19th day, the legs are well formed, the month prolonged this is the period when takes place the passing from embryo to fetus.

Nr fetuses	Weight fetuses	Weight fetuses without placenta	Length fetus
1.	11.9	4.3	4.6
2.	11.5	7.2	3.2
3.	11.7	8.1	4.3
4.	11.8	5.6	3.8
5.	10.8	5.1	3.7



Fetus at day 14



Fetus at day 20

At fetuses that reach the terminus point were made determinations regarding the development state using measurement and weight of the determination. Were determined: the weight of the fetus including the placenta, fetus with fetal cavers and placenta as well as the weight of the fetus alone. The data obtained are registered in the fallowing table.

Nr fetuses	Weight fetuses with placenta	Weight fetuses without placentas	Weight with placenta and fetal cavers (ammies and alaneida)
1.	49.91	45.39	50.51
2.	42.32	38.32	42.93
3.	36.65	33.51	37.28
4.	50.66	46.26	46.86
5.	45.55	41.02	46.16
6.	41.08	37.65	42.32
7.	42.48	38.36	43.17
8.	41.84	38.21	42.55
9.	28.05	24.32	28.80
10.	40.38	36.53	40.78

The uterine tract was extracted, the uterine carn was opened in length and was registered the disposable space for each fetus and that as about 4.36 cm. Data regarding the measures made upon female tract are given in the following table:

Full uterus	230.75g
Empty uterus with ovary and ovarian source	29.55g
Empty uterus with ovarian source but without ovary	29.10g
Empty uterus without ovaries and without ovarian source	27.89g
Empty uterus with ovary	30.92g
Ovary	0.43g
Distance between cervix and last placenta	5 cm
Distance between oviducts and first placenta	3.8 cm

Conclusions

1. After the research was noticed that in the 19th day of pregnancy the fetus is completely developed.
2. The fetal placenta weight overtakes the maternal one in the 20th day of pregnancy.
3. The fetus weight during pregnancy increase very evolving spectacularly between the 24th and the 31st day.

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Particular nuclear forms in the cells of the exocrine pancreas in nutria

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Small pieces of pancreas from two nutria were fixed in Stieve liquid mixture and then were embedded in parafin and processed for clasical histological study. Another pieces were procesed for electron microscopy studies, after a Crăciun&Horobin methodology.

Histological examination revealed a particular structure of the nuclei of acinar cells compared with clasical structure existing in the pancreas of terrestrial mammals. The nuclei have no spherical shape but a great poly-morphism, being oval with irregular outline or having the shape of S, V, and L.

The ultrastructural examination confirmed the histological results. Nuclei have deep longitudinal or transversal incision.

Key words: nutria, exocrine pancreas, morphological investigations, nucleons

Introducere

Nutria este un mamifer semiacvatic căruia îi place apa dar nu umezeala, iubeşte căldura dar suportă şi temperaturile de 8-10 grade (Severin şi col.,1966; Sîrb şi Nestorov, 1979; Bud şi col. 2003). În stare sălbatică trăieşte în Argentina, Uruguai, Paraguai, Brazilia şi Chile. În Europa au fost aduse pentru crescătorii, prima dată în Cehoslovacia în anul 1925, apoi în Germania, Franța, Polonia , Anglia etc. Fiind un mamifer adaptat la mediul acvatic, nutria a stârnit interesul multor cercetători. Studii morfologice recente au fost efectuate pe ficat (Damian, 2002) şi pe pancreasul endocrin (Miclăuş şi col, 2003). Studiul nostru şi-a propus să scoată în evidență eventuale aspecte particulare existente în pancreasul exocrin de nutrie, comparativ cu aspectele structurale clasice existente la mamiferele terestre (Fawcett, 1994).

Material şi metode

De la două nutrii (un mascul şi o femelă) în vârstă de 10 respectiv 12 luni, au fost recoltate fragmente de pancreas sub formă de felii cu grosimea de 4-5 mm, fixate în amestec Stieve şi incluse în parafină. Evidențierea unor particularități la nivelul nucleilor celulelor acinilor pancreatici a impus efectuarea de investigații electronomicroscopice. În acest scop, fragmente fixate la fel ca cele utilizate pentru examenul histologic au fost prelucrate în continuare pentru examen electronomicroscopic utilizând o metodologie pusă la punct de Crăciun şi Horobin (1989).

Rezultate şi discuţii

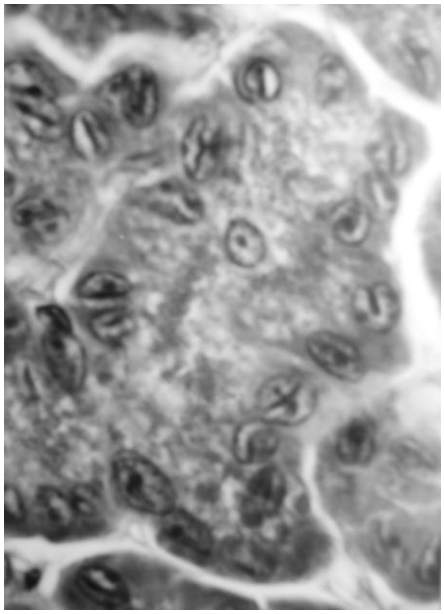
Examenul histologic a scos în evidență faptul că pancreasul la nutrie este foarte asemănător cu al majorității mamiferelor terestre, în ceea ce priveşte histoarhitectonica sa. Astfel, acesta apare format din lobi şi lobuli delimitați de septe conjunctive subțiri, prezintă o componentă exocrină şi una endocrină, cea exocrină fiind predominantă. Componenta exocrină este reprezentată de acini seroşi care, atât ca formă cât şi ca aspect sunt comparabili cu ai majorității mamiferelor.

Examenul de detaliu al nucleilor celulelor acinilor pancreatici a scos, însă, în evidență aspecte particulare, nemaiîntâlnite în pancreasul exocrin al mamiferelor terestre. Astfel, un număr relativ mare dintre nucleii celulelor acinilor pancreatici de nutrie nu au forma sferică întâlnită la alte

specii, ci prezintă un polimorfism foarte pronunţat (Fig 1-3). Aceştia au forme curioase ce variază de la ovalari, încurbați, sub formă de bastonaş, până la forma literelor S, V, L etc. Mulți dintre ei, dar mai ales cei ovalari, au aspectul bobului de fasole, apărând împărțiți aproape simetric de o membrană ce străbate nucleul de la un pol până în apropierea celuilalt pol. În cazul altora se observă două sau chiar mai multe membrane ce pătrund mai mult sau mai puțin adânc în nucleu. Aceste membrane determină o compartimentare a nucleilor respectivi, cu apariția aspectului de nuclei lobulați. Este un aspect pe care noi nu l-am mai întâlnit până acum.

Examinările electronomicroscopice au confirmat rezultatele examenului histologic (Fig. 4), evidențiind faptul că nucleii ovalari alungiți prezintă, fie câte o incizură longitudinală adâncă care aproape că îi împarte în doi lobi, fie aceştia prezintă mai multe incizuri transversale, care aproape că traversează nucleii, legătura dintre diferitele părți ale acestora făcându-se prin porțiuni înguste. Caracteristic este faptul că aceste tipuri de invaginări sunt foarte înguste, lăsând spații foarte mici între ele. Alți nuclei prezintă câte o invaginare mai largă dând nucleului un aspect încurbat, de corn sau au conturul foarte ondulat. Prin aceste incizuri suprafața de contur a nucleilor şi, implicit, suprafața de contact nucleu-citoplasmă creşte foarte mult.

Aspectul particular al nucleilor celulelor acinilor pancreatici de nutrie este greu de înțeles cel puțin pe baza unei investigații morfologice. Din acest punct de vedere, celulele în formă de trunchi de piramidă ale acinilor pancreatici ar trebui să aibă formă sferică, aşa cum se prezintă la mamiferele terestre. Nici o altă formă nu ar avantaja celulele în exercitarea funcției lor secretorii. Apariția de nuclei atât de diferiți ca formă, la celule la care aceştia în mod normal sunt sferici, trebuie privită deocamdată cu mult interes. Numărul mic de animale luate în studiu nu ne permite să apreciem dacă aceste aspecte apar doar la unii indivizi sau sunt o caracteristică de specie. Mai mult, pentru elucidarea semnificației funcționale a lor sunt necesare şi altfel de investigații, nu numai morfologice. Este cert, însă, că aceste forme bizare cresc suprafața de contact nucleu-citoplasmă ceea ce, ar putea oarecum sugera o creştere a proceselor de schimb dintre nucleu şi citoplasmă. Este desigur doar o sugestie, astfel de procese nu pot fi verificate prin investigații morfologice.



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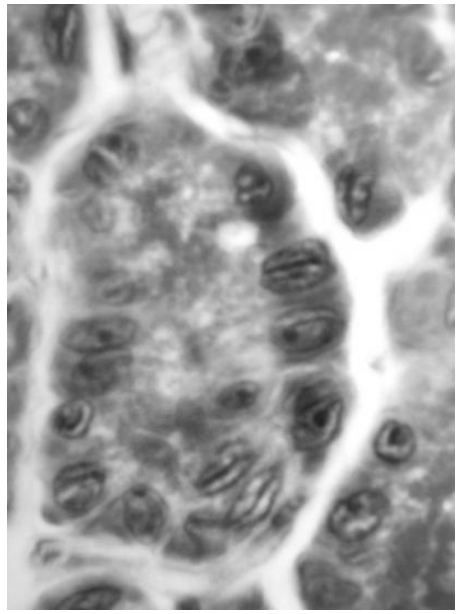

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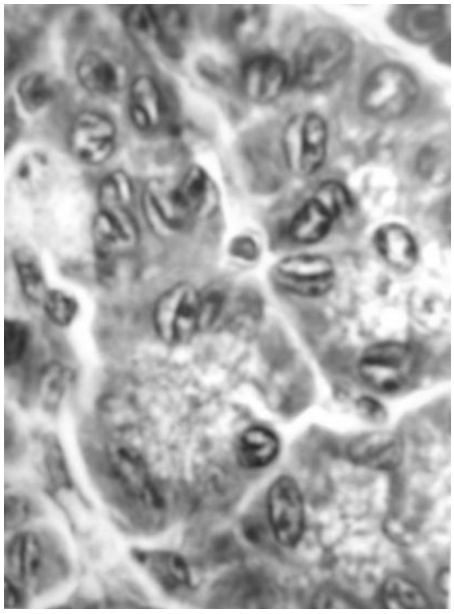

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Fig. 1-3 Ob. 63

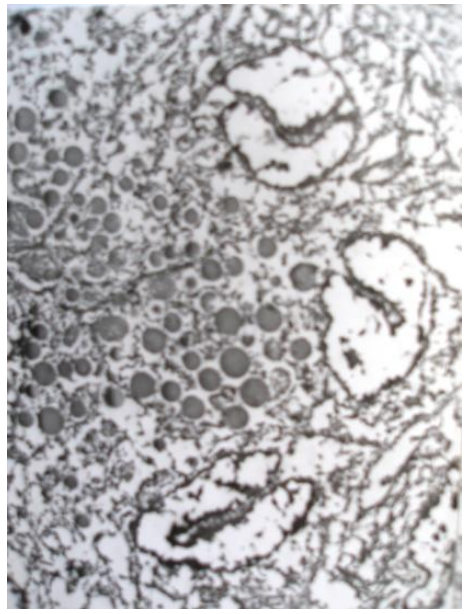

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Fig. 4 4800X

Concluzii

La cazurile investigate de noi, un număr relativ mare dintre celulele acinilor pancreatici prezintă nuclei de altă formă decât cea sferică întâlnită la celelalte specii de mamifere.

Aceşti nuclei prezintă incizuri de diferite adâncimi care au tendință de compartimentare a nucleului sub formă de lobuli nucleari mai mult sau mai puțin net conturați.

Pentru a stabili dacă aceste forme nucleare particulare sunt caracteristice pancreasului de nutrie sau apar doar la unii indivizi, este necesar să se facă investigații pe un număr mai mare de animale.

Această compartimentare nucleară creşte suprafața de schimb dintre nucleu şi citoplasmă dar semnificația funcțională a acestui aspect nu poate fi elucidată prin investigații morfologice.

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A study of the red blood cell aggregation for bovine blood

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Red blood cell aggregation is an important component of whole blood viscosity and is the major cause of non-Newtonian flow properties of blood. Aggregation of red cell has been shown to play a role in the viscosity at low shear rates, and deformability at high shear rates. Comparative animal studies showed the wide variation of whole blood and erythrocyte aggregation among different mammalian species. Horse RBCs aggregation was registered by many authors but for cow and sheep the aggregation is not reported in normal conditions. In this work the aggregation of erythrocytes in bovine blood is studied using fractal analysis. Measuring the fractal dimension for the aggregated red blood cells we can obtain a method to provide the disease of the animal.

Key Words: RBC aggregability, cow blood, fractal analysis

INTRODUCTION

Anthony van Leeuwenhoek presented the phenomena of aggregation and disaggregation of red blood cells in a mail addressed to Royal Society in September 25, 1699. J. De Haan related the aggregation of horse red blood cells in 1918 and he showed that aggregation for cow and sheep is practically absent. Red blood cell aggregation is an important component of whole blood viscosity and is the major cause of non-Newtonian flow properties of blood.

Viscometric measurements demonstrated that apparent viscosity of blood rises with decreasing shear rates. Aggregation of red cell has been shown to play a role in the viscosity at low shear rates, and deformability at high shear rates. At low shear rates, the cell layers are composed of aggregated cell, but at higher shear rates, the aggregates degrade to form thinner layers of oriented cells.

The axially migration of red blood cell and the plasma sleeving represents a phase separation, and this process a self-organisation may be considered, and may be treated as a problem of non-linear dynamic and chaos. Although considerable data are now available regarding the physiological and clinical import of this phenomenon, the specific mechanism involved in RBC aggregation have yet to be elucidated.

At present there are two co-existing “models” for RBC aggregation [1]:

1) Bridging Model, described by Baskurt et al., which hypothesises that the aggregation occurs when binding forces due to the adsorption of macromolecules onto adjacent cell surfaces exceed disaggregation forces due to the electrostatic repulsion, membrane strain and mechanical shearing

2) Depletion Model which proposes a preferential exclusion of macromolecules from RBC surface, thereby generating on osmotic gradient, a flow of fluid away from the intercellular gap, and a movement of adjacent cells.

Understanding the exact nature of the relationship between RBC aggregation and in vivo blood flow resistance is important, both from clinical and physiological point of views. Development of methods to modify RBC aggregability may start a new clinical approach to circulatory disorders; however clinicians would need to know the details of RBC aggregation-tissue perfusion relationship.

Comparative animal studies showed the wide variation of whole blood and erythrocyte aggregation among different mammalian species. Therefore, the Popel et al. data [4] showed that athletic species exhibit a consistently higher degree of red blood cell aggregation than their sedentary counterparts. Horse RBCs aggregation was registered by many authors but for cow and sheep the aggregation is not reported [1], [6].

Traditional mechanical and mathematical methods are both proved to be insufficient in describing the aggregation process. Over the last years, fractal geometry has been applied with great success to many different physical, chemical and biological systems. The concepts derived from fractal and chaos theory are fundamental to the description and modelling of phenomena in biology, from the molecular to ecosystem levels of organization (BENOIT MANDELBROT, *The Fractal Geometry of Nature.* New York, 1975). Fractal geometry is geometry of nature; it deals with irregular, complex but selfsimilar structures or natural phenomena. Many physiological systems have been found to be both spatial and temporal fractals.

In [2] Men-Zhen Kang and co-workers found that RBC aggregation shows fractal characteristics by analysing the aggregation images. Their research presents the time dependence of Information Dimension for RBC for human blood samples. A CCA model was used to reveal the relationship between the fractal dimension and the binding energy.

We also used the fractal analysis to study the properties of RBC for human and animal blood [3], [5]. In this paper the aggregation of erythrocytes in bovine blood is studied using fractal analysis.

MATERIALS AND METHOD

Samples from peripheral bovine blood were operated using May-Grüwald Giemsa colorature. With the aid of the microscope we obtained the photos. Using erythrocyte planar images, fractal dimension of the aggregates was computed by means of HarFA soft, after the purification of the cells.

In HarFA is used a modification of traditional Box Counting Method. By this modification on obtain three fractal dimensions, which characterize properties of black plane DB, black-white border of black object DBW (and this information is the most interesting) and properties of white background DW. The fractal dimension is the slope of the straight line „Black&White".

RESULTS AND DISCUSSION

Fig.1 shows the morphology of RBCs under normal conditions for cow blood when the RBCs are not aggregated and Fig. 2 shows aggregated erthrocytes.

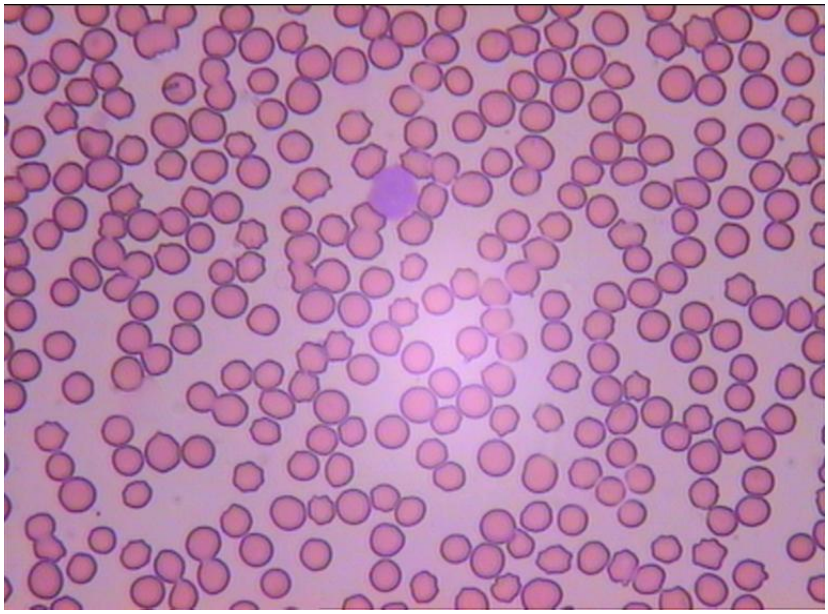


Fig.1 Erythrocytes from cow blood

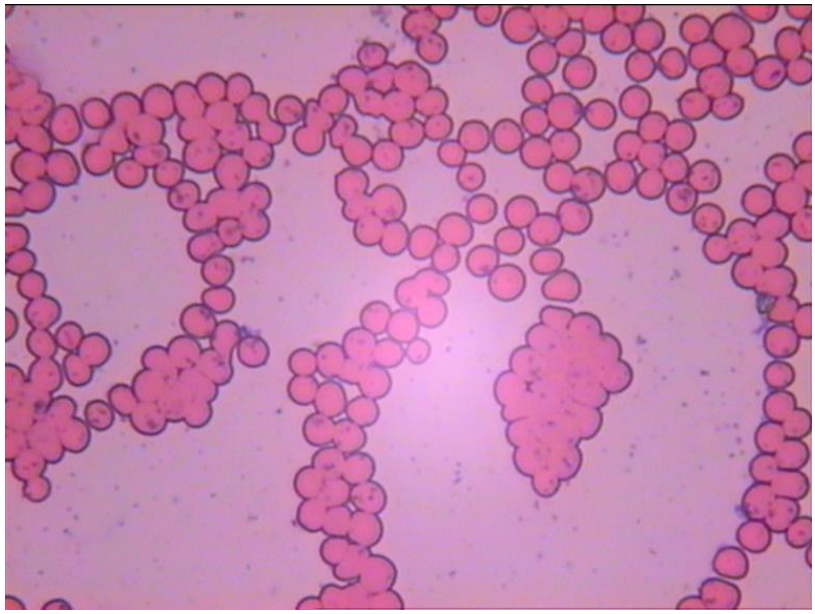
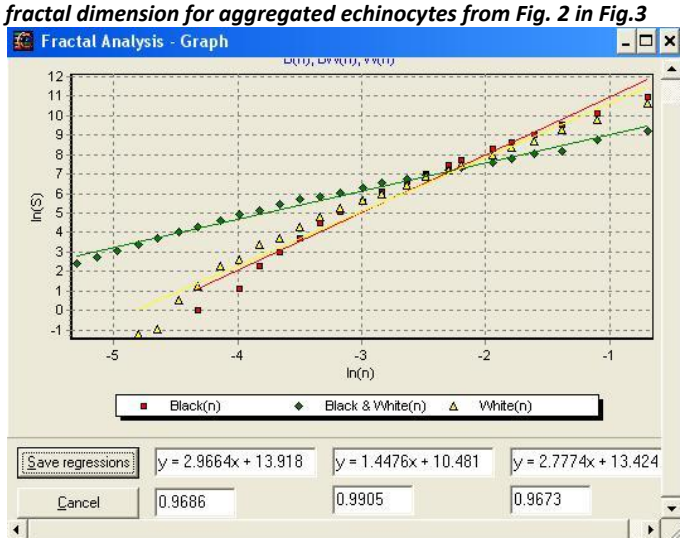


Fig.2 Aggregated erythrocytes from cow blood

The fractal dimension for aggregated echinocytes from Fig. 2 in Fig.3



For fractal dimension of the health bovine blood we obtained a value of **1.7** and for aggregated red blood cells, this means in the case of a disease, we obtained a fractal dimension of **1.45.**

CONCLUSION

We can suppose that if we develop an easy method to measure the image's fractal dimension, fractal analysis may be a good assessment of the aggregation. Measuring the fractal dimension for the aggregated red blood cells we can obtain a method to find the stage of the disease.

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Some results of the effects of mercury on animal blood

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Mercury exists in three forms: elemental mercury, inorganic mercury compounds (primarily mercuric chloride), and organic mercury compounds (primarily methyl mercury). All forms of mercury are quite toxic, and each form exhibits different health effects. The concentration of mercury in blood reflects exposure to organic mercury as well as metallic and inorganic mercury. Mercury toxic subjects exhibited an increased occurrence of rapid heart beat, irregular pulse chest pains, heart palpitations, and high blood pressure. In this work we studied effects of different heavy metals on the blood spectrum and we showed that mercury modified this spectrum; this means a small quantity of mercury can be identified from the blood sample spectrophotometric analysis.

Key Words: methyl mercury, blood spectrum, spectrophotometric analysis

INTRODUCTION

Mercury, the only metal that is liquid at room temperature has been used in different products as batteries, thermometers and so on. Although quite useful, mercury is poisonous and can contaminate the environment. There are different forms of mercury: elemental mercury, organic mercury (methyl mercury) which is very easy through the digestible tract) and inorganic mercury (mercury salts). Any type of mercury is toxic but organic mercury is most dangerous than the other forms of mercury. People can be infected with mercury by eating food (especially fish). Most of the information related to mercury poisoning was obtained from accidental exposures in the past (Minamata disease in Japan and the accidental event from Iraq in 1972).

Researchers have found that mercury affects several aspects of cardiac function, including the ability of heart muscle to contract, the electrical conduction activity in the heart, and the function of regulators of cardiac activity. Mercury toxic subjects exhibited an increased occurrence of rapid heart beat, irregular pulse, chest pains, heart palpitations, and high blood pressure [3]. By contrary, Rossoni et al. [7] showed that an acute intravenous administration of Hg^{2+} produces important hemodynamic changes but the main effect is a progressive decrease of arterial blood pressure associated with a reduction of heart rate. Mercury has a high affinity for and readily binds to the thiol or *sulfhydryl* (sulfur/hydrogen combination) sites in living tissues. There are several thiol sites in the hemoglobin molecule in the red blood cells used to transport oxygen throughout the body. When mercury accumulates in red blood cells in humans and other animals then mercury attaches to the thiol sites and the hemoglobin can't carry as much oxygen as it could. This results in decreased availability of oxygen (*hypoxia*) that is needed by all body cells and explains one way that mercury toxicity can cause chronic fatigue symptoms. The effect on heart function depends on the number of sites blocked by mercury. Another important influence of mercury on the heart function is its adverse affect on the ability of the heart muscle to contract. This is because of the ability of mercury to attach to thiol proteins, this time in the heart muscle itself. The function of muscle tissue depends on the interaction between *actin* and *myosin* and their combination to form *actomyosin*, resulting in tissue contraction. The connection of these two proteins occurs at thiol sites in the myosin molecule. If mercury attaches to those thiol sites, the muscle tissue will not be able to function. Mercury blocks the enzyme in the cell membrane that actively passes calcium in and out of the muscle cells by attaching to the thiol part of the

enzyme. Calcium is necessary for the proper function of heart muscle. Hypertension is caused by mercury preventing the passage of calcium into the heart muscle cells, thereby increasing the force of contraction.

The half-life of methylmercury in blood is relatively long (approximately 44 days) and the concentrations in newly formed hair are about 250 times higher than in blood. Once concentrated in hair, the level of methylmercury remains unchanged; measurements in consecutive hair segments are thus useful indicators of past exposure (depending on the length of the hair). It is not useful to measure urine levels because methylmercury is not excreted by the kidneys. The mercury concentration in the blood is also a good biomarker of the mercury concentration in the brain [2]. Oliveira Ribeiro and coworkers [5] evaluated and compared physiological and haematological responses of *Hoplias malabaricus* exposed to heavy metals. The authors evaluate haematological effects of metals on erythrocytes, total leukocytes and differential leukocytes counts, hematocrit, haemoglobin concentration, and red blood cell indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Results showed differences in erythrocytes, haemoglobin, hematocrit, MCV, and white blood cells counts. The number of leukocytes was increased in the presence of methylmercury, suggesting effects on the immune system. Also the MCV increased in individuals exposed to methylmercury. Christine M.Y. Choy and coworkers [1] compared blood mercury concentrations of infertile couples with those of fertile couples in Hong Kong, and examined the relationship between blood mercury concentrations and seafood consumption. Mercury concentrations in whole blood were measured by cold vapour atomic absorption spectrophotometry. As a conclusion the authors indicated that higher blood mercury concentration is associated with male and female infertility and higher seafood consumption is associated with elevated blood mercury concentrations in infertile population. We also studied the effects of different cations on the blood spectrum using molecular absorbtion spectrophotometry [6]. In this work we studied effects of different heavy metals on the blood spectrum and we showed that mercury modified this spectrum.

MATERIALS AND METHOD

To see the effects of mercury on animal blood, the following operations were performed: Blood sample from dog was drawn from the jugular vein of healthy animal located at the Faculty of Veterinary Medicine from Iasi. This blood sample was collected in test tube with EDTA as anticoagulant. Then we introduced 2 μL of blood in distilled water and 6mL solutions of 5% concentration from different salts: $HgCl_2$, $Zn(SO_4)_2$, $TlNO_3$. The spectrophotometric measurements were performed with SPECORD 200 from Analytik Jena, immediately after the solutions were prepared.

RESULTS AND DISCUSSION

In the Fig.1and Fig.2 are presented the comparative spectra for blood solutions which contain distilled water and different salts.

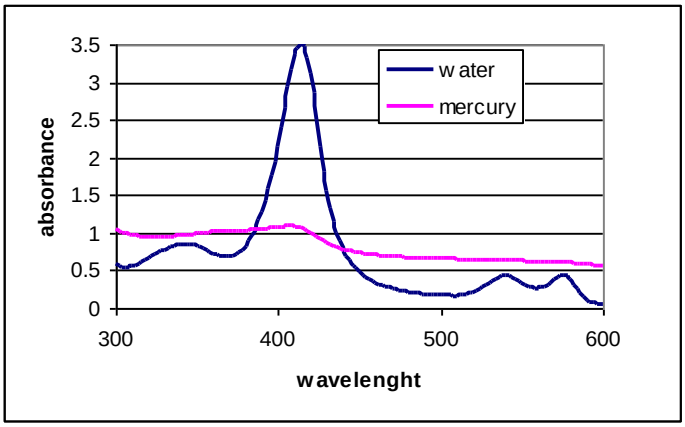

Fig.1 Comparative spectra from solutions of blood in distilled water and mercuric chloride

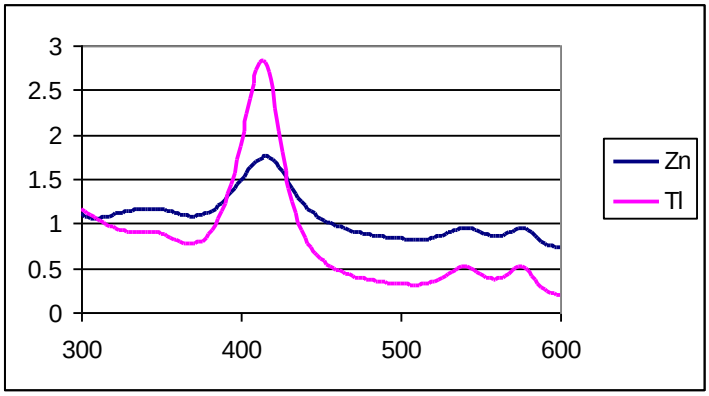

Fig.2 Comparative blood spectra from solutions that contain Zn and Tl

From the spectrophotometric analysis of the haemoglobin sperctrum and Fig.1 we can see that mercury solution influences in a different way the blood spectrum. Mercury affects the absorbtion spectrum very much; this means the absorbtion maximum that characterize the haemoglobin spectrum disappears. By contrary, the other elements from the same group, Zn and Tl doesn't change the absorbtion spectra in what the maximum of the spectra positions are concerned, it only decrease the absorbance. The absorbance is lower for solutions that contain Zn than from Tl solutions. This means the effects of these cations are negligible by comparison with mercury effects.

CONCLUSION

In this work we studied effects of different heavy metals on the blood spectrum. Our measurements showed that mercury modified this spectrum, this means mercury concentrates in erythrocytes, affects haemoglobin spectrum and possible structure of haemoglobin. The other metals, Zn and Tl don't change the absorbtion spectra and the decrease of the absorbtion is higher for Zn than for Tl. The same strong effect of mercury and small effect of Zn and Tl we obtained in the case of plant treatment with these heavy metals [4]. We can suppose that molecular spectrophotometric analysis may be a good method to identify a small quantity of mercury from a blood sample.

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Free radicals scavenging effect and antioxidant activity of *Capsicum annuum* alcoholic extract

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The ethanolic extract of chilly pepper (Capsicum annuum) was found to contain compounds with antioxidant activity. The extract was studied for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion scavenging activity. Superoxide anions were generated by the phenazine methosulphate (PMN)/NADPH system. The extract was also studied for lipid peroxidation assay by thiobarbituric acid-reactive substances (TBARS) method using rat brain homogenate. The results indicated that Capsicum annuum extract scavenging free radicals DPPH but not scavenging superoxide anion. Capsicum annuum extract has antioxidant activity on rat brain homogenate.

Key Words: chilly pepper (Capsicum annuum), antioxidants

Capsicum annuum is an herbaceous annual plant that reaches a height of one meter and has glabrous or pubescent lanceolate leaves, white flowers, and fruits that vary in length, color, and pungency. This plant is cultivated almost exclusively in Europe and Unites States. The level of pungency of the *Capsicum annuum* depends upon the concentration of capsaicinoids, primarily of capsaicin, in the fruit. The chemical composition of the *Capsicum annuum* includes pungent principles, poyphenols, carotenoids, micronutrients such as vitamins C and E. The extracts of *Capsicum* species have been reported to have antioxidant properties (3, 4).

As a medicinal plant, the *Capsicum annuum* has been used as a folk remedy for diarrhea, arthritis, muscle cramps, etc. High levels of hot pepper have induced stomach ulcers and cirrhosis of the liver in laboratory animals.

The present study aims to assess the antioxidant capacity of alcoholic extract of *Capsicum annuum.* Plant extract was tested for different free radical scavenging activity and for capacity to reduce lipid peroxidation in rat brain homogenate.

MATERIALS AND METHODS

Plant Material. The air-dried *Capsicum annuum* was powdered using a mortar and pestle. 10 g powdered was extracted with 100 ml ethanol.

Brain homogenate. Male rat (160 g) was sacrificed by cervical dislocation, and then the entire brain was processed to get 25% homogenate in cold phosphate buffer saline pH 7.4 using glass teflon homogenizer.

DPPH radical scavenging assay. Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals of plant extract was measured according to the method reported by Govindarajan and col (2). 500 µl DPPH 6mM and 500 µl of test extract was added at different dilutions (1:5, 1:4, 1:3, 1:2 and 1:0, alcoholic extract:alcohol (v:v)). Equal amount of ethanol was added to the control. Absorbance was recorded at 512 nm. The inhibitory percentage of DPPH was calculated according to the following equation:

%Inhibition = [(Absorbance control – Absorbance sample)/Absorbance control] x 100

Superoxide scavenging assay. Superoxide anions were generated by the phenazine methosulphate (PMN)/NADPH system according to the described procedure. The reaction mixture

consisted 2 ml PBS 50 mM, pH 7,4, 50 µl Na_2EDTA 0,1mM, 100 µl Nitro blue tetrazolium salt (NBT) 1,5 mM, 50 µl PMS 1mM, 200 µl NADPH 0,5 mM and 50 µl extract. Extract was added at different dilutions (1:5, 1:4, 1:3, 1:2 and 1:0, alcoholic extract: alcohol (v : v)) (5). Equal amount of ethanol was added to the control. Absorbance was recorded at 407 nm. The reaction was conducted at room temperature for 2 min and initiated by the addition of PMS. The ability of extract to scavenged anions superoxide was calculated using the following equation:

%Inhibition = [(Absorbance control – Absorbance sample)/Absorbance control] x 100

Inhibition of lipid peroxidation. The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances (TBARS) by using the standard method. (1) The experiment was conduced in PBS 50 mM, pH 7.4. 200 µl brain homogenate and 500 µl PBS were mixed with 100 µl $FeCl_3$ (1mM), 100 µl alcoholic extract and 100 µl ascorbate (1 mM). Mixture was incubated at 37^0C for 60 min. Lipid peroxidation was initiated by adding 100 µl $FeCl_3$ solution to 200 µl brain homogenate. At the end of this incubation period, 50 µl of 2% butylated hydroxytoluen (BHT) was added followed by 1 ml of 2.8 % (w/v) trichloroacetic acid and 1 ml of 1% (w/v) thiobarbituric acid (TBA). The solutions were heated in a bath at 80^0C for 20 min to develop the malondialdehyde thiobarbituric adduct $(TBA)_2$ – MDA. The $(TBA)_2$ – MDA chromogen was extracted into 2 ml butan - 1-ol and the extend of peroxidation was measured in organic layer at 532 nm. Extract was added at different dilutions (1:5, 1:4, 1:3, 1:2 and 1:0, alcoholic extract: alcohol (v : v)). Equal amount of ethanol was added to the control. Inhibition of lipid peroxidation was calculated using the following equation:

%Inhibition = [(Absorbance control – Absorbance sample)/Absorbance control] x 100

RESULTS AND DISCUSSIONS

DPPH radical scavenging. As the data shown in figure 1, the inhibitory effect of alcoholic extract of *Capsicum annuum* on free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was found to be strong and dose dependent. Crude alcoholic extract strong scavenged DPPH radicals, inhibitory effect was 87.5 ± 0.02% and for the first dilution 42.0 ± 0.01%.

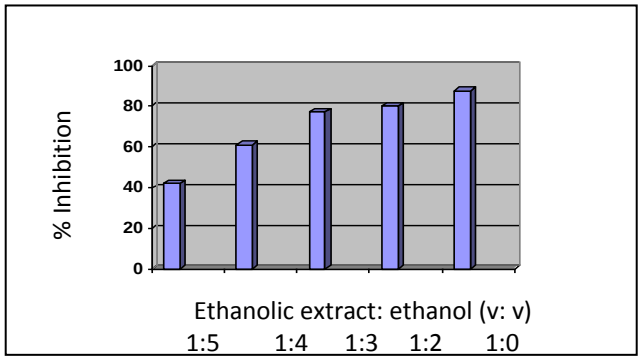

Figure 1. Scavenging of DPPH radicals by Capsicum annuum ethanolic extract

Superoxide scavenging assay.

The obtained results have shown that for the dilutions we have used, the alcoholic extract of *Capsicum annuum* does not annihilate the superoxide anion. Surprisingly, for all dilutions, the absorbance of the probes has been higher than that of the control (fig. 2). We suggest that *Capsicum annuum* release superoxide anions in the presence of system FMN/NADPH/NBT.

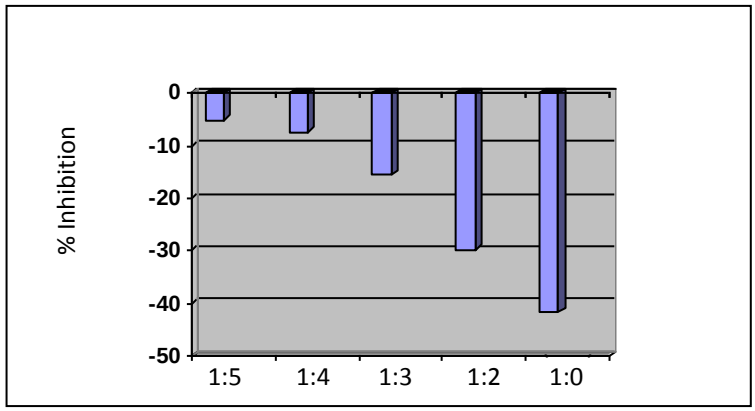

Figure 2. Effect of the ethanolic extract of Capsicum annuum on superoxide anions

Inhibition of lipid peroxidation. The ethanolic extract of *Capsicum annuum* exhibits a high degree of inhibition on rat brain lipid peroxidation. From the data (fig. 3) it was found that *Capsicum annuum* gave protection against lipid peroxidation. The protection degree depended on the concentration of the alcoholic extract. Thus, for the undiluted extract, the protection was 63.02 ± 0.01%, while for the weakest dilution – 9.3 ± 0.01%.

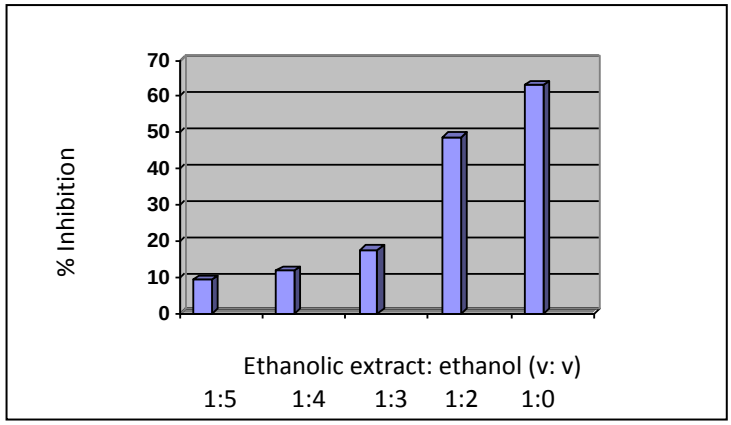

Figure 3. Effect of ethanolic extract of Capsicum annuum on inhibition of lipid peroxidation using rat brain homogenate

CONCLUSIONS

1. Ethanolic extract of *Capsicum annuum* significantly reduced the malondialdehyde content, which is a measure of lipid peroxidation.
2. Free radicals of 1.1-diphenyl-2-picrylhydrazyl (DPPH) are scavenged by ethanolic extract of *Capsicum annuum.*
3. Free radicals superoxide anions are not scavenged by ethanolic extract of *Capsicum annuum.*

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Triturus v. vittatus (Urodela) Larvae at Various Breeding Sites in Israel

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Triturus v. vittatus larvae were studied during four years at various breeding sites in northern Israel. Altitudes of the localities ranged from 212 to 740 m above sea level. The larval growth period was found to be the longest, between April and July in Dovev Pond, which is located at the highest elevation investigated (740 m above sea level). The period of larval growth and water quality varied among the various sites and differed from year to year during the study. All breeding sites contained newt larvae, which shared the site with various anuran larvae, Hyla savignyi, Bufo viridis, Rana bedriagae and Pelobates syriacus. Salamandra infraimmaculata larvae and adult newts inhabited two rain pools simultaneously, but there was very little overlap of the larval growth periods. Temperatures in the ponds increased significantly from winter to spring and ranged between 5-30ºC, with no significant differences among the ponds. In regard to all other water parameters measured, significant differences were observed among the ponds during certain periods ($p<0.05$). The pH varied from 6.5-10, and dissolved oxygen ranged between 2-27 mg/L, generally between 5-10 mg/L. Electrical conductivity (EC) varied between 150–800 μs and increased slightly over the seasons. Ammonia (NH_4) and nitrite (NO_2) concentrations were low, and were, in general, less than 1 and 0.25 mg/L, respectively. Turbidity remained relatively constant, varying between 0-150 NTU (nephelometric turbidity units).

In conclusion, large variations among the different ponds were observed with respect to the length of the newt larval growth period and the time required for the completion of metamorphosis,. However, the water parameters of these ponds, which were taken at different time periods and from ponds of various altitudes, were all in the same range.

Key Words: amphibia, Triturus vittatus, water quality, larval growth period

INTRODUCTION

The life cycle of most amphibians, and all six amphibian species located in Israel (Degani, 1982; 1986), require water bodies that are surrounded by adequate terrestrial habitat, in order to support both phases of their life. The aquatic phase is critical for the amphibian larvae to complete metamorphosis.

The six species of amphibians that exist in northern Israel are: the striped newt, *Triturus vittatus vittatus*; the fire salamander, *Salamandra infraimmaculata* (Amphibia, Urodela, Salamandridae); and four Anuran species: the tree frog, *Hyla savignyi*; the green toad, *Bufo viridis*; the water frog, *Rana bedriagae;* and the spadefoot, *Pelobates syriacus* (Degani, 1982; 1986). Israel, which offers mainly xeric habitats, unusual for amphibians, represents the southeastern limit of distribution of these species (Steward, 1969; Degani and Mendelssohn, 1983). Hence, amphibian larvae occupy a very narrow and specific ecological niche in the region, and are under severe pressure from

predators and other biotic and abiotic factors (Degani and Mendelssohn, 1983; Degani, 1986; 1996). Amphibian larvae have been found and studied in a variety of ephemeral pools, ponds and streams of hilly woodlands in northern Israel, as well as on the coastal plain, during winter, spring and summer. The biotic and abiotic factors of these water bodies appear to be the limiting factors in the distribution of the larvae.

Little is known about the ecological conditions of the natural habitats of the *Triturus vittatus.* Some aspects of the subspecies, *T. v. vittatus,* located in Israel, and of its biology and life cycle in Europe and the Mediterranean region, have been described earlier by Raxworthy (1989) and Olgon et al. (1997). Three subspecies of the banded newt, *Triturus vittatus,* are currently recognized. The *T. v. vittatus* form is distributed along the eastern edge of the Mediterranean Sea, ranging from Turkey in the north, to Israel, where it reaches its southern limit; the *T. v. cilicensis* form is found on the eastern and northeastern borders of the Mediterranean Sea; and *that of T. v. ophryticus* is located in the Caucasus, east and south of the Black Sea (Fig. 1).

In Israel, *T. v. vittatus* habitats range from the north to the central coastal plains, where habitat conditions are most extreme. The biology and life cycle of *T. v. vittatus* populations in northern Israel and the Upper Galilee have been previously described (Degani and Mendelssohn, 1983; Degani, 1986). Larvae of *T. v. vittatus*, throughout their aquatic phase, as well as adults, inhabit mainly winter pools that contain water until the beginning of the summer or sometimes during the whole year (Degani and Kaplan, 1999). The terrestrial adult newts reach the area of the pond with the beginning of the rainy season, before the ponds fill up with water, and subsequently, enter the ponds in their aquatic phase, after they are full. In the Upper Galilee, males inhabit the ponds from January to March, leaving it after mating. Females may remain in the water until May, during which they deposit between 18-68 eggs on plant or rock surfaces until they move onto their terrestrial stage (Degani and Mendelssohn, 1983). Afterwards, the larvae hatch, 19-29 days later, depending upon water temperatures. The period of activity, population parameters and food habits of mature *T. v. vittatus* in central Israel have been studied by Geffen et al. (1987), who discovered that during November–December, adult newts appear on land and enter the pond after it has filled up, remaining in the water until late February.

The Upper Galilee landscape is characterized by varying altitudes and Mediterranean forest cover, as well as exposed areas. The influence of landscape characteristics on amphibian distribution has been described and shown to have an effect on the larval amphibian assemblages (primarily the forest cover and wetland hydroperiod) (Herrmann et al., 2005; Mazerolle et al., 2005). The Upper Galilee area has been for the last 50 years, and still is, under intensive cultivation and urban use, and breeding sites of *T. v. vittatus* may possibly be affected by intensive agriculture and hydroperiod (Beja and Alcazar, 2003). The aim of the present study was to examine the ecological and biological conditions and variables of different breeding sites, located at various altitudes in northern Israel, where larvae of *T. v. vittatus* grow and reach metamorphosis.

MATERIALS and METHODS

Five breeding sites from different locations were selected. The areas of the ponds were examined from the beginning of October, based on a previous study (Degani and Mendelssohn, 1983), which reported that the terrestrial newt migrate to the area of the pond a few weeks before the rainy season.

The breeding periods of *T. v. vittatus* in winter rain pools, ponds and rock pools, in northern Israel (Fig. 1) were studied during four years (2001-2005). The elevations of these habitats ranged from 212 to 740 m above sea level (asl), representing extremes of ecological and physical conditions, such as differences in temperature and hydroperiod. At the onset of the rainy season,

when natural pools had filled up for the first time, and until the pools had dried out, migration periods of mature newts were monitored and water parameters measured every two weeks. In situ measurements of temperature, pH, dissolved oxygen and electrical conductivity (EC) were carried out, using a handheld pH meter equipped with a digital thermometer (WTW, pH315i, Germany), a handheld oxygen meter (WTW, Oxi330 set, Germany) and a handheld EC meter (WTW, Multiline P4, Germany), respectively. Water samples (0.5 litres) were collected from each breeding site and taken to the laboratory for further analysis of ammonia ($NH_{4)}$, nitrite (NO_2) and turbidity, using the Ammonium and Nitrite Cell Test (Merck 1.14739 and 1.14547, Germany) and the Spectroquant Photometer, NOVA 60 (Merck, Germany). Turbidity was determined with the Turbidimeter (HACH - Loveland, Colorado, USA).

Larvae of all amphibian species were collected throughout the season by a hand net (Degani and Mendelssohn, 1983), identified according to species and grouped by the water body vicinity, in which they were collected.

Differences in larval size, and in the time periods that larvae had spent in the pond until they had reached metamorphosis were calculated by linear regression: y = ax+b. Regressions were conducted on the mean size of a given pond, and differences of the growth trajectories were measured by comparing the slopes of the growth curves among sites. Water parameters of the various ponds, were analysed by two-way analysis of variance (ANOVA), followed by the Bonferroni post-test, with the year and breeding site, treated as independent variables, using the Graph-Pad Prism software (Graph Pad, San Diego, CA), with the level of significance between different groups set at $p<0.05$. The correlation of abiotic parameters was determined using Spearman's correlation coefficient based upon ranked seasonal medians (Hays, 1981), using the Graph-Pad Prism software, with the level of significance in the different groups set at $p<0.05$.

RESULTS

The mature newts arrived at the area of the pond, at a short time before the beginning of the rains, entering the breeding sites at the beginning of the winter, when the ponds were filling up with water, and subsequently staying in the ponds for several months. The larval growth of the *T. v. vittatus* is presented in figure 2. At the beginning, following hatching, the larvae were very small, and it was difficult to see and collect them from the ponds with the sampling methods used. The larval growth periods differed among the various ponds and over the years of the study. In all ponds, the larval growth periods were between 1.5 - 3 months, with the longest period of growth observed in larvae of Dovev Pond, which is at the highest elevation studied (Fig. 1). The calculations for larval growth were: in Dovev Pond $y=0.2936X+12.4202$, $r^2=0.9423$, $p=0.001$, in Matityahu Q. Pond $y=0.1439X+28.2460$, $r^2=0.9788$, $p=0.01$, in Pharaa Pond $y=0.3841X+8.6932$, $r^2=0.9978$, $p=0.29$ and in Nahalit Pond $y=0.6086X+13.2184$, $r^2=0.9547$, $p=0.02$ (Fig. 2). At all five breeding sites examined in the present study, where newt larvae grew and completed their metamorphosis, other amphibian larvae were found. It was discovered that the newt larvae were present in the breeding sites together with anuran larvae, *H. savignyi*, *B. viridis*, *R. bedriagae* and *P. syriacus,* but in most cases they appeared in the ponds only after the salamander larvae (*S. infraimmaculata*) had completed metamorphosis, and vacated the pools. *H. savignyi* and *B. viridis* tadpoles were observed in all breeding sites where newt larvae grew.

The size of the ponds varied from 78.5 m^2 to 1017 m^2 and the depth from 0-2.5 m. The temperatures in the various ponds increased significantly, from the winter to the spring, and ranged between 5 to 30 ºC (Figs. 3-6). During the four years of the study, no significant differences ($p>0.05$) among the various ponds were detected during the periods that newt larvae occupied the ponds, with the exception of the water temperatures in Dovev Pond, which were lower at the beginning of the growth periods, during the years 2001-2002 and 2003-2004.

Dissolved oxygen concentrations ranged between 2-27 mg/L in the different ponds, but most of the time they were constant, ranging between 5-10 mg/L, with significant differences observed only between Pharaa and Nahalit during the winter of 2001-2002 ($p<0.01$). High oxygen concentrations were detected during the larval growth period and during the completion of metamorphosis (Figs. 3-6).

The pH varied between 6.5–10, but throughout most of the time, values were lower and ranged between 7-9 at all breeding sites during the four years (Figs. 3-6), with significant differences between Amiad and Nahalit ponds in the breeding season of 2002-2003 ($p<0.05$) and between Pharaa and Nahalit, Matityahu Q. and Nahalit, and Nahalit and Dovev ($p<0.05$), as well as between Pharaa and Amiad, Matityahu Q. and Amiad, and Dovev and Amiad ($p<0.01$), in the breeding season of 2004-2005. The pH tended to vary during the first year of the study (2001-2002), mostly decreasing. This is in contrast to the other years, during which it increased. The relative pH levels tended to change in the various ponds during the larval growth periods (Figs. 3-6).

The electrical conductivity (EC) in the pools at the various breeding sites varied between 150–800 μs and increased slightly over the seasons (Figs. 3-6). During some periods, in 2001-2002, 2003-2004 and 2004-2005, the ECs between various ponds were significantly different (between Pharaa and Matityahu Q., and Pharaa and Nahalit [$p<0.05$], Nahalit and Amiad [$p<0.01$], Matityahu Q. and Nahalit, and Nahalit and Dovev [$p<0.001$] in 2001-2002; between Nahalit and Amiad [$p<0.01$], Matityahu Q. and Nahalit, and Nahalit and Dovev [$p<0.001$] in 2003-2004; between Pharaa and Matityahu Q., and Matityahu Q. and Nahalit [$p<0.05$] in 2004-2005).

The NH_4 concentrations were low in all ponds during the breeding season; in most cases, concentrations were found to be less than 1 mg/L. However, during some periods, concentrations in a few ponds increased dramatically (Figs. 3-6). Significant differences in the ammonia concentration were detected between Nahalit and Matityahu Q., Nahalit and Dovev and Nahalit and Amiad in the season of 2003-2004 ($p<0.001$).

The turbidity in the various ponds was relatively constant, varying between 0-150 *nephelometric turbidity units* (NTU) in the various breeding ponds. During some seasons in certain ponds, e.g. Matityahu Q. - 2001-2002 and Nahalit - 2003-2004 and 2004-2005, the turbidity dramatically increased (Figs. 3-6). Significant differences, between Nahalit and Pharaa, Nahalit and Matityahu Q., and Nahalit and Dovev, were measured during the season of 2001-2002 ($p<0.01$), as well as between Nahalit and Dovev, and Nahalit and Amiad, during the season of 2004-2005 ($p<0.05$).

The nitrite (NO_2) concentration was low during all years of the research at all breeding sites, varying between 0-0.25 mg/L (Figs. 3-6). Significant differences were measured during the season of 2002-2003 between Pharaa and Matityahu Q., and Pharaa and Amiad Q. ($p<0.05$)and during the season of 2004-2005 between Pharaa and Dovev ($p<0.05$).

When looking at the relationship between abiotic parameters in Pharaa Pond, a positive relationship was found between oxygen and pH levels ($r=0.8857$; $p=0.033$), temperature and turbidity ($r=0.8857$; $p=0.033$), temperature and EC ($r=0.9429$; $p=0.016$), EC and turbidity ($r=0.9429$; $p=0.016$) and between NH_4 and NO_2 ($r=0.9798$; $p=0.002$) during the breeding season of 2001-2002. During the breeding season of 2001-2002, in Matityahu Q. Pond, a negative relationship was observed between temperature and NH_4 ($r=0.9487$; $p=0.016$), and between temperature and NO_2 ($r=0.9487$; $p=0.016$). In 2002-2003, a negative relationship was recorded between NO_2 and NH_4 ($r=0.9487$; $p=0.016$). During the breeding season of 2003-2004, a positive relationship between temperature and pH ($r=0.8810$; $p=0.007$) was observed. In 2004-2005, positive relationships between temperature and EC ($r=0.6485$; $p=0.049$), temperature and pH ($r=0.6727$; $p=0.039$), turbidity and EC ($r=0.6848$; $p=0.034$) were found, whereas a negative relationship was observed between temperature and oxygen ($r=0.6848$; $p=0.034$). In Nahalit

pond, during the breeding season of 2003-2004, a positive relationship between temperature and NH_4 (r=0.8849; p=0.007), turbidity and NH_4 (r=0.8068; p=0.01) and between EC and NH_4 (r=0.8068; p=0.01) were detected, and during 2004-2005, a positive relationship between oxygen and EC (r=1.0; p=0.016), and between turbidity and NH_4 (r=0.8804; p=0.033) were observed. At Amiad waterholes, during the breeding season of 2003-2004, a negative relationship between temperature and turbidity (r=0.9286; p=0.002), and a positive relationship between turbidity and NH_4 (r=0.7271; p=0.031) were found. During the breeding season of 2004-2005, a positive relationship between turbidity and EC (r=0.9429; p=0.016) was recorded. At the Dovev Pond, during the breeding season of 2004-2005, positive relationships between temperature and NO_2 (r=0.7838; p=0.01), turbidity and EC (r=0.7016; p=0.02) and between EC and NO_2 were discovered (r=0.8003; p=0.007).

DISCUSSION

The distribution of *T. v. vittatus* is related to environmental parameters of the aquatic and terrestrial habitats, as is true of many other amphibian species (Herrmann et al., 2005). In the present study, we examined various breeding sites of this endemic species. Some water bodies of a given type contained only one urodela species, *T. v. vittatus*, residing with other anuran species, while in other water bodies of the same type, larvae of all six amphibian species were found together. This finding is in agreement with results of previous studies on amphibians in Israel (Degani, 1986; Blaustein and Margalit, 1994; 1996; Degani, 1996; Degani and Kaplan, 1999), as well as in other parts of the world (Skriver, 1988; Pavignano, 1990; Warkentin, 1992; Wassersug and Wake, 1995). The *T. v. vittatus* only breeds in rain pools, where water is available during only a few months a year, winter and spring (Degani and Kaplan, 1999). In some breeding spots , other amphibian larvae can be found together with *T. v. vittatus,* due to the fact that the conditions of the breeding sites were suitable for larval development and growth of more than one species (Degani, 1986).

The results of the present study support those conducted previously on the Upper Galilee by Degani and Mendelssohn (1983) and Degani and Kaplan (1999), and on the coastal plains of Israel, by Geffen et al. (1987), demonstrating that the period in which *T. v. vittatus* is present in the water in Israel is between December and April, in contrast to *T. v. ophryticus.* In northern Turkey, the adults of *T. v. ophryticus* usually stay in the water from early March to late October, and sometimes until November, depending on the climate and altitude (Kutrup, 2005b.).

Degani (1982; 1986), who studied in detail the various larval growth periods, including those of salamanders and newts in one pond, came to the conclusion that no competition exists between the two species. Similarily, in this study, it was observed that in breeding sites where newt and salamander larvae were present, newt larvae hatch and develop in the ponds, mostly after the *S. infraimmaculata* larvae have completed metamorphosis.

Kutrup et al. (2005a.) studied the food of the banded newt, *T. v. ophryticus* at different sites in Trabzon in Northern Turkey and discovered that the newts consume a wide variety of invertebrates during their aquatic phase. In Israel, the food of *S. infraimmaculata* and *T. v. vittatus* is very similar, being composed of various invertebrates (Degani and Mendelssohn, 1978; Geffen et al., 1987).

In this study, larvae of *S. infraimmaculata* were detected in habitats where the water temperature was below 15°C. During this period, only mature newts were observed in the ponds, as has been discovered in northern Israel previously (Degani, 1982; 1986). When the temperature rose above 15°C, the larvae of *S. infraimmaculata* metamorphosed and moved on to their terrestrial stage (Degani, 1996).

T. v. vittatus larvae, as well as Anuran tadpoles (*H. savignyi*, *B. viridis*, *R. bedriagae* and *P. syriacus*), have been observed in water bodies in Israel at temperatures between 15°C and 30°C

(Degani, 1986; 1996; Degani and Kaplan, 1999). This is in agreement with the temperatures recorded in the present study.

According to our results, it seems that breeding sites consisting of newt larvae are more suitable for tadpoles of *H. savignyi* and *B. viridis,* than for tadpoles of other amphibians found in Israel. These two species adapt to unpredictable habitats and breeding sites, where water is available for a relatively short time (Degani and Kaplan, 1999).

Very few factors, related to the habitats of *T. v. vittatus* (e.g. water temperature and hydroperiods) and affecting the aquatic phase of adult newts or growth duration of larvae, have been recorded (Degani, 1986; Geffen et al., 1987; Degani, 1996; Degani and Kaplan, 1999). The results of the present study support findings of previous investigations (Degani and Mendelssohn, 1978; Degani and Kaplan, 1999) that have shown that the breeding site size has very little effect on newt breeding, and that newt larvae can grow in breeding sites of various sizes.

The water quality range at *T. v. vittatus* breeding sites is relatively broad. However, not many studies have been conducted on water quality with regard to *T. v. vittatus,* although reports on the effects of environmental changes and pollution on amphibian distribution and survival have been published (see review Lecis and Norris, 2003; Herrmann et al., 2005). The range of various water quality parameters of ponds, in which larvae of *T. v. vittatus* grow and develop, are very similar to those of ponds in which exist other amphibians in Israel (Degani, 1986) and in other parts of the world (Baja and Alcazar, 2003; Herrmann et al., 2005). In the present study, most of the breeding sites of *T. v. vittatus* were located in rural areas, a factor, which might affect the conditions and water quality of the habitat.

Laposata and Dunson (2000) examined three species of temporary pond-breeding amphibians: the wood frogs (*Rana sylvatica* LeConte), Jefferson salamanders (*Ambystoma jeffersonianum* Green) and spotted salamanders (*A. maculatum* Gravenhorst). The results of their studies showed that the wastewater-irrigated ponds had a significantly higher median conductance, pH, Na, K, Ca, Mg and $N+NO_3$, and a low level of dissolved oxygen. In this study, we found a fluctuation in measurements, in NH_4, NO_2, EC and turbidity that might be affected by the agriculture area located near the ponds.

The EC is a parameter that shows the ion concentration in the water. At most sites, an increase in the EC from winter to summer was discovered as the ponds dried up. This result is in agreement with Degani (1982, 1986), who studied the parameters in ponds in Israel, which contain all six species of amphibians.

CONCLUSIONS

In conclusion, all of the newt breeding sites (temporary winter ponds), monitored in the study , had unstable ecological conditions and were in the process of changing, during the larval growth period. The water parameters of the different breeding sites studied, varied significantly from pond to pond, in a few instances in some of the years, when larvae were present in the water.

The periods, during which newt larvae inhabited the ponds, were at different times, during winter and spring, and at various altitudes,. At the sites located at the highest altitudes, larval growth periods were longer. The conditions during springtime and at the beginning of the summer were similar to the conditions observed at the lower altitudes at the end of the winter and at the beginning of spring. We propose that the adaptation of larval growth at the various breeding places occurs between the time when they are found in the pond until the point when they have reached metamorphosis and is not a result of the water conditions present during the larval growth period.

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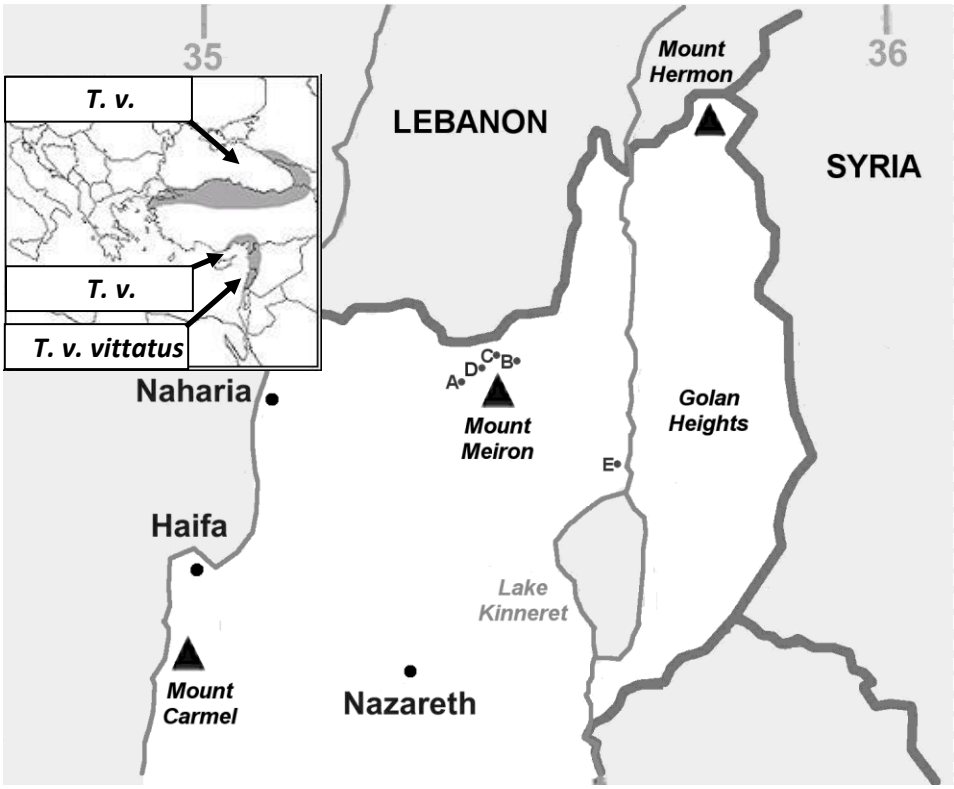

Name of Pond	Longitude	Latitude	Altitude m (ASL)
Dovev pond (A)	239158	772801	740
Nahalit pond (B)	243657	776401	665
Matityahu Q. pond (C)	242783	774855	670
Pharaa pond (D)	242784	774580	682
Amiad water holes (E)	251721	757994	212

Figure 1. Various ponds in Israel colonized with newts examined in the study.

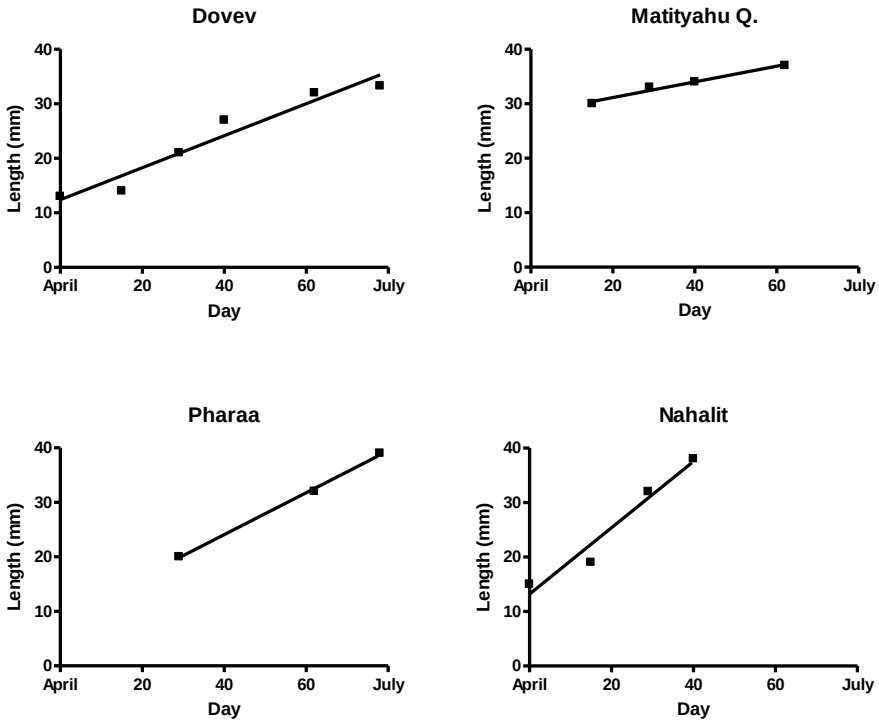

Figure 2. The growth curves of T. v. vittatus larvae from the various breeding sites. Dovev: y=0.2936*X+12.4202, r^2=0.9423, p=0.001; Matityahu Q.: y=0.1439*X+28.2460, r^2=0.9788, p=0.01; Pharaa: y=0.3841*X+8.6932, r^2=0.9978, p=0.29; Nahalit: y=0.6086*X+13.2184, r^2=0.9547, p=0.02.

Table 2. Presence of T. v. vittatus larvae at the breeding sites studied

Breeding Site	2001-2002		2002-2003		2003-2004		2004-2005	
	Months	Days	Months	Days	Months	Days	Months	Days
Matityahu Q. pond			May-July	65	April-June	60	April-June	40
Dovev pond	May-July	60	May-July	65	April-July	75	April-July	75
Pharaa pond	May-July	60			May-July	50	April-May	50
Amiad waterholes					June	35		
Nahalit pond					April-June	60		

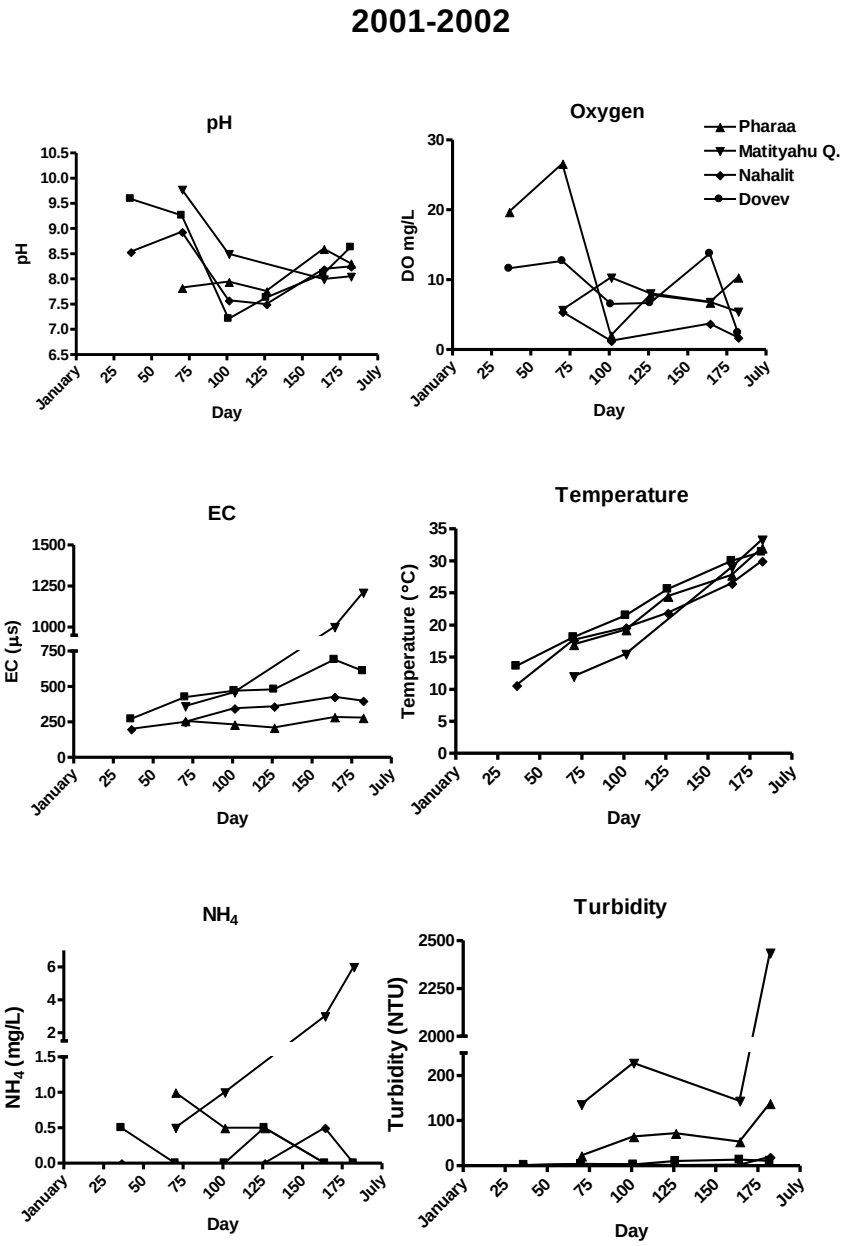

Fig. 3. The water parameters of various breeding sites where T. v. vittatus newts were present during the winter and spring of 2001-2002.

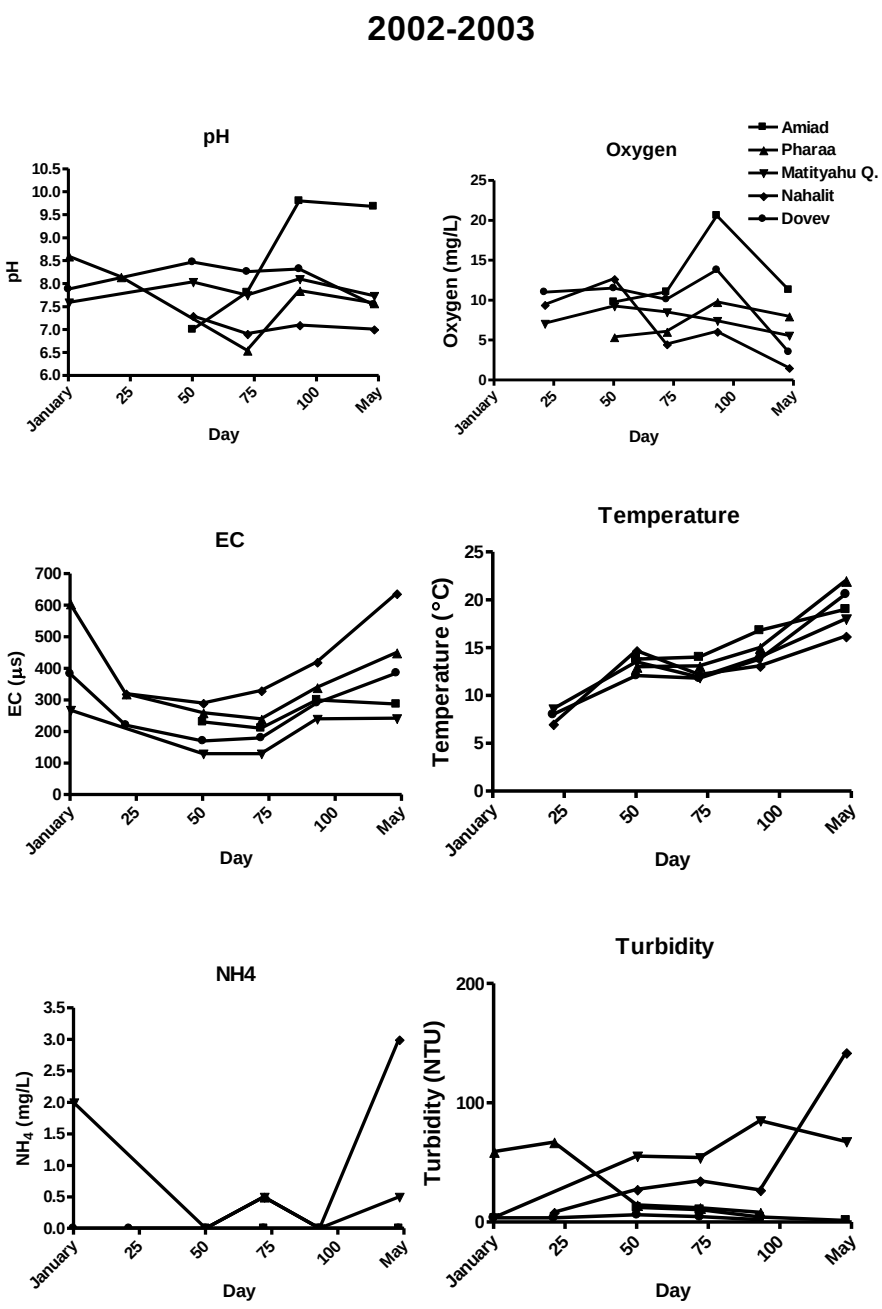

Fig. 4. The water parameters of various breeding sites where T. v. vittatus newts were present during the winter and spring of 2002-2003.

2003-2004

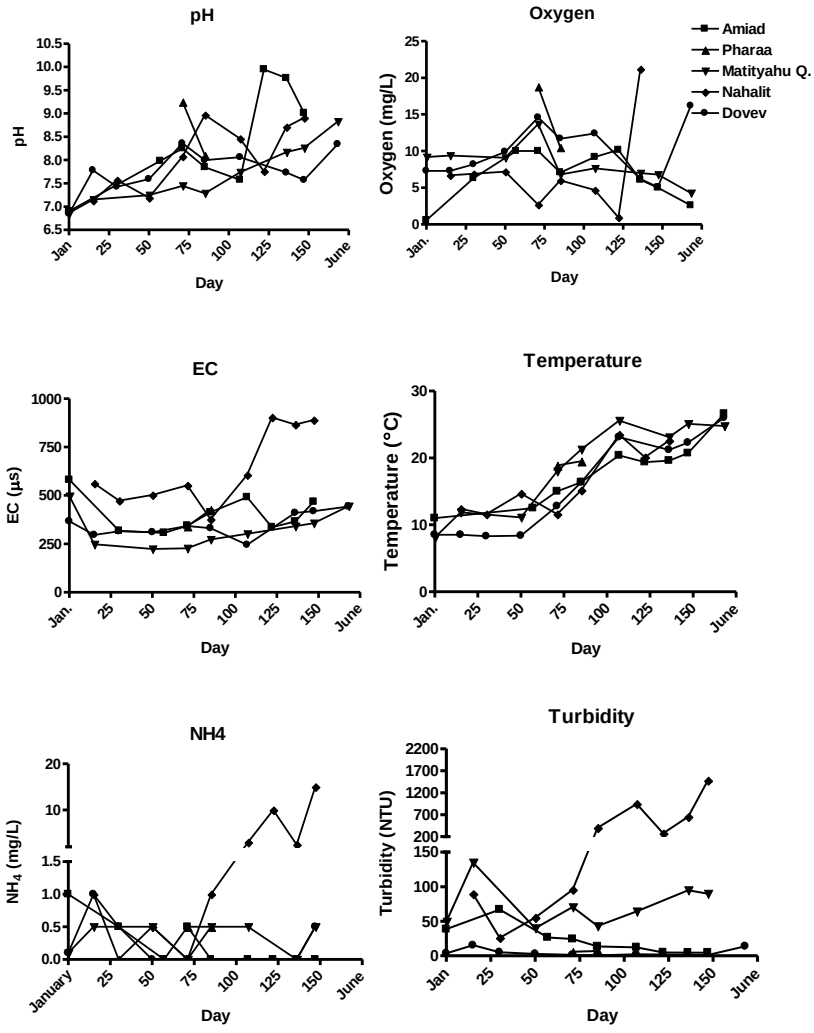

Fig. 5. The water parameters of various breeding sites where T. v. vittatus newts were present during the winter and spring of 2003-2004.

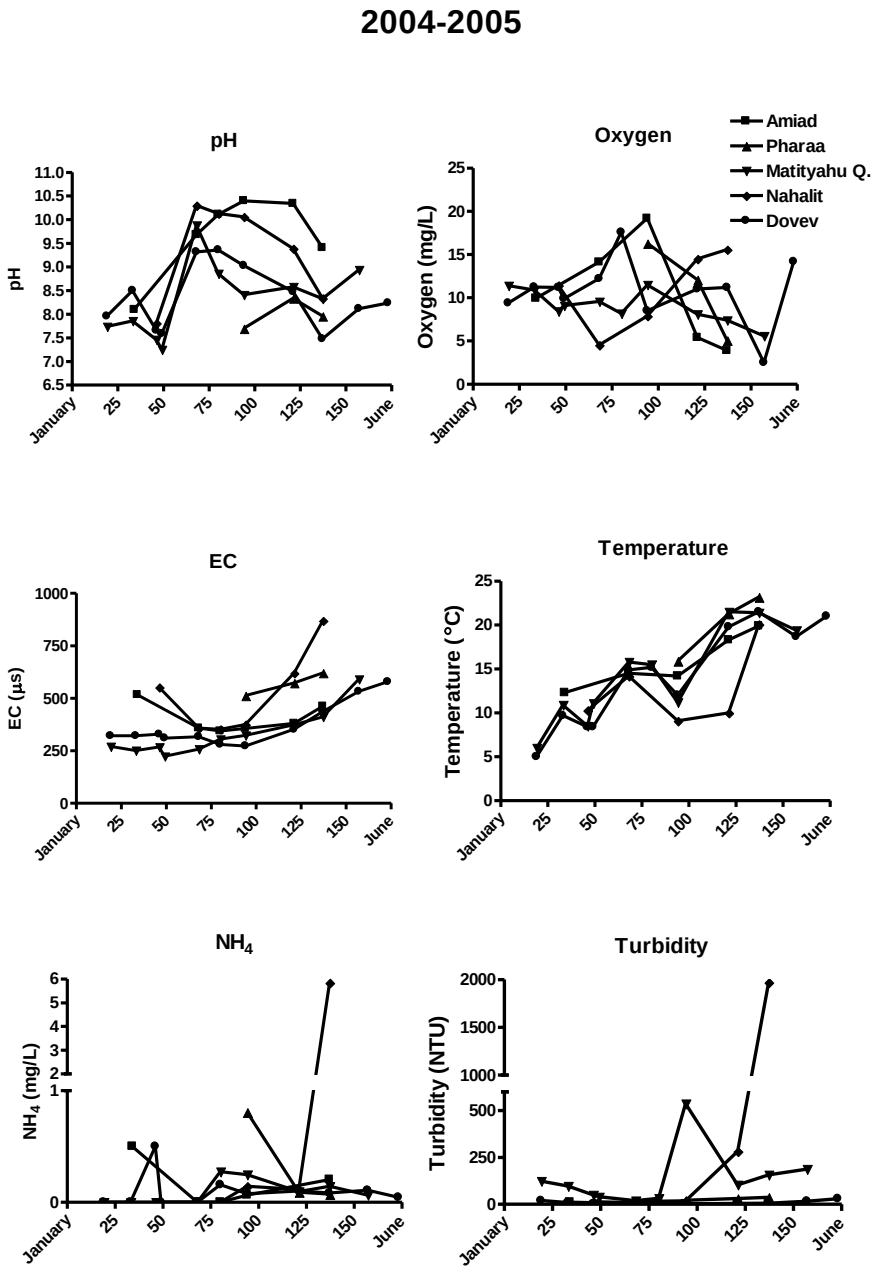

Fig. 6. The water parameters of various breeding sites where T. v. vittatus newts were present during winter and spring 2004-2005.

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The Gonadal Cycle in Males and Females of Triturus vittatus vittatus (Urodela) from the Southern Limit of Its Distribution

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In this study, we describe the gonads of mature terrestrial and aquatic Triturus vittatus vittatus males and females. The ovaries of T. v. vittatus are of the synchronic type group. Aquatic females hold a defined batch of more mature oocytes together with oocytes at different stages of development: oogonia, chromatin nucleolus, perinuclear and vitellogenic oocyte maturation. Some aquatic females were more developed than others, harboring mature oocytes during nuclear migration, as well, but most of the females contained only oocytes at different stages of vitellogenesis. In contrast, ovaries of terrestrial forms only had pre-vitellogenic and atretic oocytes.

Male gonads, consisting of seminiferous tubules, contained cysts of cells at different stages of development. In aquatic forms, spermatogonia, spermacytes spermatides and spermatozoa were observed in the gonads. The more developed specimens had lobuli packed with mature spermatozoa. On the other hand, lobuli of testes from terrestrial forms included mainly cells in the early stages: spermatogonia, spermacytes and early spermatides.

Key words. Triturus vittatus vittatus, histology, gonads, aquatic phase, terrestrial phase

INTRODUCTION

The environmental temperature and photoperiod have important roles in amphibian gonadal function, which is controlled by the gonadotropins (Fraile et al., 1988; Tananka et al., 2004).

Various aspects of oogenesis in amphibians have been described for Chthonerpeton (Berois and De Sa, 1988), Ichthyophis (Masood- Parveez and Nadkarni, 1993 a, b), Necturus (Kessel and Panje, 1968), Salamandra (Greven and Guex, 1994; Joly et al., 1994), Triturus (Fischer, 1932), Pleurodeles (Bonnafant-Jais and Men-tre, 1983) and several anuran species (e.g., Del Pino and Sanchez, 1977; Jorgensen, 1984). However, to date, there have been no detailed reports on oogenesis in *Triturus vittatus,* located at the southern border of its distribution.

The urodele species, *Triturus vittatus* (Gray, 1835; see Litvinchuk et al., 2005), is distributed throughout western Caucasus, Turkey, Lebanon, Syria, Israel, Iraq and, perhaps, Jordan. Borkin et al. (2003) have provided a list of all known records of *T. vittatus.* Litvinchuk et al. (2005) have suggested that the banded newt, *T. vittatus,* consists of two species, *T. ophryticus* and *T. vittatus,* based on trunk vertebrae count, genome size and allozyme data. The northern taxon, *T. ophryticus*, is subdivided into two geographic fragments: the "western group", populations from western Anatolian Turkey, and the "eastern group", distributed in the remaining area of Pontic Turkey and western Caucasus. According to the above criteria, the *T. vittatus* species is found in Israel.

The biology and life cycle of *T. vittatus* in Europe and in the Mediterranean region have been described by Raxworthy (1989) and Olgun et al. (1997). As indicated by their data, there are two

known subspecies in the genus, Triturus: *T. v. vittatus* along the eastern edge of the Mediterranean Sea from Turkey to Israel, where it reaches its southern limit, and *T. v. ophryticus* in the Caucasus, east and south of the Black Sea.

The banded newt, *T. v. vittatus*, is an endangered species in Israel (Geffen et al., 1987). Moreover, other species and subspecies of this genus are endangered in other regions of the world.

At the southern limit of the *T. v. vittatus* distribution (in Israel), environmental conditions are the most extreme. Limiting factors are likely to be breeding sites and the dryness of the terrestrial habitat. The biology and life cycle of *T. v. vittatus* in northern Israel and the Upper Galilee have been described by Degani and Mendelssohn (1983) and Degani (1986, 1996), while a population in central Israel has been reported by Geffen et al. (1987). *T. v. vittatus* inhabit mainly winter pools that contain water only until the beginning of the summer, although occasionally they have water year-round (Degani and Kaplan, 1999). Like other newts, *T. v. vittatus* require water bodies surrounded by an adequate terrestrial habitat to support both life phases. If either habitat is damaged, a population may be unable to survive.

The present study examines the morphological variations in gonads of *T. v. vittatus* males and females in both phases, the aquatic and terrestrial stage.

MATERIALS and METHODS

Specimens

Eighteen mature Triturus specimens, six males and 12 females were obtained from five ponds (one male from Nahalit Pond, two males and two females from Matityahu Q. Pond, three males and ten females from Dovev Pond) located at different altitudes in the Upper Galilee of northern Israel (Fig. 1. and Table 1.). As previously described by Degani and Mendelssohn (1983), adults migrate to these water bodies during the mating season (beginning of winter) to breed. There is a large adult population at all three locations (Degani and Kaplan, 1999). Specimens were sampled randomly from the entire area of the water body by hand net.

Histological analysis

The gonads of the aquatic phase were sampled from males and females in the winter ponds shortly after the newts entered the ponds, and from those in the terrestrials phase, once the newts had left the pond to live a terrestrial life. The ovaries and testes were divided into a number of sections that were fixed in Bouin fixative (Sharon et al., 1997). Paraffin blocks were prepared, sectioned at 5–8 μm and stained with haematoxylin and eosin (HE). Haematoxylin stains cellular organelles (nucleus, cytoplasm, yolk, collagen etc). Thus, the stain allowed for the determination of the oocyte oogenetic stages.

RESULTS

Aquatic female gonads held a defined batch of more developed oocytes, together with oocytes at different stages of development (Fig. 2). The stages were characterized as follows: oogonia, chromatin nucleolus stage, perinuclear stage and vitellogenic oocyte maturation. Some aquatic females were more developed than others, harboring mature oocytes as well, during nuclear migration, but most of the females contained only oocytes at different stages of vitellogenesis. In contrast, ovaries of terrestrial forms had only pre-vitellogenic and atretic oocytes (Fig. 2).

Male gonads consisted of seminiferous lobules, containing cysts of cells at different stages of development. Spermatogonia, spermacytes, spermatides and spermatozoa were observed in the gonads of aquatic forms (Fig. 3), and more developed specimens had lobuli packed with mature spermatozoa. On the other hand, lobuli of testes from terrestrial forms included mainly cells of the early stages: spermatogonia, spermacytes and early spermatides (Fig. 3).

DISCUSSION

In the newt, *T. vittatus,* spawning and fertilization occur once a year (Degani and Mendelssohn, 1983). However, very little information has been published on its oogenesis and spermatogenesis, which must become adapted to various breeding places, in which reproduction occurs under impermanent conditions (Degani and Kaplan, 1999). Oogenesis is the process whereby oogonia, that multiply by mitosis, are transformed into mature oocytes. In the present study, oocytes of newts in the aquatic phase appeared in two different stages, as oogonia and in the advance vitellogenesis stage. These oocytes seemed to be ready for the process of maturation. This finding supports the hypothesis that the maturation of oocytes occurs only after the female enters the pond (Fig. 4).

There are many studies on oogenesis in Urodela. In Urodela, Bonnafant-Jais and Mentre (1983) showed that the ovary of *Pleurodeles waltlii* contains oocytes at all stages, all year-round. This situation differs from that observed in *T. vittatus*. In *Triturus torosa*, yolk deposition takes place during aestivation and migration to the breeding sites, 5–6 months before ovulation (Miller and Robbins, 1954). Adams (1940) stated that oocyte development in *T. viridescens* is synchronized; thus, the ovarian mass is low during the summer and high from fall to spring. The ovarian cycle in other subspecies of *Salamandra salamandra* depends on the season (as in *S.s. terrestris*) or on gestation (as in *S.s. fastuosa*), with synchronized oocyte development (Joly et al., 1994). On the other hand, in *S. infraimmaculata,* oocytes of previtellogenic and vitellogenic stages are found throughout the year and during the reproductive cycle, with the percentage of vitellogenic oocytes remaining constant. Therefore, the ovarian cycle, apart from oocyte maturation, does not depend on the season or on gestation. Oocyte maturation, however, does depend on the season, starting at the onset of rainfall and increasing toward the end of winter (March–April) (Sharon et al., 1997). This is an adaptation to extreme and unpredictable climatic conditions. It allows the female salamander to be ready to ovulate practically at any time, beginning with the end of the hot, dry summer, when temperatures drop and rain starts falling. It is an alternative way to enable a nonviviparous urodele to survive in a xeric environment. Ovulation in *Salamandra salamandra* has been described only once (Joly, 1986).

In the life cycle of *T. vittatus* newts in Israel, as has been described by Degani and Mendelssohn (1983), reproduction takes place during the aquatic phase of these newts, which occurs in winter, In this phase, the ovary contains mature oocytes, and the testes are found in advanced spermatogenesis. Tananka et al. (2004) observed that gonadotropins in male newts (*Cynops pyrrhogaster*) are affected by various temperatures, and that the LH effect was more potent at 8 ºC than at 18 ºC.

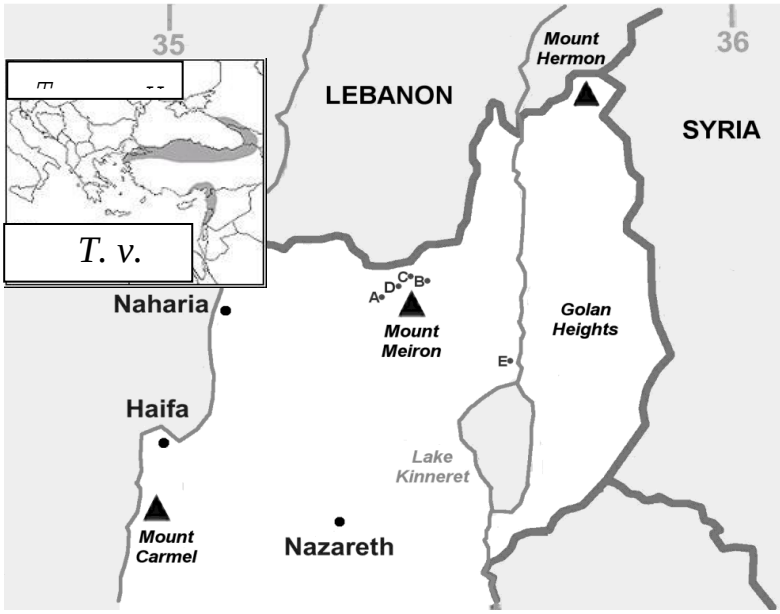
Males of T. vittatus transfer to aquatic phase just as the rain pool fills with water (Degani and Mendelssohn, 1983). In the present study we found that at the aquatic phase the testis consist of sperms that are in the last stage of spermatogenesis - spermatozoa. In other words the T. vittatus male gonad cycle is suitable to their life cycle as described in Fig. 5.

CONCLUSIONS

In conclusion, this study demonstrates that the reproduction cycle of the male and female newt is adapted to the winter pool, with its unpredictable conditions. After the transfer of newts from terrestrial to the aquatic phase, oocytes in the advanced vitellogenesis stage may mature within a short time before spawning and in the testis, sperm reach its final stages of spermatogenesis and transforming to the spermatozoa form.

Table 1. Size of adult newts at the breeding sites studied

Breeding Site	Sample size		Male		Female		Period in the ponds
	Male	Female	Weight	Length	Weight	Length	
Matityahu Q. Pond	3	7	4.27 ± 1.75	9.90 ±1.49	4.07 ± 1.02	9.64 ± 1.14	Jan-March
Dovev Pond	6	11	5.33 ± 0.96	10.87 ± 0.64	3.15 ± 0.4	9.09 ± 0.6	Jan-March
Pharaa Pond	1	4	6.4	10.00	3.93 ± 0.4	8.67 ± 0.58	Jan-March
Amiad waterholes	3	2	4.27 ± 1.85	10.17 ± 0.29	3.35 ± 1.06	8.50 ± 0.71	Dec-April
Nahalit Pond	2	2	5.35 ± 0.21	10.00	3.20 ± 1.41	8.25 ± 1.06	Jan-March



Name of Pond	Longitude	Latitude	Altitude m (ASL)
Dovev Pond (A)	239158	772801	740
Nahalit Pond (B)	243657	776401	665
Matityahu Q. Pond (C)	242783	774855	670
Pharaa Pond (D)	242784	774580	682
Amiad water holes (E)	251721	757994	212

Figure 1. Various ponds in Israel colonized by newts examined in the study.

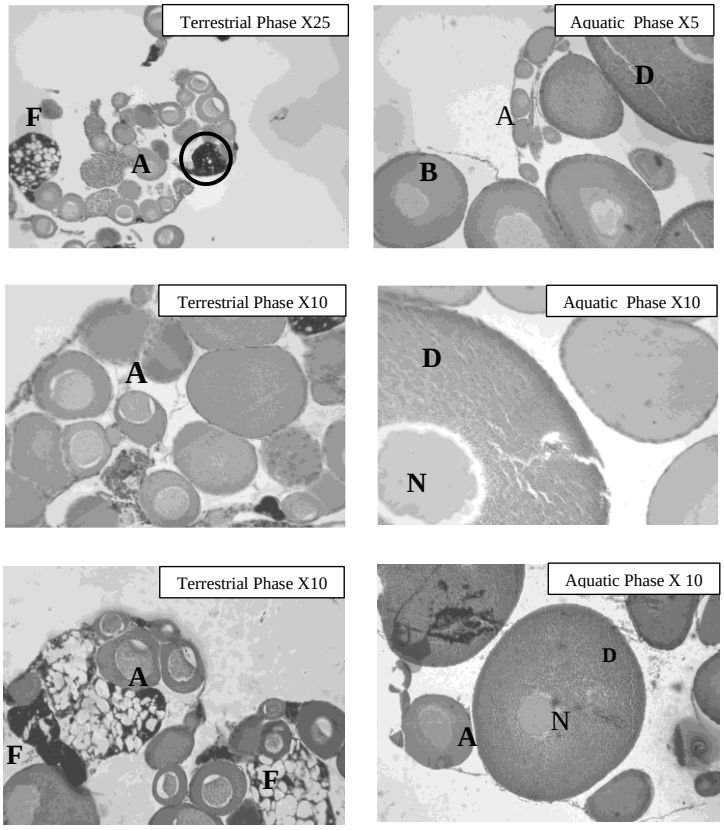


***Figure 2**. Ovary of terrestrial and aquatic female newts. A: oocytes in oogenia, B and D: oocytes in vitellogenesis in deferent size, F: oocytes in Artesia, N: neuclear.*

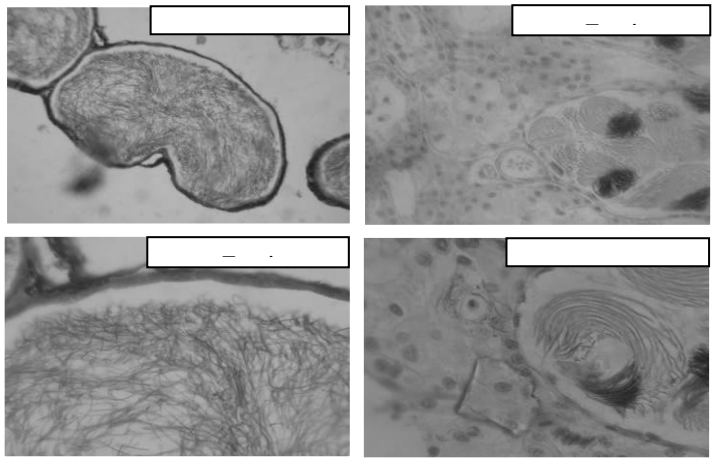


***Figure 3**. Testis in the aquatic phase. A and B are spermatophores in the testis, and C and D are different stages of cells spermatogenesis. F sperm, E various cells in the testis during spermatogenesis.*

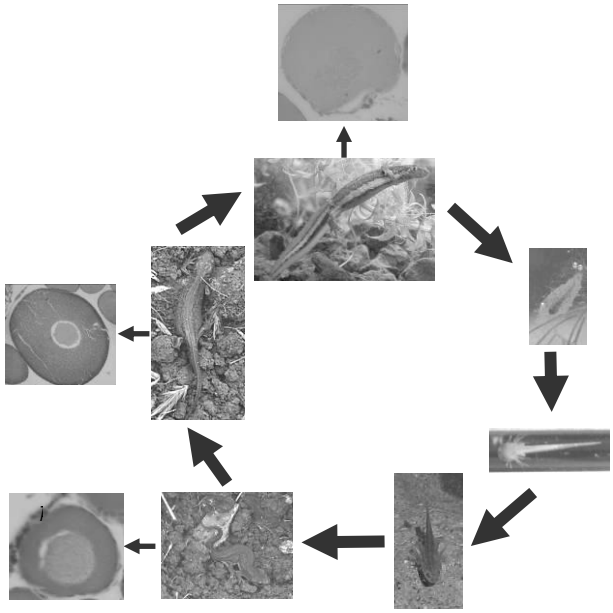


Figure 4. *Ovary changes during the life cycle of T. v. vittaus. When females are in the terrestrial phase, ovaries contain only pre-vitellogenic (PV) oocytes. The group of oocytes move on to the vitellogenesis (V) stage just before the females are ready to move to the water for the aquatic phase. During the aquatic phase the germinal vesicular brakes down and matured oocytes are ready for fertilization (M). This is when spawning occurs.*

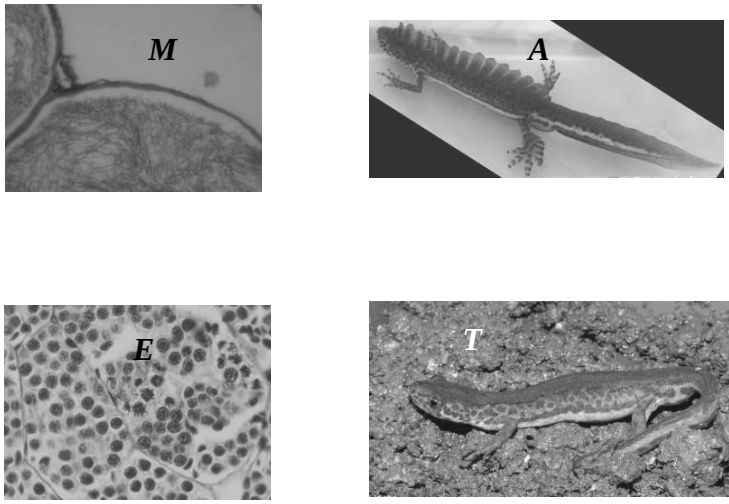


Figure 5. *Early stage spermatocytes (ES) exist in testes of males at the terrestrial phase (TP). Spermatogenesis takes place before they enter the water, while they are passing on to the aquatic phase (AP); seminiferous lobules are packed with many mature spermatozoids (MS). Fertilization takes place a short time after males and females have entered the pond.*

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Aspect respecting some joints of pelvic limb in *Struthio camellus*

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Construction principles of the ostrich hindlimb are similar to those found in some hoofed animals such as horses and are generally characterized by an inevitable combination in motion of those joints lying distally of the hips. The hindlimbs of the ostrich are on the one hand well adapted supporting and carrying the body weight and on the other hand enable exact movements during bipedal locomotion.

Key Words: ostrich, hindlimb, joints

Matherials and methods

Hindlimbs of 8 ostriches (males and females) of various ages were dissected. For a better visualization it was used the SMZ - 2T Nikon with photo device. For identification, description and payoffs homologation it was used N.A.A. – 2005.

Results

The researches followed the morphologycal aspects of tibio-tarso-metatarsal joint. This joint is achieved between the trochlea of distal epiphysis of tibia, with two articular surfaces of tarsal bone and articular plateau of proximal extremity of metatarsal bones. The last is formed by tow glenoidal cavities, completed by one meniscus, placed caudo-lateraly.

The collateral ligaments are shapely. The lateral collateral ligament consist of 3 cruciate ligaments. The medial collateral ligament is single and is longer than the first.

The meniscus has a semilunar shape and it has a cranial cornu, a body anda caudal cornu.

The cranial cornu and the body have a convex and thick lateral border and a concav and thin medial border. In the last are conected the proximal with the distal faces.

The caudal cornu is more developed transversally, and taking a piramidal shape. So, it performed three faces:

anterior articular face, slightly concave that and larged the lateral articular surface of tarso-metatarsian bone,

caudal face, slightly covex regarding the digital flexor tenons,

a ventral face, slightly convex regarding the caudal part of lateral articular surface of tarso-metatarsal bones.

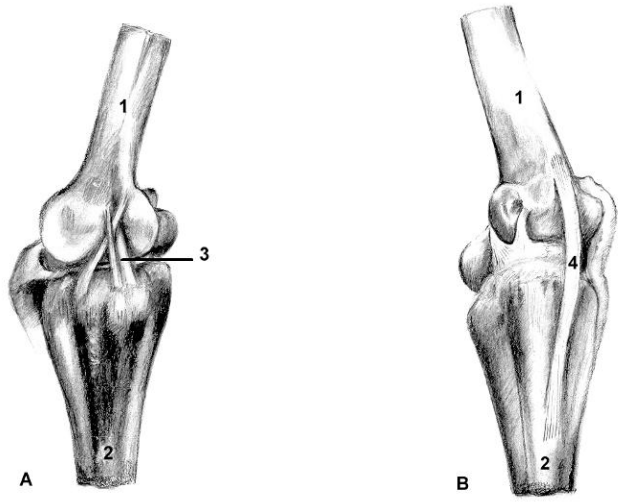

Fig. 1 Tibio-metatarsal joint in Struthio camelus A- lateral view, B- medial view
1-distal extremity of tibia; 2- proximal extremiti of metatarsal bone; 3- lig. colaterale laterale; 4- lig. colaterale mediale.

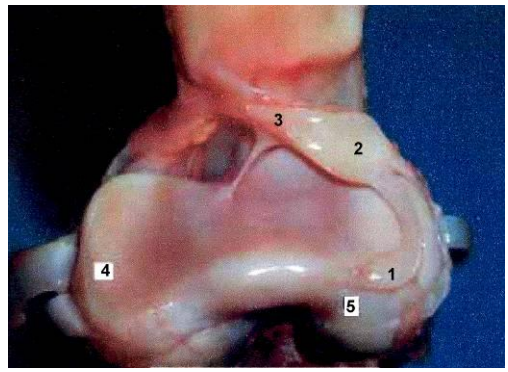

Fig 2 The left tibio-tarso-metatarsal joint (dorso-cranial view)
lateral cornu of meniscus; 2- body of meniscus; 3- medial cornu of meniscus; 4-lateral edge of articular surface of tarso-metatarsal join; 5- ligament of lateral cornu;

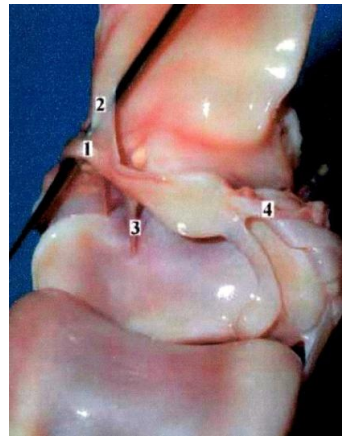

Fig. 3 The left tibio-tarso-metatarsal joint (dorso-cranial view, after flexion)
1-ligam mediale; 2- ligam. dorsale; 3- ligam. ventrale; 4-ligam. laterale

The three faces are rejoined at the level of three borders:

- proximal border is thin, and if cleaved the articular and caudal faces,
- cranio-ventral border cleaved the articular and ventral face,
- caudo-ventral border, slightly ronded cleaved the caudal and ventral border.

On the extremities of meniscus they are the fastening ligaments.

The caudal cornu has:

- a medial ligament in extension of this witch are insert on the inner face of articular capsule,
- a dorsal ligament,
- a ventral ligament, thik, witch carry on the caudo-ventral border, to lead cranially and to insert in a little notch from the caudal border of articular surface of tarso-metatarsal bone,
- a lateral ligament witch carry on this extremity and it reached the articular capsule.

The cranial cornu has a single ligament witch braced the meniscus from the articular plateau of tarso-metatarsal bones.

Conclusions

In Struthio camelus the tibio-tarso-matatarsal joint have the following particularities:

1. The medial collateral ligament is single while the lateral collateral ligament is formed by three bundles.
2. In this species is exclusive present the lateral meniscus.
3. The lateral meniscus is formed by cranial cornu the body and caudal cornu.
4. The medial cornu is better reprezented and is braced from four ligaments.
5. The latteral have only one ligament.

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Evaluation of the biochemical parameters relevant for the hepatic function on the background of the therapy with *Hipophäe rhamnoides*

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Among the xenobiotics that exert their aggressive toxicity as parental derivative free radicals, is to be found sterigmatocystin, a micotoxin chemically related to aflatoxin B1 and classified as human carcinogen class I. The present study is part of a more ample experiment that deals with the reduction of the toxicity of sterigmatocystin using vegetal products with antiradicalic action. The experiment included four lots of five white rats each, Wister line. The first lot was the reference lot, while the second one served for the experimental reproduction of cronical sterigmatocystin intoxication. Besides the sterigmatocystin dose, the animals from the third lot were given ascorbic acid, a nonenzymatic antioxidant. The fourth lot was treated with Hipophäe fructus, along with the sterigmatocystin dose. In the end, the animals were sacrificed and the blood was analyzed for biochemical investigations with important relevance upon the hepatic function and integrity. The investigated hepatic cytolisis indices, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase from blood samples emphasized a significant improvement of the liver integrity for the lot of animals treated with Hipophäe fructus. The results obtained for the biochemical parameters accountable for the liver proteosynthetic capacity (acetyl cholinesterase and total protein) did not suggest an efficient protective effect of the investigated phytopreparate.

Key Words: sterigmatocystin, antiradicalic action, Hipophäe fructus, hepatic cytolisis indices

INTRODUCERE

Numeroase xenobiotice îşi exercită toxicitatea sub forma unor radicali liberi derivaţi de la compuşii parentali, specii chimice deosebit de reactive, care provoacă declanşarea unui lanţ de reacţii chimice ce afectează prevalent componenţi ai membranelor celulare şi subcelulare (1). Printre acestea se numără şi sterigmatocistina, micotoxină furo-furanică ce acţionează hepatotoxic sub forma unui radical liber provenit de la epoxi-sterigmatocistină, metabolitul principal din ficatul animalelor ce au ingerat micotoxina parentală (2,3).

Incidenţa crescută a sterigmatocistinei în alimente de origine vegetală şi nutreţuri din zona Moldovei (4) necesită căutarea unor remedii naturale antitoxice, lipsite, pe cât posibil, de efecte secundare. Datorită conţinutului ridicat în acid ascorbic, flavonoide, carotenoide, substanţe cu acţiune antiradicalară (5), *Hipophäe rhamnoides* poate constitui un valoros agent chemopreventiv ce ar putea preveni legarea radicalului epoxidic al sterigmatocistinei de structurile de elită ale celulei (ADN, ARN, enzime) (6).

MATERIAL ŞI METODĂ

Modelul experimental redat în această lucrare monitorizează eventualele efecte antioxidante/antitoxice ale unei soluții extractive 5% din *Hipophäe rhamnoides* în intoxicația cu sterigmatocistină. Pentru fidelitatea aprecierii efectului amintit s-a luat ca etalon o soluție de acid ascorbic 5%, dat fiind conținutul ridicat în această vitamină a cătinei şi sugestia că acțiunea antitoxică se datorează într-o proporție importantă funcției enolice a acestui antioxidant. Experimetul cuprinde patru loturi de şobolani albi din linia Wistar în vârstă de 4 luni cu o masă ponderală medie de 176 g. Primul lot se compune din 5 animale şi constituie lotul de referință. Cel de al doilea lot, alcătuit din 5 şobolani, serveşte pentru reproducerea intoxicației cu sterigmatocistină. Pentru aceasta, animalelor din acest lot li s-a administrat o doza *pro die* de 8 ppm din micotoxină hepatotoxică. Al treilea lot serveşte pentru evaluarea acțiunii antiradicalare a cătinei. Cele cinci animale din acest lot au primit pe lângă doza obişnuită de sterigmatocistină (8 ppm) şi infuzie 5 % de *Hipophäe rhamnoides* (10 ml pe zi în apa de băut). Animalele ultimului lot, în număr de 5, au fost tratate concomitent cu doza obişnuită de sterigmatocistină şi cu soluție injectabilă 5% de acid ascorbic (10 ml *pro die* în apa de băut). La sfârşitul experimentului, care a durat 6 săptămâni, sângele recoltat de la animalele sacrificate a fost supus investigației biochimice. Pentru a aprecia starea de integritate a hepatocitului s-a testat activitatea a trei enzime: alanil aminotransferaza (ALT), aspartat aminotransferaza (AST) şi lactatdehidrogenaza (LDH), iar pentru testarea capacității proteosintetice a ficatului s-au investigat concentrația de proteine totale şi activitatea colinesterazei serice (ChE). Soluția extractivă apoasă din pseudo-drupele de *Hipophäe rhamnoides* s-a preparat conform farmacopeei în vigoare (7) şi s-a administrat animalelor infuzie proaspătă, preparată *ex tempore*, în apa de băut. Activitățile enzimelor serice şi concentrația de proteine totale au fost determinate prin teste standardizate (8,9,10).

REZULTATE ŞI DISCUŢII

Rezultatele obținute în urma cuantificării activității celor trei enzime, folosite ca indicatori de citoliză sunt redate în tabelul 1, fig.1 şi fig 2. Studiul acestor date evidențiază o creştere semnificativă a activității alanil aminotransferazei pentru lotul intoxicat cu sterigmatocistină (36,45 UI), față de lotul de referință (18,38 UI), în timp ce activitatea enzimei lotului care a primit concomitent cu micotoxina hepatotoxică infuzie 5% de *Hippophäe fructus* înregistrează o valoare semnificativ scăzută (22,78 UI) față de lotul tratat exclusiv cu sterigmatocistină. Din evoluția activității acestei enzime, considerate marker de citoliză hepatică, reiese intervenția antitoxică/antioxidantă a principiilor active din cătină. Referitor la lotul care a primit ca agent antioxidant soluție de acid ascorbic se observă o valoare mai apropiată de valoarea lotului intoxicat cu sterigmatocistina (30,79 UI), ceea ce poate sugera faptul ca principiile active din *Hippophäe fructus* posedă un efect antioxidant superior soluției medicamentoase de vitamina C. Evoluția celei de a doua aminotransferaze, aspartat aminotransferaza evidențiază o creştere accentuată a activității acesteia în sângele lotului agresat de prezența sterigmatocistinei (54,60UI), față de lotul de referința (33,53 UI). În cazul lotului ce a beneficiat de protecția fitopreparatului de *Hippophäe fructus*, valoarea acesteia este semnificativ diminuată față de lotul intoxicat şi neprotejat (34,52UI). Pentru animalele tratate cu soluție 5% de acid ascorbic activitatea enzimei se cifrează la 44,37 UI, valoare crescută față de a lotului de referință, dar semnificativ scăzută față de lotul tratat exclusiv cu micotoxină difuranică, ceea ce nu poate ignora intervenția antiradicalară a acestei vitamine. Efectul antioxidant/antitoxic al principiilor active din fructul de cătină este susținut şi de variația celei de a treia enzime, lactat dehidrogenaza, aşa cum reise din tabelul 1 şi fig 2. Astfel, de la valoarea de 2,99 µmol/ml care este înregistrată de lotul de referință creşte la 9,33 µmol/ml pentru lotul tratat exclusiv cu sterigmatocistină, ca apoi să scadă semnificativ la 4,29 µmol/ml, valoare apropiată de cea normală, pentru lotul protejat cu infuzie de *Hippophäe*

fructus. Lotul ce a fost protejat cu soluția de acid ascorbic a atins valoarea de 5,99 µmol/ml, valoare echidistantă între lotul de referință şi lotul protejat cu infuzie de fruct de cătină.

Tabel 1

Evoluția indicatorilor de citoliză

LOTURI	ALT [UI]	AST [UI]	LDH [µmol/ml]
Lot 1	18,88±1,929	33,53±3,706	2,99±0,598
Lot 2	36,45±1,306	54,60±3,017	9,33±5,276
Lot 3	22,78±2,999	34,52±3,212	4,29±2,320
Lot 4	30,79±0,997	44, 37±2,907	5,99±2,997

ALT = alanil aminotransferaza; AST = aspartat aminotransferaza; ChE = colinesteraza

O situație contrară dezvăluie rezultatele obținute în urma investigării parametrilor biochimici ce furnizează informații depre capacitatea de proteosinteză a ficatului.

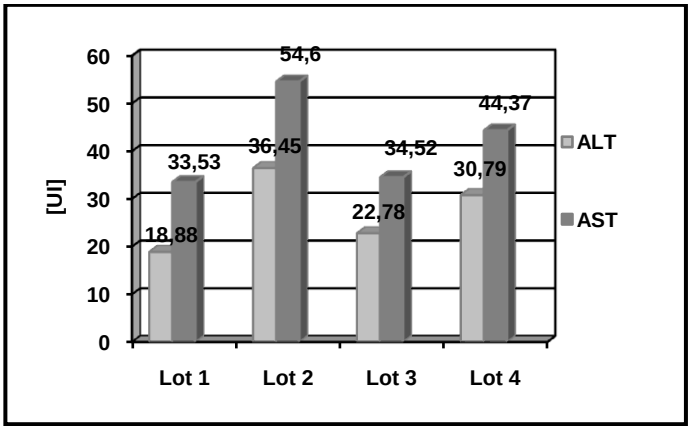

Fig. 1 Valoarea activității aminotransferazelor serice

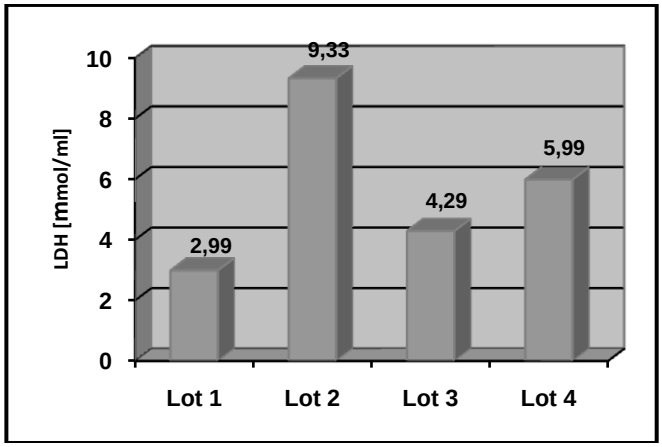

Fig. 2 Valoarea activității lactat dehidrogenazei

Aşa cum reiese din tabelul 2 şi fig. 3, activitatea colinesterazei serice, enzimă sintetizată în ficat, înregistrează o evoluție aleatorie, care contrazice evoluția indicatorilor de citoliză ce sugerează existența efectului antiradicalar/antitoxic al principiilor active (flavonoide, vitamina C, carotenoizi) din *Hippophäe fructus.* Astfel, activitatea colinesterazei din serul lotului de referință atinge valoarea de 255,95 UI, scăzând la 210,17 UI la lotul pe care s-a reprodus intoxicația cu sterigmatocistină, aşa cum era de aşteptat. În mod paradoxal activitatea enzimei din serul animalelor tratate cu infuzie de *Hippophäe fructus* se situează sub valoarea înregistrată la lotul intoxicat (199,55UI), iar pentru lotul tratat cu soluție injectabilă de acid ascorbic ca antioxidant colinesteraza scade dramatic la 182,78UI.

Tabel 2

Variația parametrilor referitori la capacitatea proteosintetica

LOTURI	Ch E [UI]	Proteine totale serice [g‰]
Lot 1	255, 95±11,422	53,67±1,99
Lot 2	210,17±17,203	36,39±3,02
Lot 3	199,55±13,24	40,99±2,97
Lot 4	182,78±19,69	41,55±3,09

ChE = colinesteraza

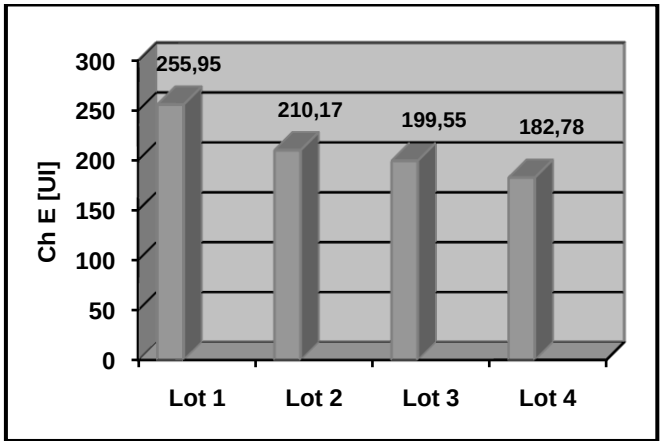

Fig. 3 Valoarea activității colinesterazei serice

Studiul proteinelor totale serice (tabel 2, fig. 4) evidențiază o scădere deosebit de semnificativă a concentrației acestora din sângele animalelor tratate exclusiv cu sterigmatocistină la 36,39 g ‰, ceea ce sugerează o reducere a potențialului proteosintetic al ficatului. O ameliorare a funcției proteosintetice a ficatului poate fi ilustrată de valoarea uşor augmentată a concentrației proteinelor totale din sângele animalelor din lotul tratat cu fitopreparat de *Hippophäe fructus* (40,99 g ‰) şi din sângele lotului protejat cu acid ascorbic (41,55 g ‰).

CONCLUZII

1.Variația activității transaminazelor serice (AST, ALT) sugerează existența unui semnificativ efect antitoxic al fitopreparatului pe bază de *Hippophäe fructus* în intoxicația cu sterigmatocistină;

2.Efectul antioxidant/antitoxic al principiilor active din *Hippophäe rhamnoides* este superior soluției medicamentoase de acid ascorbic, superioritatea fiind datorată prezenței flavonoidelor;

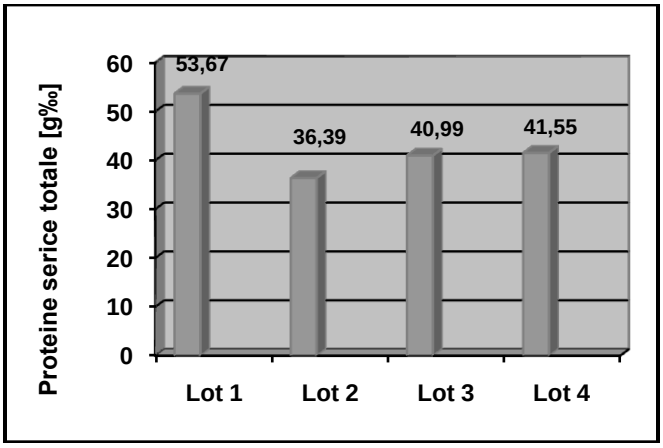

Fig. 4 Concentrația de proteine totale serice

3.Evoluția lactat dehidrogenazei confirmă intervenția chemopreventivă a soluției extractive de *Hippophäe fructus* în impactul cu micotoxina ;

4.Variația colinesterazei infirmă existența unui efect benefic atât a fitopreparatului pe bază de cătină, cât şi a soluției de acid ascorbic ;

5.Concentrația de proteine totale serice poate constitui un argument pertinent în susținerea efectului antiradicalar/antitoxic al fructului de cătină ;

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Researches regarding the antiradicalic effect of some non-vitaminic antioxidants

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The exceeding accumulation of the oxygen reactive species in the cell is considered to be a pathological state known under the syntagm of oxidative stress. The purpose of the present paper is represented by the control of the oxidative stress using substances able to annihilate the oxygen reactive species. The experiment led in this paper evaluates the antioxidant effect of yeast (Saccharomyces cerevisiae), a natural product containing glutathione, the tripeptide able to repeal the reactivity of free radicals by oxidizing its free thiol group. The experiment used four lots of white rats. The experimental model allowed the evaluation of the antioxidant effect of the yeast using glutathione as standard. The activity of two enzymes was quantificated in order to evaluate the antioxidant effect: seric catalase and superoxid dismutase. The biochemical investigations emphasize the existance the antiradicalic effect of the yeast.

Key words: antioxidants, glutathione, Saccharomyces cerevisiae, superoxid dismutase, catalase

INTRODUCERE

Stresul oxidativ, definit ca o acumulare celulară excesivă de specii reactive ale oxigenului (SRO), se asociază multor stări patologice. Prezența celulară a SRO poate avea surse endogene (respirația mitocondrială, procese redox din peroxizomi, fagocitoza, peroxidarea lipidelor nesaturate, biosinteza prostaglandinelor şi leucotrienelor) şi surse exogene (acțiunea radiațiilor UV asupra organismului în scopuri diagnostice sau terapeutice, acțiunea radiațiilor ionizante (fig. 1)(1,2).

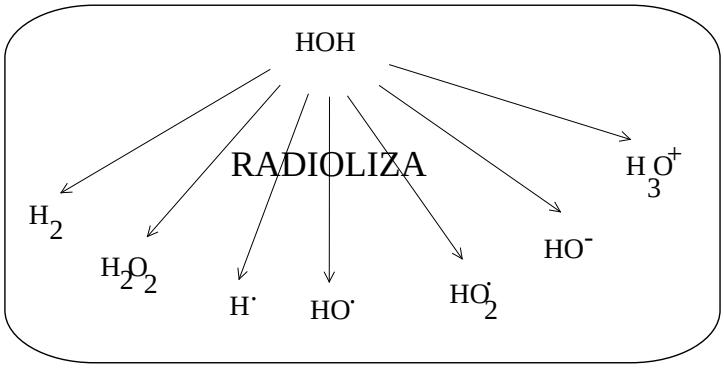

Fig. 1 Radioliza apei

Toate procesele fiziologice producătoare de SRO au motivaţii clar definite şi sunt prevăzute cu sisteme antioxidante (3). Din păcate organismul viu nu posedă sisteme de anihilare a SRO rezultate din surse endogene declanşate de instalarea anumitor patologii sau din surse exogene (4). În aceste condiţii se apelează la antioxidanţii vitaminici (acid ascorbic, tocoferoli, vitamina K_1, caroteni) şi nevitaminici (polifenoli, fitosteroli, glucosinolaţi, indoli, alil-sulfizi, coenzima Q_{10}, seleniu etc.)

MATERIAL SI METODĂ

Prezenta lucrare se înscrie în încercările de a găsi antioxidanţi naturali pornind de la modele de sisteme neenzimatice specifice organismului viu care au capacitatea de a inhiba sau preveni producerea excesivă a SRO. Glutationul, o tripeptidă naturală, ocupă un loc de seamă în arsenalul antioxidant al organismului datorită unei grupări sulfhidrice, capabile să neutralizeze reactivitatea crescută a radicalilor liberi (5). Pornind de la acest argument s-a imaginat un model experimental (tabel 1) care să evalueze potenţialul antiradicalar al drojdiei de bere, produs ce se evidenţiază nu numai prin valoarea multivitaminică ci şi prin conţinutul ridicat în glutation. Experimetul derulat pe o perioadă de 4 săptămâni cuprinde 4 loturi de şobolani albi (*Ratus norvegicus*), linia Wistar în vârstă de 6 săptămâni, având o masă ponderală medie de 143,55 g. Primul lot, alcătuit din 5 animale, a constituit lotul de referinţă. Cel de al doilea lot, format din 5 şobolani, a fost lotul de control pentru stresul oxidativ (lot control 1). Animalelor acestui lot li s-a administrat pentru inducerea stresului oxidativ ulei de floarea soarelui cu indice peroxidic ridicat în doză *pro die* de 10 ppm. Animalele din cel de al treilea lot, în număr de 5, au fost tratate concomitent cu ulei de floarea soarelui cu indice peroxidic crescut (doză *pro die* 10 ppm) şi glutation ca agent antioxidant în doză zilnică de 5 ppm (lot control 2). Ultimul lot, constituit, de asemenea, din 5 şobolani, a fost tratat cu doză identică de inductor al stresului oxidativ (ulei de floarea soarelui cu indice peroxidix mare) şi cu drojdie de bere ca agent antioxidant în doză *pro die* de 8 ppm (lot experimental). La finalul experimentului, sângele recoltat de la animalele sacrificate a servit investigaţiilor biochimice. Pentru aprecierea stresului oxidativ şi a efectului antioxidant al drojdiei de bere s-a cuantificat activitatea catalazei serice şi a superoxid dismutazei. Activitatea catalazei s-a efectuat prin metoda standardizată bazată pe principiul metodei Bach-Zubkova, iar pentru superoxid dismutaza s-a folosit metoda spectrofotometrică descrisă de Fried (6).

Tabel 1

Modelul experimental

Loturi	***Oleum Helyanthi* Doza *pro die***	**Glutation Doza *pro die***	**Drojdie de bere Doza *pro die***	**Investigaţie biochimică**
Lot de referinţă	-	-	-	SOD, catalaza
Lot control 1	10 ppm	-	-	SOD, catalaza
Lot control 2	10 ppm	5 ppm	-	SOD, catalaza
Lot experimental 1	10 ppm	-	8 ppm	SOD, catalaza

REZULTATE ŞI DISCUŢII

Rezultatele obţinute în urma cuantificării activităţii superoxid dismutazei din serul animalelor incluse în experiment sunt redate în tabelul 2 şi fig 2. Din studiul acestor date se observă că activitatea acestei enzime pentru lotul de referinţă se cifrează la valoarea de 321 U/g. Valoarea activităţii SOD pentru lotul de control 1, ce a fost tratat doar cu ulei de floarea soarelui cu indice peroxidic ridicat este diminuată cu aproape 100 U/g, ceea ce s-ar justifica prin consumarea metaloenzimei în reacţia cu radicalul liber O_2^- (226 U/g). Pentru lotul de control 2, lot ce a beneficiat de protecţia glautationului ca agent antioxidant, activitatea superoxid dismutazei

creşte semnificativ faţă de a lotului de control 1 la 275 U/g, valoare inferioară, totuşi, lotului de referinţă. O valoare apropiată de a lotului de control 2 (271 U/g) se înregistează şi pentru lotul experimental, ceea ce semnifică existenţa efectului antiradicalar al drojdiei de bere.

Tabel 2

Evoluţia superoxid dismutazei serice

Loturi	SOD (U/g)		
	Minima	Media	Maxima
Lot de referinţă	300	321	350
Lot control 1	205	226	250
Lot control 2	235	275	245
Lot experimental 1	225	271	350

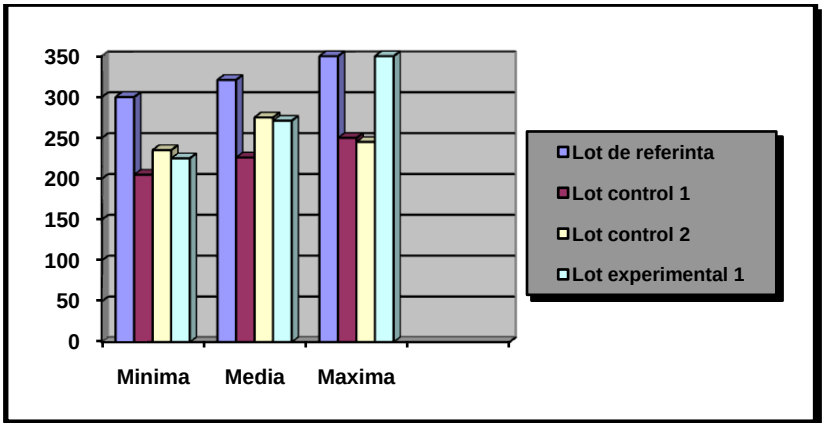

Fig. 2 Variaţia superoxid dismutazei

Investigaţiile biochimice referitoare la activitatea celei de a doua enzime, catalaza, enzimă ce acţionează în tandem cu superoxid dismautaza au dus la valori ce sunt redate în tabelul 3 şi fig 3. Studiul acestor date dezvăluie o evoluţie asemănătoare cu a superoxid dismutazei. Astfel, dacă pentru lotul de referinţă cifra catalazică este 3,01, acelaşi indicator înregistrează o scădere dramatică pentru lotul de control 1, această scădere putând fi efectul indirect al asaltului radicalilor liberi. O ameliorare semnificativă a activităţii catalazei în sensul apropierii de valoarea normală se constată la lotul de control 2, lot ce a beneficiat de protecţia antiradicalară a glutationului (2,44). Şi mai semnificativă este valoarea cifrei catalazice pentru lotul tratat cu drojdie de bere, valoare augmentată la 2,58, dar care este, totuşi, inferioară valorii caracteristice lotului de referinţă.

Tabel 3

Evoluția catalazei serice

Loturi	Catalaza (cifră catalazică)		
	Minima	Media	Maxima
Lot de referință	2,95	3,01	3,20
Lot control 1	1,05	1,24	1,80
Lot control 2	2,00	2,44	2,80
Lot experimental 1	2,10	2,58	3,05

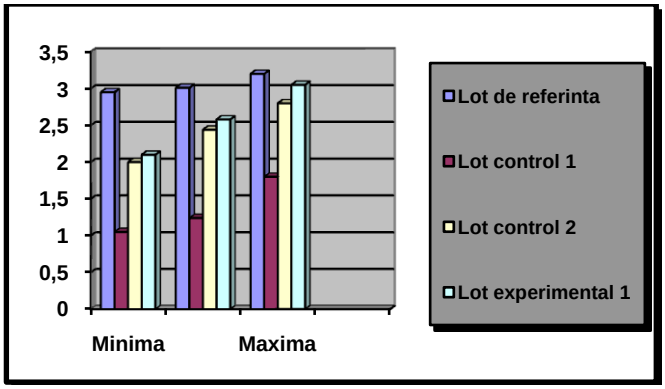

Fig. 3 Variația catalazei serice

CONCLUZII

- Activitatea superoxid dismutazei înregistrează o scădere dramatică pentru lotul de control 1(tratat cu ulei cu indice peroxidic ridicat) față de lotul de referință;
- Superoxid dismutaza atinge o valoare semnificativ crescută pentru lotul de control 2 (protejat cu glutation ca agent antioxidant);
- Activitatea superoxid dismutazei din serul lotului tratat cu drojdie de bere este apropiată de a lotului protejat cu glutation ;
- Evoluția catalazei din serul lotului de control 1 evidențiază o diminuare semnificativă față de lotul de referință ;
- Cifra catalazică a lotului tratat cu drojdie de bere se cifrează la o valoare uşor apropiată valorii lotului protejat cu glutation, ceea ce sugerează existența efectului antioxidant al acesteia.

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Morphological aspects in subclinic cardiac sarcocystosis in roe - deer (*Capreolus capreolus*)

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At a roe - deer shot at an organized hunt ton a hunting field of AGVPS Suceava, we observed a slight stasis in lungs, liver and kidneys.

Histological examination of the myocard pointed to a moderate parasitic invasion with Sarcocystis spp. (most probable S. bovicanis).

Structural modifications consist in the apparition, inside common cardiac fibres, of parasitophoric vesicles with ovoid sarcocysts with a translucid wall and a granular, intensely haematoxilinic content (the bradizoites).

Local mezenchimal reaction consists only of micronodular lymphohistioplasmocitar hiperplasia.

The principal intern organs present a slight ectasy and hematic overload of the venous circulatory sector.

Key Words: cardiac sarcocystosis, roe - deer (Capreolus capreolus)

The anatomical peculiarities about the brown bear skull

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The anatomical peculiarities about the brown bear represent a real scientific interst for morphological domain because the bear is an important animal for the professional haggard interest and the bear skull is a valuable trophy. Analyzing the morphological aspects we can remark much similitude between the bear and carnivour skulls. Very relevant in question are the external sagital crest,, nuke crests that are the excessive development, the zygomatic arch with his strong detaching that remember about the powerful temporal muscles. Laterally, the jaw bone has the developed canine socket, on the diastematic space are three rudimental premolars followed by three very strong molars. The articular condyle of the temporal bone appears such a gutter cavity with the main jointed axis is transversal oriented and the retroarticular process is high. Mandible points out through dorsal arching of its body that has a strong canine socket. The horizontal branch is thick, laterally and medial has the osseous crests detached near the base of the condyle. The mandible condyle is demicylindrical, is more transversal elongated and its jointing surface is at the same level with mandible mastication molars surface. The coronoid process hight and width, is caudal curved, the superficial sigmoid notch separates the condyl by coronocondylar process.

Key words: anatomy, bear, mandible, skull

MATERIAL AND METHODS

The study was made on the bear skulls, after theirs hunting. The osseous pieces were boiling prepared or cut out the adjacent tissues.

In this matter the pieces was submitted to the description of the form bones, length and development of the osseous processes and jointing surfaces. All of this was treated in comparison with the same structures had met at the reference species, the peculiarities discovered being photo illustrated.

INTRODUCTION

The brown bear is an important animal concern the professional haggard interest. The study about the anatomical particularities of the skeleton and especially on the brown bear skull is very interesting for the morphological discipline, or makes a solution concern the trespass litigation. The importance of this study is obtrusive by describing the morpho-functional aspects in relation with the habitual behavior (1, 4).

RESULTS AND DEBATES

The skull of brown bear presents the longitudinal axis (between the incisor plane and occipital protuberance), doubly than the transversal axis (between the zygomatic arches plan).

Dorsal face of the brown bear's skull is obtrusive by the round aspect of the anterior part of nasal bones. The dorsal face of the incisive bones, nasal processes and anterior limit of nasal bones delimit together an oval orifice, oblique dorso-cranial that is in fact the anterior openings of the nasal cavities (fig.3).

Dorsal, the nasal portion of the frontal bones are flat. Laterally, the zygomatic process is triangular, reduced, its base has, cranial and caudal, two vascular orifices. The parieto-temporal lines are detached by the base of zigomatic processes and then make together only sagital external crest. It is high, sharp and long and has the ending near the occipital external protuberance. The zygomatic arches are very strong detached, they are caudal continued by an osseous blade that rounded the ear external duct (4). Then, these are ventral prolonged by retrotympanic processes, thick and very developed, and in dorsal side with the nuke crests. The temporal grove is parasagital orientate, aspect that confirms the force in contraction of the temporal muscle (fig. 1, 2, 3).

Lateral face of the brown bear's skull. Laterally view, the skull and mandible are framed into quadrangle with the bigger border is with 40% longer than the shorter border. The canine socket is developed.

Orbit is incomplete limited, laterally is rounded by a small zygomatic process of the frontal bone and by the frontal process of the zigomatic bone. The infraorbital tubercle is like a mamelon, under it are present two lacrimo-nasal orifices (fig.2, 3).

The maxilar hiatus has the following orifices: infraorbital orifice, sphenopalatina and palatine major. The infraorbital orifice is 2-3cm off by the other (fig.6).

Dorso-ventrally, the orbital hiatus presents the following orifices: etmoidal foramen, optic orifice, orbital fissure, round orifice, cranial sphenoid wing orifice tied through the canal by the caudal orifice.

The ear duct appears such a gutter with the dorsal opening, is bounded by an osseous blade that arrived from the base of the zygomatic process of temporal bone. It is caudal prolonged with the very developed retrotimpanic process that outsize the paracondilar processes.

At the base of zygomatic process of the temporal bone, antero-ventral by the ear duct, is visible the oval orifice. The zygomatic arch, that is detached more caudal than cranial, is large and for it made was participated the process zygomatic of temporal bone ventral jointed with the temporal process of zygomatic bone, cranial jointed with the zygomatic process of jaw bone.

Ventral face of the skull at brown bear. The roof of the mouth is quadrangular in shape, has several inconstant line, anterior is limited by the upper incisors, laterally by each strong conic canines, three premolars reduced (rudimentary aspect) and three molars. The first molars has tubercles ordered like a club, the following molars have the bunodont aspect at masticate faces. The last molar is doubled longer than width (fig. 4,5).

Anterior, the roof of the mouth has two oval palatine fissure, vascular impress and only interincisiv orifice. Caudal, by the last molars, is located the maxilar tubercle that appears like an osseous crest, axial planed by a vascular grow.

The guttural opening is low and wide because the vomer bone is detached nearly the ending of the third cranial part of the oral cavity.

The horizontal blades of the palatine bones are caudal continued by an thick and rounded ventral caudal processes that bounded the guttural opening together with the pterigoid bones. These have a triangular and tabular hook form.

The jointing surface of the temporal bone isn't like a condyle form because the retroarticular process is high and jointing face is continued on it. In consequence, the articulary face is transformed into a cavity (fig.5).

The tympanic bulla is triangular and large.

Caudal face of the brown bear's skull.

The external protuberance of the occipital bone is triangular in shape, caudal displaced. Laterally, it is continued by the nuke crests, high and sharp, and ventrally, by a developed osseous blade (2,3). The caudal displaced till the jointing plan gives the half moon aspect of the horizontal part of the occipital. The nuke face has an axial osseous crest with the same profile (fig.1, 2).

The occipital condyls have a biconcave aspect, bounded by osseous crests. The occipital orifice is wide, the dorsal border has two osseous thorny separated by one notch.

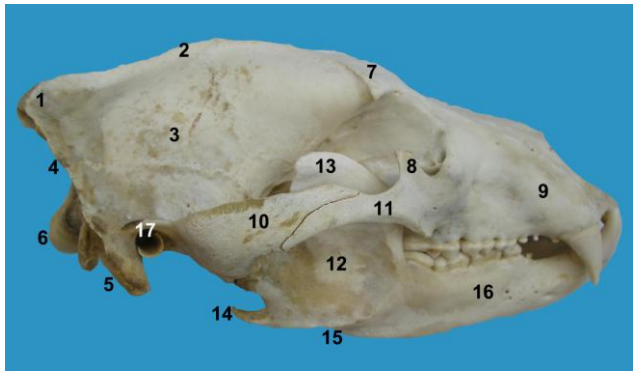

Figure1. Skull and mandible of the brown bear.

1. external occipital protuberance, 2. external sagittal crest, 3. temporal muscle fossa, 4. nuchal crest, 5. retroauricular process, 6. occipital condyle, 7. zygomatic process of frontal bone, 8. orbital process of zygomatic bone, 9. canine socket, 10. temporal bone, 11. temporal process of zygomatic , 12. masseter fossa, 13. coronoid process, 14. angular process, 15. vascular notch, 16. mental foramen, 17. external acoustic meatus.

Figure 2. Lateral view of brown bear skull.

1. occipital condyle, 2. external occipital protuberance, 3. retroauricular process, 4. paracondylar process, 5. . external acoustic meatus, 6. nuchal crest, 7. external sagittal crest, 8. temporal bone, 9. temporal process of zygomatic, 10. frontal process of zygomatic bone, 11. zygomatic process of frontal bone, 12. temporal fossa, 13. canine alveole, 14. maxilar hyatus, 15. pterygoid bone.

Figure 3. Dorsal view of brown bear skull.
1. external occipital protuberance, 2. nuchal crest, 3. external sagittal crest, 4. frontal, 4`. temporal line, 5. zygomatic process of frontal bone, 6. frontal process of zygomatic bone, 8. temporal fossa, 7. zygomatic arch, 8. nasal, 9. infraorbital foramen, 10. incisive, 11. palatine fissure, 12. incisive foramen.

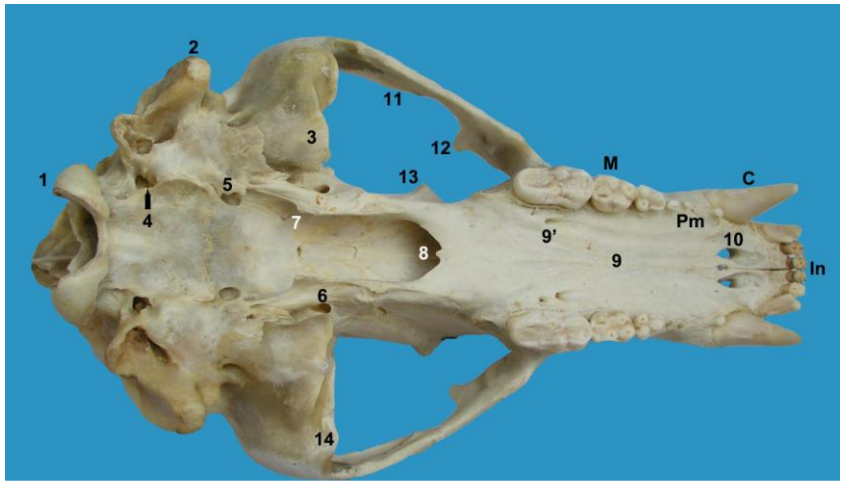

Figure 4. Ventral view of brown bear skull.
1. occipital condyle, 2. retroauricular process, 3. retroarticular process, 4. jugular foramen, 5. foramen lacerum, 6. oval foramen, 7. pterygoid bone, 8. choanal region, 9. hard palate, 9`. palate foramen, 10. palatine fissure, 11. zygomatic arch, 12. frontal process of zygomatic bone, 13. zygomatic process of frontal bone, 14. articular condyle of temporal bone.
I – incisive, C – canine, Pm – premolar, M – molar.

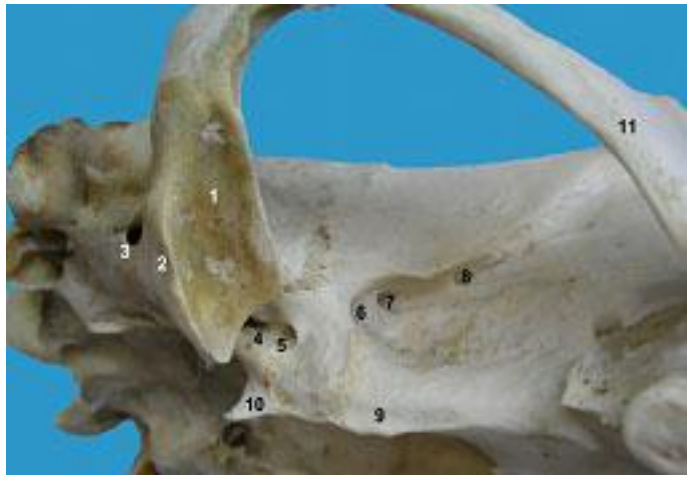

Figure 5. Hiatus orbital of brown bear skull.
1. condyle of temporal bone, 2. retroarticular process, 3. jugular foramen, 4. oval foramen, 5. caudal alar foramen, 6. rostral alar foramen, 7. orbital fissure, 8. optic canal, 9. palatine bone, 10. pterygoid bone, 11. zygomatic arch.

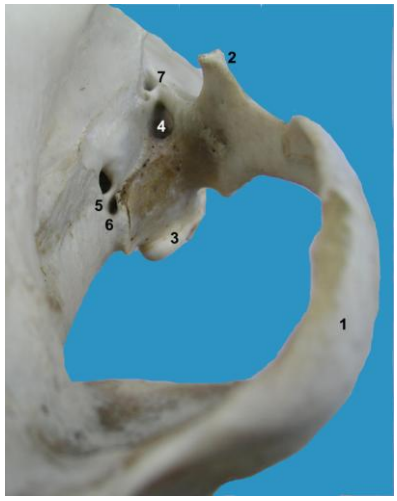

Figure 6. Maxilary hiatus of brown bear skull.
1. zygomatic arch, 2. frontal process of zygomatic bone, 3. maxilla bone, 4. maxilary foramen, 5. sfenopalatiny foramen, 6. palatine foramen, 7. foramen lacrimal.

The mandible at brown bear

The body of mandible is curved, the horizontal branch is thick and right. It has three incisor sockets, only very strong canine socket, at some bears in the diastemal space are present the rudimentary premolars, bat the last premolars is always present (fig.7).

From the last premolar till canine socket, there are the 5-7 orifices, 2-3 of them are larger then the rest (3). Axially, both mandibles are jointed together and this is transformed in time in sinostosis. The horizontal branches have a caudal divergent orientation, bat the molar masticate plans are parallel.

The curbed branch of mandible has a caudal and angular process that has 1,5-2 cm length and hook shape being separated through the notch (that makes three to four circle arch) by the

mandible condyle. The descent of the mandible condyle till the molars level is a very characteristic aspect of the species that have a powerful force in mandible (fig.7, 8).

The coronoid process is quadrangle representing the most of the curved face of mandible (2, 4). Laterally it has a deeply masseteric fosse, ventral bounded by a harsh line cranial continued till base by the angular process (fig. 8).

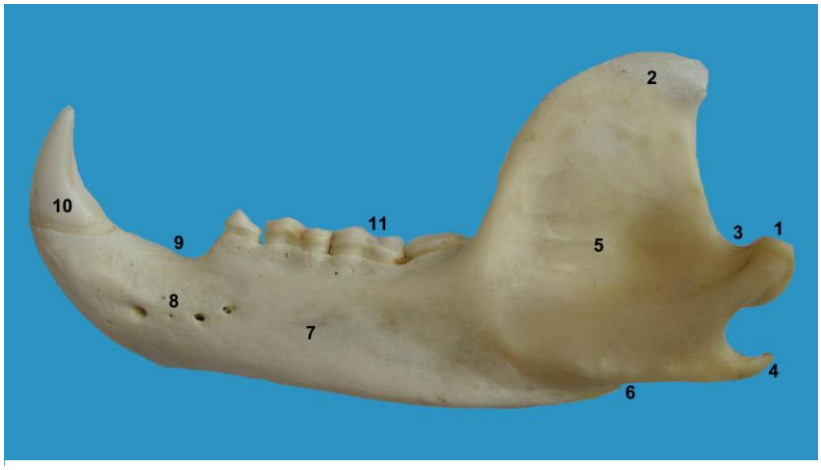

Figure 7. Lateral view of brown bear mandible.
1. condylar process, 2. coronoid process, 3. coronocondylar notch, 4. angular process, 5. masseter fossa, 6. vascular notch, 7. horizontal part, 8. mental foramina, 9. interdental space, 10. canine, 11. molars.

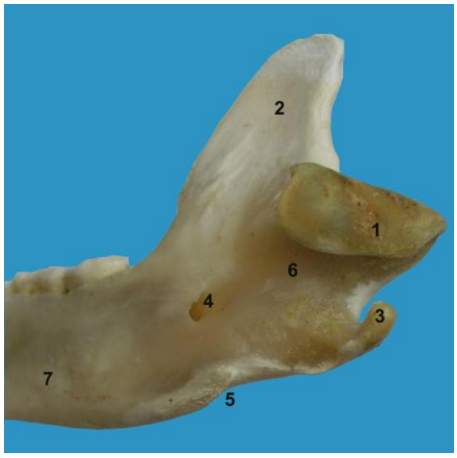

Figure 8. Vertical part of mandible – view medial.

1. condylar process, 2. . coronoid process, 3. angular process, 4. mandibular foramen, 5. vascular notch, 6. pterigoid fossa, 7. horizontal part.

Conclusions:

1. The temporal fosse, deeply, limited by the axial nuke crests and the dorsal border of the zygomatic arch, denoting a very powerful temporal muscle.
2. Like carnivorous, the orbit is incomplete limited by the zygomatic process of the frontal bone, and the frontal process of the zygomatic bone.
3. The jointing face of the condyle of mandible appears such a gutter transversal elongated because the jointing surface is prolonged on the retroarticular process that is very developed.
4. The jointing face of the mandible condyle is such a half of cylinder, is transversal elongated. This aspect demonstrate that the mandible make a moving only in vertical plan.
5. The coronoid process is very high, surpassing the condyle that descents till the masticate molars level.

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The anatomical peculiarities of the squirrel's skull (*Sciurus Vulgaris*)

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Being a rodent, the squirrel is an example of the species reaction to the living conditions by adapting to the locomotion type, preserving, at the same time, the common rodent characteristics. Having very developed nuke muscles, that guarantee the control during the jumping among the trees, the occipital bone is poor when it comes to the processes of muscles insertion, they are reduced to the nuke line and five round faces and to the paracondilar processes absence_that appear as a hardly visible tubercle. The squirrel's skull has a very little guttural hole because the wings of the sphenoid are reduced. The mandible is particular because of the solid mandible angle, the developed of the coronoid process and the mandible condyle location at the same level with the mandible molars.

Key words: squirrel, skeleton, skull, rodent, mandible

Material and work methods

The study of the morpho-functional peculiarities of the squirrel's skeleton was achieved through dissection and preparation of the osseous pieces after being cleared away from theirs adjacent tissues, following up the length and the aspect of each bone formations. The observed peculiarities were photographed, described and compared with the ones found to other species, following up their roles, according to the anatomical fundamental principles, in terms of cause and effect.

Introduction

Being integrated into the bigger Rodent Order, the squirrel is a representative example for the species adapted to the habitual conditions, for the adaptation of the skeleton morphologic characteristics that are dependent on the moving type among trees branches. Like the apendicular skeleton, the skull and the back bones have the morphological characteristics somewhere between the other terrestrial rodents and the felines, for example. These peculiarities are represented by the preservation of some rodent characters: at the skull, for example, by the prehension apparatus and the mastication type, but also by other aspects more resemblant to the feline skull, by the adaptation to the moving type.

Results and debates

Laterally viewed, the squirrel skull is also particular through the convex aspect of the neurocranium and the absence of the occipital bone from the external protuberance (Fig. 1, 2). Although the squirrel has developed cervical muscles, the nuke crest is low, it is reduced to the osseous semicircle outline that goes to the level of the occipital jointing condylus (1, 4).

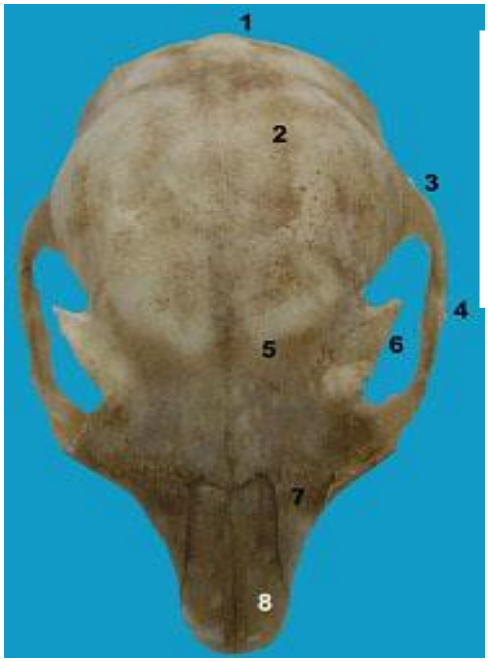


Fig.1 Dorsal aspect of the skull at squirrel
1-occipital bone, 2- temporal bone, 3- zygomathic process of temporal bone , 4 – zygomatic arch, 5 – frontal bone, 6- process orbital process of frontal bone, 7- incisive bone, 8- nasal bone

The areas for the muscles insertion on the occipital bone are represented by the five pisses of relief, with the same size, smooth and circular in shape, both dorso-ventral and transversal diameter are curved and they are separated by the fine groove (2, 3). These get to the jointing plane of the occipital bone. These aspects cause making an equal amplitude of the muscles force: both to the extensor muscles and the lateral rotator muscles of the head, all of them having an important role in coordinating and controlling the correct position of the body, especially during the jump moving from one tree branch to another (4, 5). The maximum extension of the head is allowed by the very low height of the spinossus process of the axis bone (Fig. 2).

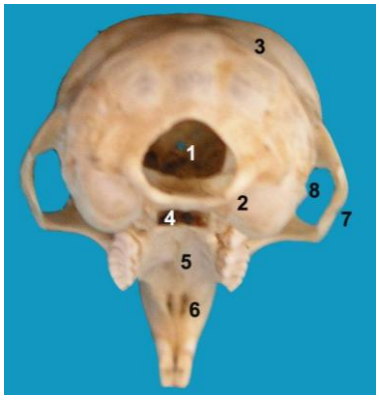


Fig. 2 Caudal aspect of the skull at squirrel
1- occipital foramen, 2- occipital condyle, 3- nuchal cresta, 4- choanal region, 5- hard palate, 6- palatine fissure, 7- zygomathic arch, 8- orbit

The other peculiarity of the squirrel skull is the absence of the paracondylar processes (1). These have a tubercular and reduced aspect and situated toward the dorsal edge of the occipital condylus. Laterally, between each condyle and paracondylar process each triangular space for muscular insertion is formed (Fig. 2, 3)

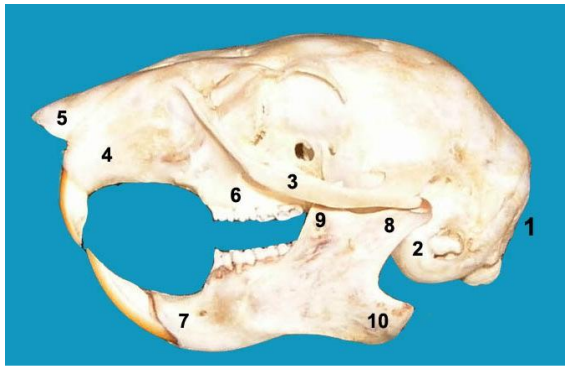


Fig. 3 The lateral view of the skull and mandible at squirrel
1- occipital bone, 2- tympanic bulla, 3- zygomathic arch, 4- maxilar bone, 5- nasal bone, 6- alveolar process of maxilar bone, 7- incisor process of mandible, 8- condylar process, 9- coronoid process, 10- angular process,

Most of the jointing surface of the condyl is developed in the horizontal plane, it lacks the vertical amplitude for moving. The occipital hole is approximately round (3). The basioccipital is flat and it hasn't any muscular relief (Fig. 2, 4).

Dorsally viewed, the cranium presents the evident convexity of the temporal bones. The zygomatic processes of the temporal bones are very slightly detached laterally, presenting on the longitudinal axis the articular surface (condylus temporalis) for jointing with the condyl of the mandible. The temporal fossa is hidden, the orbita isn't wholly formed, orbital process of the frontal bone being formed by an osseous blade, caudally oriented and pointed. (Fig. 1, 5)

Ventrally viewed, the squirrel skull presents a very short guttural hole because the wings of the sphenoid bone are reduced. The chonae opening is more cranial with 0,3mm in comparison with the guttural opening, this aspect being possible because the palatine bones, that don't have the pterigoid processes, prolong caudally the osseous palate (4, 5). Therefore, between the presphenoid and the palatine bones the nasopharinx is limited (Fig. 2, 4). The orbital hiatus has the following openings: the etmoidal hole, the optic nerve foramen, the orbitalis fissure and the cranial alaris orifice.

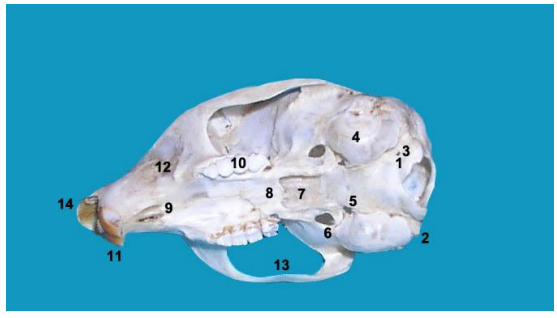


Fig. 4.Ventral view of skull at squirrel
1- occipital condyle, 2- paracondylar process, 3- hipoglosal canal, 4- tympanic bulla, 5- foramen lacerum, 6- oval foramen, 7- choanal region, 8- hard palate, 9- palatine fissure, 10- molars, 11- incisors, 12- infraorbital fissure, 13- zygomathic arch,14- nasals

The hiatus of the jaw bone has a large sphenopalatine orifice, the palatine fissure is approximately rounded by the palatine bone and the alveolar process of the last jaw molar.

The jaw bone is very developed. The zygomathic process appears like a horizontal detachment, under the form of an osseous blade in shape at the basis of which we can find, the very narrow infraorbital orifice, like a fissure. (Fig. 2, 4)

The incisive bones are very massive, they have an evident development, delimitating the lateral part and half of the roof of the nasal cavity (Fig. 1, 4, 5).

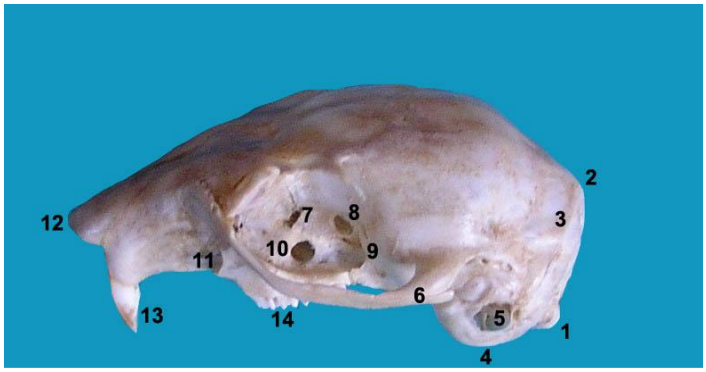


Fig. 5 Lateral view of squirrel's skull

1- occipital condyle, 2- external occipital protuberance, 3- nuchal crest, 4- tympanic bulla, 5- external acoustic meatus, 6- zygomathic arch, 7- etmoidal foramen, 8- optic canal, 9- orbital fissure, 10 maxillary foramen, 11- infraorbital fissure, 12- nasal bone,

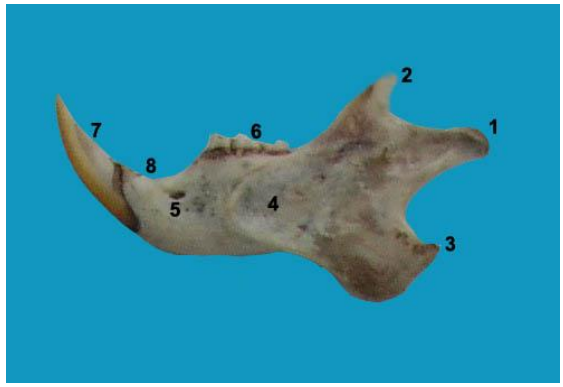
The alveolar process of the incisive bone is very deep, going to the basis of the jaw bone. The incisors have unlimited rise and a chisel form. The peculiarity of the prehension mechanism is given by the fact that the squirrel doesn't break the hard shell of the fruits, she rubs cutting out layer by layer the shell. This process doesn't need a special extra force developed by the musculatures but it is produced by the propulsion and retraction movement of the mandible. (Fig. 3)

The mastication process is completed by breaking the food into little pieces between the molars. The abrasion surfaces of the jaw molars have a convex cranio-caudal aspect, being bounded by the molar crests (3,6).

The mandible is special by the massiveness of its angle. The condyl of the mandible is placed near the abrasion plane of the mandible molars and it develops a big force when raising the mandible. (Fig. 6)

The development of the corono-condylaris process much over the molars, it develops a force of the temporal muscle for cutting the food like the power pincers, between the tops of the mandible incisor and coronocondylar process it is formed a semicircle.

The development of the fosse maseterica up to the first mandible molar, the expansion and the depth of the pterigoid fosse denote the implication and development of the mastication muscles. Another characteristic of the squirrel mandible is represented by the angular process that is flat, quadrangle in form, that having the places for insertion of the retractor and descending mandible muscles. (Fig. 6)



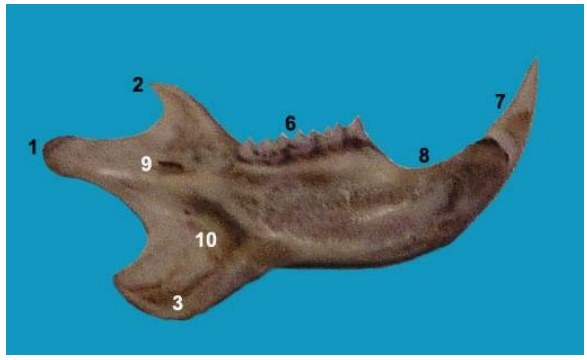


Fig. 6 Mandible at squirrel

1- condylar process, 2-coronoid process, 3- angular process, 4- masseter fosse, 5- mental foramen, 6- molars, 7- incisor, 8- interdental space, 9- mandibular foramen, 10 ptherigoids fosse.

Conclusions:

1. Although the squirrel has developed cervical muscles, the nuke crest is short, it is reduced to the osseous semicircle line.
2 The squirrel skull is characterized by the absence of the paracondilar process that has a tubercular and reduced aspect
3. Most of the jointing surface of the condyle is developed in the horizontal plane, and it lacks the vertical amplitude for moving.
4. The squirrel skull presents a very short guttural hole because the wings of the sphenoid bone are reduced.
5. Mandible is particular by the massiveness of the mandible angle, the development of the coronoid process and the mandible condyle location at the same level with the mandible molars.

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Oxidative stress in experimenthal hyperlipidemia

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The goal of this work was the evaluation of the lipid peroxidation by the quantitation of the plasma MDA, and of the antioxidant enzymatic defensive by the quantitation of both SOD and GPX activities in rats and rabbits fed an hyperlipidemic diet. Blood samples were obtained at the end of the experiment from each lot fed a standard diet (control) and an hyperlipidemic diet (L_1 lot) . The statistic analysis of the investigated blood values showed significant variations between L_1 lot and control. The investigation of the aforementioned markers showed that rats were more tolerant to the dietary-induced oxidative stress than rabbits.

Key words: cholesterol, atherogenic diet, oxidative stress, rabbits, rats

INTRODUCTION

Risk factors in atherosclerosis enhance lipid peroxidation process that increase the tissue free radical levels and cause a dismetabolic condition known as the oxidative stress (4).

The goal of this work was the evaluation of the lipid peroxidation (by quantitation of MDA), as well as that of the antienzymatic enzymatic defensive (by quantitation of SOD & GPX activities) in rats and rabbits fed an hyperlipidemic diet.

MATHERIALS AND METHODS

Two ten-subject lots of healthy albino rabbits, aging 10-12 months and weighing 2,5-3 kg each, were formed: $L_1$and M lots.

- Subjects in the L_1 were daily fed a standard diet to which 0,5 g cholesterol and 2 g methilthiouracil in 2 ml of sunflower seed oil for each kg of body weight was added
- Subjects in the M lot were daily fed the standard rabbit diet.

Twenty Wistar healthy rats were divided into two lots, to whom differentiated diets were given, as follows:

- Standard diet: semisintetic, balanced, containing 19% crude protein and 1% fats for the *control lot(M)*;
- Atherogenic diet: standard diet supplemented with 40% sunflower seed oil, 2% cholesterol, 0,4% methithiouracil and 1% dehidrocolic acid for the *L_1 lot* .

During the twelve- week experimental period, subjects in all lots were fed and watered *ad libitum*. At the end of the trial blood samples were collected in heparinated tubes to obtain plasma and red cells.

Three oxidative stress markers were investigated: malonil dialdehide (MDA); glutathion peroxidase (E.C. 1.11.1.9.) (GPX); superoxid dismutase (E.C. 1.15.1.1.) (SOD). Plasmatic MDA was quantitated by Ohkawa method (9), a spectrophotometric method using a coloured derivative of the thiobarbituric acid.

Plasmatic GPX was quantitated by yhe Fukuzawa method (10) using a DNTB derivatise whose absorbtion was measured at 412 nm against the blank.

Haematic SOD activity was determined by the Minami method (11), a spectrophotometric method that uses nitroblue tetrasolium as an oxidative agent whose inhibited reduced form, formasane, was measured at 540 nm.

The analytical results were statistically processed by the "t" Student test and were expressed as mean value ± standard error deviation.

RESULTS AND DISCUSIONS

Tabel 1

Values of some oxidative stress markers in rabbits and rats fed an hyperlipidemic diet

Lot	*Rats*			*Rabbits*		
	Plasma MDA (μmol/l)	***Red cell SOD (U_{SOD}/l)***	***Plasma GPX (IU/l)***	***Plasma MDA (μmol/l)***	***Red cell SOD (U_{SOD}/Ht)***	***Plasma GPX (IU/l)***
L_1	*4,4±1,05*	*4,8±0,05*	*112,5±5,49*	*5,1±0,37*	*4,5±0,28*	*138,3±9,23*
M	*1,9±0,70*	*5,1±0,08*	*95,1±11,39*	*2,5±0,33*	*5,8±0,15*	*125,7±12,6*
Statistic significance	*p<0,001*	*p<0,001*	*p>0,2*	*p<0,001*	*p<0,001*	*p<0,1*

The results are shown in tab. 1 in which plasma MDA concentrations, red cell SOD and plasma GPX activities are presented as X ± SED of ten subjects in each lot (rabbits/ rats) at the end of the experiment.

In general, the variation of the absolute concentration values were lower in rats as compared to rabbits (12) confirming previous reports (13, 14) that the developement of the atherogenic process is much slower in the former :

- Subjects in the L_1 lot had significantly higher (p<0,1) MDA concentrations (4,490±1,054) as compared to those in the control (1,990±0,707).
- The haematic values of SOD at the end of the trial were of the same dimension order and statistical significance as those in the rabbit trail; L_1 lot showed significantly lower (p<0,001) mean value as compared to control. These values indicated that subjects in the L_1 lot were more exposed to the oxidative stress than those in the control.
- The GPX activity at the end of the 12-week trial showed nonsignificant differences between experimental lot and the control. Overall, the GPX activity was significantly lower in rats (112,500±5,491 in the L_1 lot as compared to rabbits (138,350±9,231 respectively).

CONCLUSIONS

1. MDA mean values at the end of the atherogenic experiment were significantly higher in L_1 lot as compared to the control
2. SOD mean values at the end of the experiment were significantly lower in the L_1 lot as compared to the control
3. GPX mean values were nonsignificantly different in either of the two experimental lots as compared to the control.
4. Levels of the investigated oxidative stress markers showed that rats were more tolerant to the dietary-induced oxidative stress than rabbits.

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Researches regarding the influence of copper on mastitis in dairy cows

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Antioxidant nutrients (vitamin E, vitamin C, Selenium, Copper, Zinc, β-caroten) are essential for theprotection of immune cells during an inflammatory response. In a healthy mammary gland, the number of somatic cells is up to 200.000 per ml of milk, and in the hours follwing the an intramammary infection, it can rise up to a few millions.

To evaluate the importance of Copper on the frequency and evolution of mastitis in cows, a study was conducted within the Research and Development Station for the Breeding of Cows, Dancu, Iasi. 16 clinically healthy cows in the same period of lactation were selected, 8 of which were given 2 gramms of Copper Sulphate per os, per week, according to NRC 2001, and the other 8 were the control lot. At the beginning of the study, 12 cows, 6 of which from the “copper” lot, had a Somatic Cell Count higher than 200.000 / ml of milk.

After 2 months of administration of Copper Sulphate in the “Copper” lot, only 2 cows reacted positive to the R-Mastitest subclinical mastitis detection test, the bacteriological test showing Streptococcus spp. and Staphylococcus spp.

Key Words: mastitis, dairy cow, somatic cells, bacteriological exam

Tuberculosis- a common epidemiological approach for human and veterinary

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Tuberculosis is an infectious disease, with an enzootic character situated in antropozoonosis category. It is met at humans and also to several species of domestic and wild mammals, as well as to birds, but the most important for animals pathology is bovine tuberculosis.

Some developed countries succeed in the struggle against bovine tuberculosis, which allowed them to obtain "free disease" status, such as Germany and Netherland. Other countries, such as France, is trying to obtain the "free disease" status, but there are other countries, like Romania, that are facing with this disease for very long time, without making substantial progresses, in the control of this disease, although the incidence of the disease is at a relative low level.

At the moment, according to the World Health Organization statistics, Romania is on the top of the list, at number four, in Europe, concerning the incidence of human tuberculosis. Therefore, 130 cases at 100.000 inhabitants are appearing every year, which means almost 30.000 diseased people in the whole country (the European mean is 30 diseased people at 100.000 inhabitants). There are reported 5 new cases in children every day, 82 new cases in adults and 5 people dies every day because of this disease.

The Romania tuberculosis epidemiological parameters have the highest values from the entire Europe.

Testing the number of animals which has been confirmed positive for tuberculosis by lab tests (anatomy and pathology tests, direct bacteriologic tests, histological tests, biological tests on guinea pigs) during the period between 2001-2006, we noticed that the incidence of tuberculosis in animals decreased up to the 2004 years, but the incidence of tuberculosis in humans is higher.

Key words: tuberculosis, epidemiology, control, physician, veterinarian

Introducere

Tuberculoza, o boală infecto-contagioasă, având caracter enzootic încadrată în categoria antropozoonozelor, se întâlneşte atât la om cât şi la numeroase specii de mamifere domestice şi sălbatice, precum şi la păsări, iar cu importanță deosebită pentru patologia animală este tuberculoza bovină.

Unele țări dezvoltate au înregistrat succese remarcabile în lupta contra tuberculozei bovine, fapt care le-a permis obținerea statutului de țară indemnă la această boală, cum ar fi Germania şi Olanda. Alte țări sunt în curs de obținere a acestui statut, de exemplu Franța însă cele mai multe, printre care şi România, se confruntă cu tuberculoza de foarte multă vreme, fără ca în ultimii ani,

să se înregistreze progrese semnificative privind eradicarea, cu toate că incidența bolii este la un nivel relativ scăzut.

În ceea ce priveşte acțiunile de monitorizare a tuberculozei în populația de bovine din România, acestea au fost continue şi susținute, iar controlul şi eradicarea bolii au fost cuprinse în „Programul Strategic de supraveghere, prevenire si control al bolilor la animale, al celor transmisibile de la animale la om, protecția animalelor şi protecția mediului”, actualizat în fiecare an şi aprobat prin Ordin al Preşedintelui Autorității Naționale Sanitare Veterinare şi pentru Siguranța Alimentelor.

Cu toate că nu este cunoscută cu exactitate, din literatura de specialitate se estimează, că în raport direct cu incidența tuberculozei bovinelor, frecvența tuberculozei umane produsă de *Mycobacterium bovis* este cuprinsă între 1 şi 5%. Aceasta constatare nu este concludenta deoarece este necesară tipizarea tuturor izolatelor umane (identificarea genului de *Mycobacterium*), un aspect al bacteriologiei tuberculozei neutilizat în țara noastră pe scara largă. Fără această informație, contribuția animalelor în transmiterea bolii la oameni nu oferă date reale si nu se poate formula o concluzie asupra relației om-animale in transmiterea tuberculozei.

Lupta împotriva tuberculozei trebuie dusă simultan la animale şi la om, pe un front larg în care să fie mobilizați prin lege să coopereze atât medicii umani, cât şi cei veterinari întrucât efectivele indemne sau asanate pot fi recontaminate printr-o sursă de infecție umană după cum în multe gospodarii în care sunt animale tuberculoase se pot depista şi cazuri de tuberculoză umană.

Material şi metodă

În abordarea epidemiologică a tuberculozei s-a recurs la efectuarea unor anchete retrospective, analizând datele existente la Autoritatea Națională sanitară Veterinara şi pentru Siguranța Alimentelor şi Institutul de Diagnostic şi Sănătate Animală in perioada 2001-2006 care concentrează şi prelucrează informațiile primite de la direcțiile sanitare veterinare şi pentru siguranța alimentelor județene precum şi date de la Ministerul Sănătății Publice, Autoritatea de Sănătate Publică.

Prelucrarea datelor s-a făcut în conformitate cu metodele epidemiologiei descriptive şi analitice, fiind realizate reprezentări sub formă de tabele, hărți şi grafice. Acestea au permis să se facă evaluări privind evoluția tuberculozei prin studierea interrelatiilor dintre cei trei factori (surse epidemiogene, modalități de transmitere şi populația receptivă) care guvernează apariția şi dezvoltarea procesului epidemiologic şi formează împreună „triada epidemiologică” sau „lanțul epidemiologic”

Rezultate

În România pentru diagnosticul tuberculozei bovine, se folosesc, în mod curent, două teste tuberculinice testul intradermic unic (TU) care utilizează tuberculină bovină şi testul comparativ simultan (TCS) care foloseşte tuberculină bovină şi tuberculină aviară.

Testarea periodică a bovinelor pentru depistarea animalelor infectate şi evitarea introducerii acestora în exploatațiile libere de tuberculoză a fost efectuată până in anul 2002 la toate bovinele de pe întreg teritoriul țării în vârstă de peste 6 luni iar din anul 2003 începând de la vârsta de peste 6 săptămâni prin test tuberculinic unic.

Decelarea hipersensibilității de tip întârziat la animalele infectate este testul cel mai utilizat în diagnosticul tuberculozei bovine şi, în ciuda unor limite privind sensibilitatea şi specificitatea, a reprezentat şi reprezintă încă fundamentul programelor prin care se speră să se ajungă la eradicarea sau la reducerea semnificativă a incidenței acestei boli.

Pentru stabilirea statusului real al animalelor, ideal este ca sensibilitatea şi specificitatea testelor tuberculinice să aibă valori cât mai apropiate de 100%, ceea ce ar asigura succesul programelor de asanare a bolii.

În practică, însă, în funcție de un complex de factori intrinseci sau extrinseci, valorile acestor parametri cunosc variații semnificative, dar pot atinge, uneori, valori maxime de 95-96% sau chiar de 98-99% .

Un aspect important privind diagnosticul alergic al tuberculozei este reprezentat de frecventele reacții fals pozitive dintre care unele sunt reale, putând fi denumite nespecifice, iar altele, aparente, explicabile prin nedecelarea leziunilor specifice, care de cele mai multe ori au dimensiuni submacroscopice, ori nu sunt depistate dacă examenul post-mortem se face superficial.

Sensibilizarea nespecifică față de tuberculină este indusă de factori foarte numeroşi, putând fi diferențiate reacții paraalergice generate de alte micobacterii decât M. bovis şi reacții pseudoalergice datorate unor procese patologice infecțioase, parazitare, micotice, virale, unor stări fiziologice particulare sau calității produsului revelator folosit.

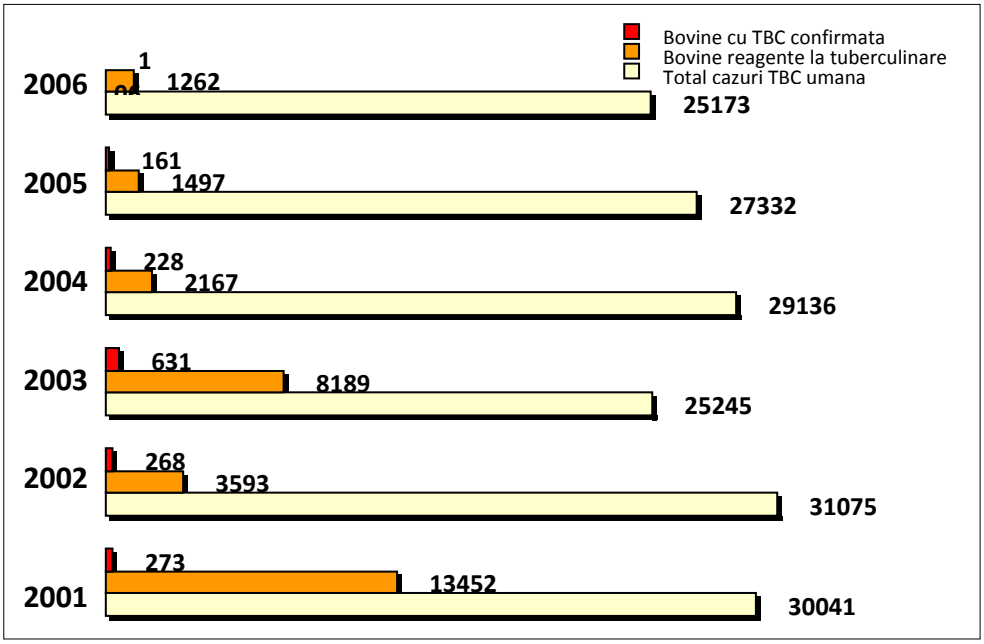
În prezent, conform statisticilor Organizației Mondiale a Sănătății, România este printre primele 4 țări din Europa în ceea ce priveşte incidența tuberculozei umane. Astfel, anual apar 130 de cazuri la 100.000 de locuitori, adică aproximativ 30.000 de bolnavi în toată țara (media europeană este de 30/100.000 locuitori), zilnic sunt raportate 5 cazuri noi de tuberculoză la copii, 82 de cazuri noi la adulți şi 5 decese din această cauză. Indicatorii epidemiometrici ai tuberculozei înregistrează în România cele mai ridicate valori din Europa.

În tabelul de mai jos sunt redate comparativ cazurile de tuberculoză la om şi animale în perioada 2001-2006

Analizând numărul de animale cu tuberculoză confirmată în urma examenelor de laborator (examen anatomopatologic, examen bacterioscopic, examen histologic, bioproba pe cobai) in perioada 2001-2006, am observat că incidența tuberculozei la animale a scăzut începând cu anul 2004 insă la om incidența s-a menținut la un nivel ridicat.

De asemenea s-a constatat ca există un mare număr de animale reagente la TCS comparativ cu cazurile confirmate iar cauza poate fi numărul mare de reacții fals pozitive care se datorează unor cauze sensibilizant-nespecifice, utilizării unor tuberculine cu calitate îndoielnică sau insuficient verificată si efectuarea unor investigații de laborator prin teste cu sensibilitate scăzută.

JUDET	2001			2002			2003			2004			2005			2006		
	om	bovine		om	bovine		om	bovine		om	bovine		om	bovine		om	bovine	
	total cazuri TBC	Reagente TCS cap	Confirmate TBC cap	total cazuri TBC	Reagente TCS cap	Confirmate TBC cap	total cazuri TBC	Reagente TCS cap	Confirmate TBC cap	total cazuri TBC	Reagente TCS cap	Confirmate TBC cap	total cazuri TBC	Reagente TCS cap	Confirmate TBC cap	total cazuri TBC	Reagente TCS cap	Confirmate TBC cap
AB	342	85	3	309	14	9	355	19	4	351	20	2	367	3		340		
AR	553	183	14	637	90	9	572	53	5	643	71		647	35	2	693	266	8
AG	793	38	4	934	272	6	809	14	5	870			749			635		
BC	958	9		986	8		819			1071	2		964			896		
BH	657	135	3	865	70	41	628	70	32	662	74	30	613	28	3	493	27	5
BN	266	11		248			262			267			265			253		
BT	573			621	1	2	654	6	4	841	22	2	670		1	595	42	16
BV	583	111	16	550	5	5	440	18		474	20	1	447	50		370	25	
BR	448	713	1	442	27	9	394	1028	3	475	39	4	427	45	7	376	20	1
BZ	520			563	16	3	487		2	509	12		507	7		415	9	
CS	515	277	2	491	3		393	5		412	3		448	37		390	14	
CL	515	185		598	3		373		1	461	2		401	65	1	397	33	
CJ	738	2268	1	732	417		552	1083		614	515		477	23		453	2	
CT	1308	433	5	1408	124	81	1154	239	149	1360	56	1	1246	17	1	1133	11	1
CV	102	171	58	112	70	52	82	422	223	107	116	65	111	295	34	91	21	9
DB	789	955		827	4		699	305	1	800	6		674	189		647	281	
DJ	1214	217	19	1182	33	10	1044	18	7	1207	8		1234	15	1	1216	35	
GL	1237	8	4	1328	13	2	851	27	12	1035	7	8	1090	2		937	12	
GR	519	197		551	2		409			530	2		490			464		
GJ	531			574			461			541	11		562			473!		
HR	199	1363	3	137	801	2	157	2934		148	190	4	162	48		152	40	
HD	636			642	437		573			677	0		579			580		
IL	362	141	70	379	50	5	296	12	5	371	15	2	341	3	2	344	16	3
IS	1258	27		1236	255		973			1161	2		1232			1142		
IF	678	191	16	633	10	3	491	2	1	569	6		527	1		474		
MM	740	254		689	119		536	29	14	707	188	42	516	29	4	545	9	3
MH	398	65	48	555	11	7	432			533			519			444		
MS	688	2328		651	8		607	12		753	12		717	8		641	4	
NT	892	6		823			560			723	32		766			864	1	
OT	1065	99		985	11		754	18	9	897	49		905	57	22	771	44	2
PH	913	411		994	247		852	579		954			886			957		
SM	546	996		580	286		532	1127	108	596	287	45	483	343	78	472	203	41
SJ	351	575	3	314	10	5	284	17	4	269	21	2	272	10		235	3	
SB	263	207		245	21		284			~~340~~	33		322			287	1	
SV	858	2	1	889	1		828			823			694			668	5	
TR	767	59	2	827	21	14	658	86	37	701	36	5	643	17	4	587	41	13
TM	1106	209		1311	3	3	931	13	3	1058	45	13	1018	6	1	937	10	
TL	409	238		467	57		346			398			373	12		317		
VS	720	244		824	34		635	17	2	729	258	2	646	146		640	85	6
VL	472			535			452			541			577			415		
VN	481	34		504	39		416	36		552	7		468	1		409	2	
Mun. B	3078	7		2897			2210			2433			2297	0		2026		
TOTAL	30041	13452	273	31075	3593	268	25245	8189	631	29163	2167	228	27332	1497	161	25173	1262	106



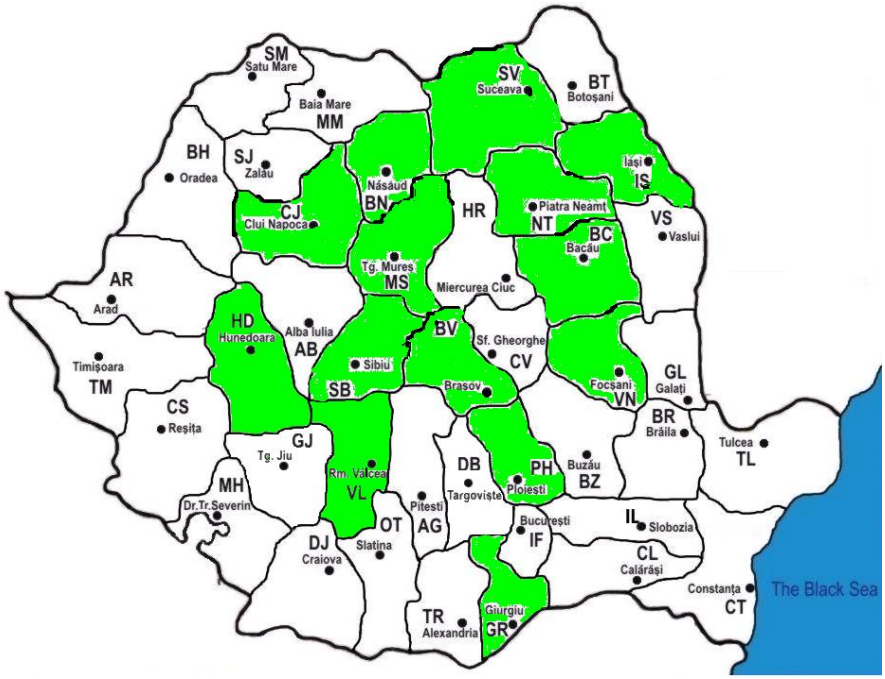
Exprimarea statistica a cazurilor de tuberculoză la animale şi om pe anii 2001-2006

In ceea ce priveşte tuberculoza bovină s-a constatat că datorită acțiunilor de control şi eradicare aplicate pe teritoriul României, județele Hunedoara şi Vâlcea au devenit indemne, iar în județele, Bacău, Bistrița, Braşov, Giurgiu, Iaşi, Mureş, Prahova, Vâlcea şi Vrancea s-a înregistrat o descreştere continuă a incidenței tuberculozei bovine.

Întrucât, în perioada 2001 – 2006, procentul de infecție cu *Mycobacterium bovis* a efectivelor de bovine din România este sub 0,2%, Autoritatea Națională Sanitară Veterinară şi pentru Siguranța Alimentelor va aplica începând cu anul 2008, următoarea metodologie de supraveghere, monitorizare şi eradicare a tuberculozei bovine:

a) efectuarea unei singure tuberculinări pe an, în trimestrul 2, în județele: Alba, Arad, Argeş, Bihor, Botoşani, Brăila, Buzău, Caraş-Severin, Călăraşi, Constanța, Covasna, Dâmbovița, Dolj, Galați, Gorj, Harghita, Ialomița, Ilfov, Maramureş, Mehedinți, Olt, Satu-Mare, Sălaj, Teleorman, Timiş, Tulcea, Vaslui;

b) eliminarea testării alergice la bovinele din următoarele județe în care nu s-au înregistrat animale infectate în ultimii 5 ani: Bacău, Bistrița Năsăud, Braşov, Cluj, Giurgiu, Hunedoara, Iaşi, Mureş, Neamț, Prahova, Sibiu, Suceava, Vâlcea, Vrancea şi Municipiul Bucureşti.



- ***judeţe in care s-au eliminat testarile alergice***

In tabelul de mai jos sunt redate datele epidemiologice pe perioada 2002-2006, din care rezulta procentul de bovine tuberculinate din efectivul total şi procentul de bovine pozitive din total tuberculinate precum si numărul de bovine abatorizate si cele cu rezultat pozitiv.

Din acest tabel rezultă că România a făcut eforturi deosebite privind testarea alergică a efectivelor de bovine cu procente apropiate de 100% însă această activitate nu a fost dublată şi de un efort la fel de consistent privind aplicarea celorlalte masuri din programul de eradicare fapt ce ne menţine încă cu un nivel ridicat al incidenţei tuberculozei bovine comparativ cu celelalte ţări din Uniunea Europeană.

Date epidemiologice privind tuberculoza bovină în perioada 2002-2006

Anul	*Nr. total de bovine (a)*	*Nr. total de bovine supuse programului de testare (b)*	*Nr. total de bovine testate (b)*	*Nr. Total de bovine testate individual (c)*	*Nr. De bovine pozitive*	*Tăieri*		*Indicatori*	
						Nr. de bovine tăiate cu rezultat pozitiv	*Nr. total de bovine tăiate (d)*	*% testate din bovine supuse programului de teestare*	*% prevalenţa bovine pozitive*
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9=(4/3)x100*	*10=(6/4)x100*
2002	*2644027*	*2326694*	*2309022*	*2309022*	*964*	*565*	*215316*	*99.2*	*0.04*
2003	*2727826*	*2607494*	*2604273*	*2604273*	*1360*	*1065*	*250105*	*99.8*	*0.05*
2004	*2680179*	*2587736*	*2558734*	*2558734*	*810*	*558*	*234100*	*98,8*	*0.03*
2005	*2733997*	*2606226*	*2595718*	*2595718*	*771*	*400*	*149235*	*99.5*	*0.029*
2006	*2754119*	*2612521*	*2558945*	*2558945*	*538*	*541*	*167989*	*97.94*	*0.021*

(a) Nr. total de animale

(b) Include bovinele testate individual sau supuse unui test de grup (ex. testul de lapte din tanc)

(c) Include numai bovinele testate individual

(d) Include toate bovinele pozitive abatorizate si de asemenea bovinele negative abatorizate supuse programului.

În acest context considerăm că pentru eradicarea tuberculozei bovine este necesară aplicarea în mod unitar a următoarelor măsuri:

- testare si dirijarea imediată la tăiere a bovinelor pozitive;
- prevenirea răspândirii bolii prin controlul mişcării permițându-se doar mişcarea animalelor indemne;
- inspecții post-mortem şi după abatorizare a tuturor bovinelor in direcția tuberculozei;
- asigurarea trasabilității prin buna funcționare a sistemului de înregistrare şi identificare;
- asigurarea de fonduri financiare pentru despăgubirea animalelor tăiate pentru supraveghere.

Se estimează, că în raport direct cu incidența tuberculozei bovinelor, frecvența tuberculozei umane produsă de *Mycobacterium bovis* este cuprinsă între 1 şi 5%. Însă această constatare nu este concludentă deoarece este necesară tipizarea tuturor izolatelor umane (identificarea genului de *Mycobacterium*), un aspect al bacteriologiei tuberculozei absent în țara noastră. Fără această informație, contribuția animalelor în transmiterea bolii la oameni nu oferă date reale şi nu se poate formula o concluzie asupra relației om-animale în transmiterea tuberculozei.

În esență, principalii factori de risc pentru populați umană sunt:

- contactul fizic strâns între om şi animalele infectate; în țara noastră 40% din populație trăieşte în mediul rural şi lucrează în agricultură;

- practicile privind igiena alimentară prin consumul de produse lactate crude sau numai acidulate care pot fi contaminate cu *M. bovis* a reprezentat mult timp principalul mod de transmitere a tuberculozei de la animale la om manifestată îndeosebi prin limfadenită cervicală, forme abdominale şi alte forme extrapulmonare.

Concluzii

- Lupta împotriva tuberculozei trebuie dusă simultan la animale şi la om, pe un front larg în care să fie mobilizați prin lege să coopereze atât medicii umani, cât şi cei veterinari întrucât efectivele indemne sau asanate pot fi recontaminate printr-o sursă de infecție umană după cum în multe gospodarii în care sunt animale tuberculoase se pot depista şi cazuri de tuberculoză umană;
- In perioada 2001- 2006, nu s-a diagnosticat tuberculoza bovină in unele județe insa incidenta tuberculozei la om este mare ceea ce poate sa conducă la doua concluzii respectiv ca bovinele nu sunt sursa de infecție pentru oameni din județele respective sau ca nu au fost comunicate date reale privind diagnosticul tuberculozei bovine mai ales prin examene paraclinice (TU, TCS);
- Considerăm ca pentru reuşita unui program de eradicare a tuberculozei bovine la nivel național se impune o evaluare a riscului pentru a determina modul corespunzător de acțiune deoarece costurile unui astfel de program sunt foarte mari, necesarul de medici veterinari pentru efectuarea de testări tuberculinice este de asemenea mare iar acestea pot influenta fezabilitatea programului;
- Prevenire transmiterii infecției de la animale la om este un obiectiv major care poate fi realizat numai prin grija pentru menținerea unei sănătăți publice adecvate, prin programe eficiente de educație privind inclusiv măsurile cele mai corespunzătoare de igienă;
- Este necesară o inițiativă legislativă comună Autorității Naționale Sanitare Veterinare şi pentru Siguranța Alimentelor precum şi a Ministerului Sănătății şi Familiei privind controlul tuberculozei compatibil cu programele existente în Uniunea Europeană şi care să conducă la eradicarea acestei boli în România.

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11. Ordinul ANSVSA nr. 104 din 19 octombrie 2005 pentru aprobarea Normei sanitare veterinare privind criteriile pentru planuri naţionale de eradicare accelerată a brucelozei, tuberculozei şi leucozei enzootice bovine;
12. Ordinul ANSVSA nr.105 din19 octombrie 2005 pentru aprobarea Normei sanitare veterinare care introduce măsuri naţionale pentru eradicarea brucelozei, tuberculozei şi leucozei bovine
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14. www. who. int.

The energetic profile in cows with ketosis

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Tests on the energetic profile made on a batch of 15 Holstein Frize dairy cows diagnosed with ketosis showed hypoglycemia (38,0±2.2mg/dl) and lower average values of the alkaline reserve (21.5 ±0,5mEq/L) and serumal cholesterol (72.1±3.8mg/dl) as compared to the average values in clinically healthy cows and to the limits of reference.

The mean values of the lipemia (512,0±9.3mg/dl), serumal triglycerides (69.5 ±3.1mg/dl), lactic acid (19.0±1.8mg/dl), pyruvic acid (1,4±0.3mg/dl) and β-hydroxybutyrate (16.5±2.1mg/dl) were higher than the average values in clinically healthy cows and the limits of reference.

These results are arguments for the implication of the liver in the pathogenesis of the condition.

Ketosis in dairy cows is characterised by excessive accumulation of ketone bodies in the blood as a result of a disorder of the glucide - lipid metabolism. Therefore, the sanguin biochemical determinations of the energetic profile have a special importance because they can give essential information in cases of energetic deficiency and implicitly ketosis.

Key words: ketosis, dairy cow, energetic profile

Materials and methods

The researches were made on a batch of 15 Holstein Frize dairy cows diagnosed with ketosis and with an average milk production of 24.0±3.0 liters of milk/day.

To assess the energetic profile, blood samples were collected in test tubes without anticoagulant to express sanguin serum. Afterwards, using the EOS 880 Plus semiautomatic biochemical analyser and specific kits we have determined the parameters of the energetic profile: glucose (GLU), lactic acid (AcL), pyruvic acid (AcP), cholesterol (CHOL), total lipids (TL), triglycerides (TGL) and β-hydroxybutyrate (BHB) as a representative of ketone bodies.

Determining the β- hydroxybutyrate is relevant for assessing the ketonemia, knowing that it is the end of the metabolic line and it represents 78% of the total of ketone bodies, while the aceto-acetate, being unstable, turns rapidly into β- hydroxybutyrate or acetone. After determining the β- hydroxybutyrate, we have calculated the total value of the ketonemia using the formula: CC=100xBHB/78.

The same determinations were made for a number of 10 clinically healthy cows of the same batch, with a similar physiological status.

The data obtained were processed statistically, calculating the arithmetical average (x) and standard deviation of the arithmetical average (Sx) as compared to the values of reference found in literature.

Results and discussions

The values of the energetic profile in cows with ketosis are showed in Table 1.

Table 1.

The average values of the energetic profile determinated on cows with ketosis

Parametri ai profilului energetic	Unităţi de exprimare	Valori medii de referinţă	Vaci clinic sănătoase (n=10)	Vaci cu cetoză (n=15)
Glucoză (GLU)	**mg/dl**	**62,0±12,0**	**49,3±2,4**	**38,0±2,2**
Acid lactic (AcL)	**mg/dl**	**13,0±7,0**	**15,2±1,5**	**19,0±1,8**
Acid piruvic (AcP)	**mg/dl**	**0,5±0,3**	**0,9±0,2**	**1,4±0,3**
Colesterol (CHOL)	**mg/dl**	**100,0±50,0**	**129,0±6,0**	**72,1±3,8**
Lipide totale (TL)	**mg/dl**	**300,0±150,0**	**440,0±6,2**	**512,0±9,3**
Trigliceride (TGL)	**mg/dl**	**30,0±15,0**	**43,1±2,9**	**69,5±3,1**
Betahidroxibutirat (BHB)	**mg/dl**	**sub 14,4**	**5,2±1,4**	**16,5±2,1**
Corpi cetonici (CC)	**mg/dl**	**5,0±4,0**	**6,6±0,6**	**21,1±3,0**
Rezerva alcalină (RA)	**mEq/L**	**24,5±2,5**	**23,0±1,0**	**21,0±0,5**

In cows with ketosis, the average value of the glycemia was 38.0±2.2 mg/dl. This value is lower not only than the average values of reference but also than those of the clinically healthy cows. Lower average values were also observed in what concerns the alkaline reserve (21.0±0.5mEq/l) and the serumal cholesterol (72.1±3.8mg/dl).

The average value of the lipemia was higher, respectively of 512,0±9.3mg/dl, than the average value of reference and that found for the clinically healthy cows. Higher average values were also found for serumal triglycerides (69.5±3.1mg/dl), lactic acid (19.0±1.8mg/dl), pyruvic acid (1.4±0.3mg/dl), β-hydroxybutyrate as a representative of the ketone bodies (16.5±2.1mg/dl), and ketone bodies in general (21.1±3.0 mg/dl).

The decrease of the glycemia is most frequently a result of a poor synthesis of volatile fatty acids (AGV) in the rumen, especially the propionic acid that is essential for gluconeogenesis in case of an insufficient glucid intake. In the absence of AGV, to produce energy the body uses especially lipids that will be mobilised from reserves and biodegraded in the liver. The biodegradation of lipids to produce energy will lead to a rise of the level of triglycerides in the serum as well as in the liver, leading to its fatty loading. This explains the increase of the triglyceridemia as a result of the increase of the lipemia and a decrease of the glycemia.

The lipids are biodegraded to acetylcoenzyme A, which, in the absence of the oxaloacetate used in the process of gluconeogenesis, can't be used completely in the cycle of the tricarboxylic acids and therefore will follow the path of forming ketone bodies. This is the origin of the increase of the plasmatic concentration of ketone bodies. In these circumstances there is a tendency towards the increase of the metabolic acidity, as the ketone bodies being acid, especially the β-hydroxybutyric acid and acetylacetic acid, they retain the alkaline salts. Therefore the average values of the alkaline reserve in the cows with ketosis were lower than those of the clinically healthy cows, as well as those of reference.

The average value of the lactic acid situated towards the upper limit of reference values could be associated with the decrease of the alkaline reserve and implicitly with the metabolic acidosis and not with the excessive intake of easily soluble glucides, or intense effort over a short period of time.

We blame the increased average level of pyruvic acid on the deterioration of the liver's functions and the impossibility of its use in metabolic pathways.

The lower average values of the cholestherolemia can be explained by the fact that the peak of lactation and the lipidic liver distrophy, physiologic-metabolic states found in ketosis, alter the function of synthesis of the liver and lead to hypercholestherolemia. This state is counterbalanced by the intake of green fodder that produces hypocholestherolemia. Thus the cholestherolemia was within the limits of the values of reference found in literature.

Conclusions

1. The tests on the energetic profile made on cows with ketosis showed hypoglycemia (38.0±2.2mg/dl), lower average values of the alkaline reserve (21.0±0.5 mEq/l), and serumal cholestherol (72.1±3.8mg/dl) as compared to the average values found in clinically healthy cows as well as to the limits of reference.
2. The average values of the lipemia (512.0±9.3mg/dl), serumal triglycerides (69.5±3.1mg/dl), lactic acid (19.0±1.8mg/dl), pyruvic acid (1.4±0.3mg/dl), β- hydroxybutyrate as a representative of the ketone bodies (16.5±2.1mg/dl), and of the ketone bodies (21.1±3.0mg/dl) in general were higher than the average values found in clinically healthy cows and the limits of reference.
3. Hypoglycemia, hyperlipemia, hyperketonemia, the increased concentration of lactic and pyruvic acids in cows with ketosis are arguments for the implication of the liver in the pathogenesis of the condition.

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The hematological profile in cats with infectious peritonitis

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The haematological profile in cats diagnosed with feline infectious peritonitis showed a decrease of the hemoglobin (Hb= 7.5±1.6g/dl), packed cell volume (PCV= 28.3±2.1%), mean corpuscular hemoglobin (MCH=12.3±0.3pg) and mean corpuscular hemoglobin concentration (MCHC=26.5±0.4%), and the mean cell volume was unmodified (MCV=46.4±0.4μ^3). These values prove the existence of a normocytic, hypochromic, hypoplastic anemia.

In the leucocytic formula we noticed neutrophylia (N=74.0±0.8%), lymphopenia (L=16.0±0.2%) and the increase of the number of basophiles (B=2.0±0.1%). This fact shows the existence of an active inflammatory process, confirmed by the presence in the coloured blood smear of trombocyte aggregates and segmented and vacuolised neutrophils.

Key words: cat, feline infectious peritonitis, hematologic profile

Materials and methods

The researches were made on 18 cats, male and female, of different ages and breeds, diagnosed with feline infectious peritonitis (PIF). After specifying the diagnosis through echographic examination and peritoneal punction with the presence of inflammatory fluid, blood samples were taken in order to establish the haematological and sanguine morphological profile.

For this, we used the „ABC-vet" automatic haematological analyser to determine the hemoglobine (Hb), packed cell volume (PCV), number of erythrocytes (NrE), number of leucocytes (NrL), number of platelets (Tr) and erythrocytic constants: mean cell hemoglobin (MCH), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC). The erythrocyte sedimentation rate (ESR) was determined through the Westergreen method, and the morphological exam of the blood was made on blood smears coloured May- Grunwald- Giemsa, also calculating the leucocytic formula.

The results were processed statistically, calculating the arithmetical average (x) and standard deviation of the arithmetical average (Sx) as compared to the average values of reference found in literature.

Results and discussions

The results of the haematological profile are showed in tables 1 and 2.

Table 1

Haematological profile of cats with infectious peritonitis

	Determined parameters				Calculat parameters		
	Hb	PCV	NrE	NrL	MCV	MCH	MCHC
Units	g/dl	%	10^6/µL	10^3/µL	μ^3	picograme	%
Average reference values	8,0-15,0	30,0-45,0	5,0-10,0	5,5-19,5	39,0-55,0	13,0-17,0	30-36
Average opteined values	7,5±1,6	28,3±2,1	6,1±0,9	11,7±1,3	46,4±0,4	12,3±0,3	26,5±0,4

In cats with infectious peritonitis, we have obtained lower values as compared to those of reference in what concerns the Hb (7.5±1.6g/dl), PCV (28.3±2.1%), MCH (12.3±0.3pg) and MCHC (26.5±0.4%). Also, the NrE (6.1±0.9 10^6/µl) was within the average reference values, but was close to their lower limit, and the MCV (46.4±0.4μ^3) was within the physiologic limits of the species.

These results prove the existence of a normocytic, hypochromic, hypoplastic anemia.

Table 2

Platelets, erythrocyte sedimentation rate and leucocytic formula

	ESR	Tr	NrL	*Leucocytic formula*				
				N	E	B	M	L
Units	mm/h	10^3/µL	10^3/µL	%	%	%	%	%
Average reference values	3,0	300,0-700,0	5,5-19,5	35,0-75,0	2,0-12,0	rare	1,0-4,0	20,0-55,0
Average opteined values	9,0	640,0±2,4	11,7±1,3	74,0±0,8	7,0±0,6	2,0±0,1	1,0±0,1	16,0±0,2

In cats with infectious peritonitis, Tr (640.0±2.4 10^3/µl) and NrL (11.7±1.3 10^3/µl) had average values within the limits of those of reference.

As for the leucocytic formula we noticed a tendency towards neutrophylia (74.0±0.8%), lymphopenia (16.0±0.2%) and the increase of the number of basophiles (2.0±0.1). This requires the existence of an active systemic inflammatory process, which is also confirmed by the higher ESR (9.0 mm/h) and the presence in the coloured blood smear of aggregated platelets and the segmented, vacuolised neutrophyls (fig. nr. 1).

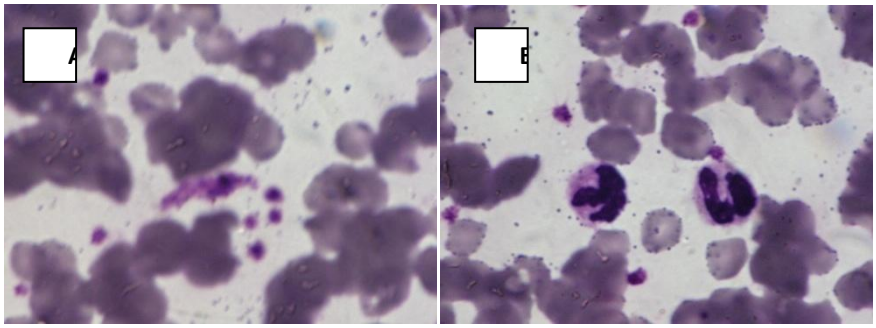

Fig. nr. 1. A-Aggregated platelets; B-Segmented and vacuolised neutrophyls

Moreover, the May-Grunwald-Giemsa coloured blood smear does not show reticulocytes, which confirms the hypoplastic anemia.

Conclusions

1. In what concerns the red cell series of the blood, in cats with infectious peritonitis we found a decrease of the Hb (7.5±1.6g/dl), PCV (28.3±2.1%), MCH (12.3±0.3pg), MCHC (26.5±0.4%) and the MCV (46.4±0.4μ^3) was unmodified. These values prove the existence of a normocytic, hypochromic and hypoplastic anemia.
2. In the leucocytic formula showed neutrophylia (74.0±0.8%), lymphopenia (16.0±0.2%) and an increase in the number of basophiles (2.0±0.1). This proves the existence of an active inflammatory process.
3. In the coloured blood smear we noticed aggregated platelets and segmented and vacuolised neutrophyls, which confirms the evolution of the inflammatory process.

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Study regarding the dynamics of the uterine inflammatory disorders and their influence on some reproductive values

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Sterility is not a disease, but a syndrome corresponding to different disturbances of the reproductive function (Bîrțoiu A.; Seiciu Fl., 2004, Bogdan Al.,1981, Boitor I.,1987, Cernescu H., 2004, Drugociu Gh., Seiciu Fl., Boitor I, Bilcea P., 1977, Gluhovschi N., Drugociu Gh., Seiciu Fl. 1972, Groza I., 2006, Grunert G.A.,1994, Mînzatu I., Bogdan Al., 2003, Mircu C., 2001, Runceanu L., 1995, Runceanu L., Cotea C., 2001, Seiciu Fl., 1987).

The research we conducted between 2004-2006, taking in evidence 576 females ; followed the diagnosis of two morphoclinical types of endometrial affections: the acute cataral endometritis and the acute purulent endometritis.

Key Words: cow, endometritis

Material şi metoda de lucru.

Cercetările au fost efectuate în cadrul unei ferme de creştere a vacilor pentru lapte, pe un număr de 297 vaci care au prezentat afecțiuni la nivelul mucoasei uterine . Femelele au fost întreținute şi exploatate la un nivel corespunzător.În cadrul fermei se practică însămânțările artificiale.

Rezultate şi discuţii

În urma cercetărilor efectuate se înregistrează un număr mediu de 75,3 cazuri/an cu diferențe apropiate în anii 2004 şi 2005 (114 şi 123 cazuri) şi reletiv mai scăzut în 2006 (60 cazuri).

Tabel nr.1

Evoluția endometritelor acute şi cronice în anii 2004 – 2005 - 2006

	Total femele	Total cazuri		Endometrită acută				Endometrită cronică			
Media pe cei 3 ani		Număr vaci	%	Cataral		Purulentă		Cataral		Purulentă	
				Număr vaci	%	Număr vaci	%	Număr vaci	%	Număr vaci	%
	587	75	17	27	5,12	47	8,03	14	2,47	6	1,02

Endometritele cronice purulente înregistrează în medie un număr mult mai mare de cazuri, 6 cazuri ce reprezintă 1,02 % cu valori apropriate : 9 cazuri în 2004, ceea ce reprezintă 1,56%, 6 cazuri în 2005, ceea ce reprezintă 0,89% şi 3 cazuri în 2006, ceea ce reprezintă 1,02%. Apariția acestor forme de evoluție primară se poate datora ca şi în cazul endometritei cronice latente subinvoluției uterine sau ruperii echilibrului dintre organism şi agenții patogeni, datorită întreruperii tratamentului.

Evoluția cazurilor de endometrite acute şi cronice pe trimestre în anii 2004 -2005 - 2006

În lunile martie, aprilie şi mai 2004 se înregistrează un număr de 75 cazuri, în 2005 se înregistrează 51 de cazuri şi în 2006 se înregistrează 32 cazuri.

Tabel nr. 2

Anii	Total femele în evidență	Total cazuri		Din care pe trimestre							
		Număr vaci	%	I		II		III		IV	
				Număr vaci	%	Număr vaci	%	Număr vaci	%	Număr vaci	%
2004	575	114	19,7	28	4,86	72	12,52	5	0,87	9	1,56
2005	670	123	18,3	31	4,62	58	8,56	17	2,53	17	2,53
2006	490	60	12,2	16	3,26	29	5,91	9	1,83	6	1,22
Media pe trei ani	578	75	16,7	26	4,24	53	9,03	13	1,74	10	1,77

Sterilitatea şi infecunditatea lezională constituie urmarea directă a unor tulburări funcționale ale aparatului genital. Leziunile de la nivelul organelor genitale determină infecunditatea, fie în forma existenței lor ca atare, fie în urma tulburărilor funcționale pe care le antrenează în anumite condiții ; leziunile de la nivelul tractusului genital, pot determina sterilitatea definitivă.

În condițiile de creştere şi exploatare a vacilor din ferma în care se fac cercetările, un rol major în instalarea infecundității este atribuit agresiunii infecțioase specifice sau nespecifice, precum furajarea dezichilibrată în unele perioade.

Principalii indicatori de reproducţie la vacile cu endometrită.

La vacile cu endometriă purulentă cronică SP înregistrează 156 zile în anul 2005 şi valori inferioare respectiv 148 de zile în 2006 şi 149 de zile în 2004.

Valorile Ig sunt mai mari, 3,9 în anul 2005 şi mai mici 3,5 şi respectiv 3,6 în anii 2006 şi 2005.

Dintre endometritele cronice purulente în perioada studiată s-au diagnosticat două cazuri de piometru la care s-au obținut un SP de 104 zile, CI de 1331 zile şi respectiv SP de 960 zilz şi CI de 1220 zile.

Tabel nr. 3

Nr. Crt.	Specificare	2004			2005			2006			Media multianuală		
		SP	CI	Ig	SP	CI	Ig	SP	CI	Ig	SP	CI	Ig
1.	Endometrită acută catarală	207	491	4,9	227	511	5	185	470	3,6	206,3	491,3	4,3
2.	Endometrită acută purulentă	191	470	3,8	232	516	4,5	185	476	4	202,6	487,6	4,1
3.	Endometrită catarală cronică	316	598	5,1	379	663	6,9	35	635	6,4	348,3	633,3	6,1
4.	Endometrită purulentă cronică	149	434	3,6	156	441	3,9	148	433	3,5	151	436	3,6

CONCLUZII

1. Urmărind frecvența endometritelor constatăm că cel mai frecvent a fost diagnosticată endometrita purulentă acută 8,03 % din 578 de vaci luate în evidență, urmată de endometrită cataralâ acută 5, 12 %, endometrită purulentă cronică 1,025.
2. Urmărind evoluția endometritelor acute şi cronice în dinamică pe trimestre şi luni, constatăm că cel mai mare număr de endometrite s-au înregistrat în lunile martie, aprilie şi mai, 2004 - 70 cazuri, 2005 – 51 cazuri şi în 2006 - 36 cazuri . Procentual, situația cazurilor de endometrită este următoarea : în 2004 – 61%, în 2005 – 41% şi în 2006 – 53%. Acest procent ridicat al cazurilor de endometrită în lunile martie, aprilie şi mai , se explică printr-o slăbire a reacției organismului şi implicit a mucoasei uterine la reacția agenților patogeni, scăderea tonicității musculaturii uterine, datorită stabulației prelungite, furajării precare pe timpul stabulației şi limitatrea plimbării femelelor gestante şi fătate recent.
3. Trecerea în forma clinică de endometrită acută (cataralâ purulentă) în endometrită cronică arată că o importanță deosebită trebuie acordată diagnosticului în forma acută şi instituirea unui tratament judicios deoarece se observă că în medie s-au obținut vindecări numai la 78% din endometritele catarale acute şi 76,3%din endometritele purulente acute.

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Qualitative interrelationship regarding TNG, pH and glucose during preservation of boar semen

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Significant inequalities regarding the semen quality between certain laboratories who process boar semen were observed. The reserches , performed on semen doses from two farms, followed three parameter:total number of germs (TNG), pH and level of glucose. The analises were simoultaneously en route of semen preservation. The germs left-over after dilution are multipling, therefore TNG grows progressive during preservation. The glucose level decrese dinamic and is pronounced on high TNG. The pH values are relative constant and tight related to the microorganisms activity. The glucose-fermenting microorganisms use the glucose from the diluant and come in tropfic competition with the spermatozoons. Because of the metabolit products eliminated ,the pH of the medium tends to acidify and this way the semen dose quality changes.

Key words: semen, glucose, germs, pH

The fertility of a boar depends on the semen quality. This idea stands on the findings refering to the property of the ejaculate, the chemical composition in correlation with spermatozoon fecundant capacity but also whith the microbiological load [4,8]

The boar semen contains simple and complex sugars (glucides); these sugars are important as a source of energy and for spermatozoons movement [5,6]

The energetic substances are located into the spermatozoon or in the seminal plasma; demonstrating the existence of two energetic sources. The metabolic processes are present on the spermatozoon but also on the seminal plasma. Spermatozoons internal metabolism consist of aerobic phosphorilation of his own energetic sources that is the plasmalogenous [3]

Fructose is one of the main source of energy for boar spermatozoons and the amount is between 9 and 40 mg/100 ml crude semen.

Glucose is the main source of energy on diluants, with values between 3 and 60 g at 1000 ml distilated water [1-3, 11].

Many workings reveal the significance of studing the factors that can influence the semen biological value, amongst wich the presence of microbian flora plays a keyrole. [1-3, 17]

The total number of germs (TNG) from crude semen we fiind it between 18.800 and 325.300 depending upon hygiene at harvest and the genital tract health status. The spermatozoon conglomeration was often associated with bacterial contamination. The most frequent isolated bacterias were: *Bacillus spp., Actinobacillus sp., Staphylococus spp., Flavobacterium spp., Klebsiella spp., Pseudomonas sp., Micrococcus sp., E. Col, Citrobacter sp., Proteus spp., Actinomyces sp., Serattia spp., Enterobacter sp., Bacillus sp., Streptococcus spp.* [1,7,11,14,17]. Normally, the pH of crude, fresh semen is situatted between 7,2 and 7,5 ; if the values are changing, the spermatozoons are begining to lose their fecundant capacity and viability, leading to decresed quality semen nozzles.

Materials and methods

The research was done on boar semen for artificial inseminations that emanate from two farms. Farm no.1 is an intensive breeding unit and farm no. 2 is a semi-intensive breeding unit.

The samples were submitted on general and particular (biochemical, microbiological) examinations to determine their quality .

The examinations have been performed in different laboratorys (reproduction, pathology of reproduction, bacteriology, micology) from Iasi Veterinary School and in the Microbiologic Lab of the Iasi County Sanitary-Veterinary and Food Safety Laboratories.

TNG (total number of germs) was detrmined through succesive serial dilutions procedure and typify was done using microenzimatic „miniApi" tests.

To determine glucose we used „EOS880 Plus" analizer and the pH was identified with „WTW INOLB".

Results and discussions

The glucose level(mg/dl) on fresh semen had values within 38,7 and 70,4 (farm 1) , the average 53,56 and within 28,8 and 63,2 (farm 2), the average 43,84. we could observe that after semen dilution, the concentration of glucose raised until the average of 526,18 on farm 1 and 509,17 on farm 2(table no. 1).

Table no.1

Level of glucose(mg/dl) in crude and dilutted semen

Sample nr.	Crude semen		Semen after diluttion	
	Farm 1	Farm 2	Farm 1	Farm 2
1.	42,3	28,8	521,6	542,9
2.	70,4	31,1	509,3	480,4
3.	48,1	63,2	524,4	530,2
4.	50,6	38,6	518,4	513,5
5.	38,7	47,3	528,2	494,3
6.	53,2	53,1	536,9	509,9
7.	62,1	30,7	534,6	520,3
8.	66,9	36.4	532,3	503,7
9.	48,7	51,3	525,7	516,4
10.	54,6	57,9	530,4	480,1
x	53.56	43.84	526.18	509.17

One of the main roles of glucose is to ensure the nutrients and energy in the preservation period.

Glucose is also a source of nutrition for germs in semen; that brings the trophic competition between germs and spermatozoons and by their metabolism, substratum will change, resulting in poor quality semen nozzles.

The diluants used to prepair the semen nozzles are numerous and diversified by: producer company, ingredients, quality; and they always must have an antibiotic. After dilution, if all the germs are not be blasted, they find a proper environment to grow and develop, making the semen quality even more poor.

The glucose (mg/dl) concentration in 17^0C preserved nozzles, declines progressive. On farm no.1 the average glucose is 527,5 after dilution; 527,1 at 24 hours; 517,7 at 48 hours; 513,8 at 72

hours; 509,5 at 96 hours. On farm 2 the averages were: 526,3 (at T0); 505,2(at T1); 482.0(at T2); 463,8 (at T3) as shown in table nr. 2.

The dinamic of glucose concentration reduction represents his consumption by the spermatozoons and it is tight lynked with the diluant quality and the semen microbiological load.

Table no.2

The dinamic of TNG, glucose level and pH

Time / Farm	Sample no.	T0			T1			T2			T3			T4		
		TNG	Gluc	pH	TNG	Gluc	pH	TNG	Gluc	pH	TNG	Gluc	pH	TNG	Gluc	pH
Farm 1.	1.	-	531,6	7,5	10	529,8	7,4	30	527,4	7,1	60	525,1	6,9	130	522,3	6,6
	2.	-	539,3	7,2	20	537,2	6,9	40	530,8	6,5	90	523,8	6,5	250	521,7	6,4
	3.	-	523,6	7,0	10	521,7	7,0	10	520,1	7,0	20	517,1	6,9	40	510,2	6,7
	4.	-	519,9	6,9	40	517,2	6,8	70	514,2	6,6	160	508,3	6,5	390	504,8	6,5
	5.	-	519,3	6,9	10	509,3	6,7	60	503,4	6,5	240	501,6	6,4	420	495,6	6,4
	6.	-	531,4	7,0	10	515,2	6,9	30	510,4	6,9	50	507,0	6,7	80	502,5	6,7
	x	**-**	**527.5**	**7.1**	**16.7**	**521.7**	**7.0**	**40.0**	**517.7**	**6.8**	**103.3**	**513.8**	**6.7**	**218.3**	**509.5**	**6.6**
Farm 2.	1.	-	514,1	6,8	320	503,7	6,5	720	490,8	6,1	1620	477,3	5,5	-	-	-
	2.	-	542,9	7,3	270	513,5	6,9	580	493,4	6,4	1270	486,2	5,7	-	-	-
	3.	-	531,4	7,2	250	509,9	7,0	510	488,3	6,6	2310	478,1	5,3	-	-	-
	4.	-	515,3	7,1	430	490,4	6,6	690	456,7	6,0	2060	416,7	5,4	-	-	-
	5.	-	527,8	7,2	370	508,3	6,7	560	480,6	6,0	2370	460,9	5,5	-	-	-
	x	**-**	**526.3**	**7.1**	**328,0**	**505.2**	**6.7**	**612,0**	**482.0**	**6.2**	**1926,0**	**463.8**	**5.5**	-	-	-

(T1=24h, T2=48h, T3=72h, T4=96h)

In farm no.2 , the diluant was made at place and it contained only one antbiotic. In farm no.1 the diluant came from a company producer wichdoes not specifies the ingredients, but we know that a cocktail of antibiotics is used.

We can see the level of glucose on farm 2 decreses progressive at considerable smaller values than farm 1.

This inequality comes from a higher TNG. We mentionate as important that after T3 determinations, all the spermatozoons had died. Spermatozoons dead and conglomeration occured because of the medium changes via acidification.

Average pH values varied from 7,1 (at T0) to 6,6 (at T4) in farm 1; and from 7,1 to 5,5 (at T3) in farm 2 (fig. No 1.).

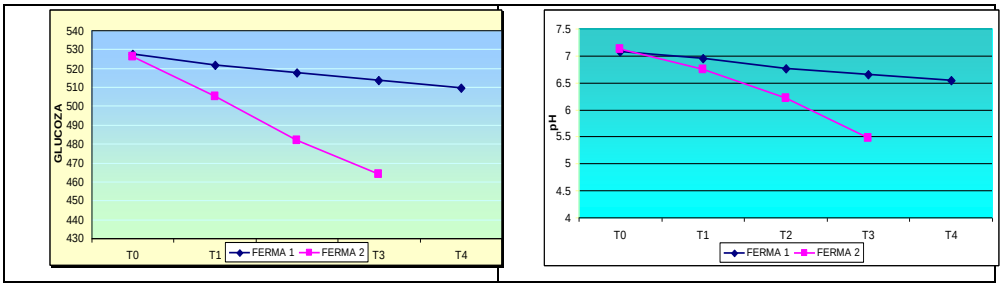

Fig.no 1.

The dinamic of glucose and pH in doses (diluted semen)

It can be observed a tight correlation between TNG, pH and glucose consumption; so, at high TNG level, the pH comes down by acidification and the level of glucose decreses.

After semen nozzles microbiological examination we found some resistant bacteries that waren't affected by the diluant antibiotics and various of funguses (table no.3).

Table no.3

The microorganisms indentification

Bacteries	Funguses
Escherichia spp.	*Cladosporum*
Staphylococus spp	*Penicillium*
Proteus spp.	*Fusarium*
Streptococus spp.	*Aspergillus*
Pseudomonas spp.	*Mucor*
Klebsiala spp.	*Alternaria*
Bacillus spp.	*Geotrichum*
Actinomices spp.	*levuri*

From the isolated bacteriums, 81,7% have glucose fermentation as a principal biochemical property. That explains the decrese of the glucose level in the semen nozzles with higher TNG. As higher the number of germs per ml, as lower the concentration of glucose will decrease.

Conclusion

1. The level of glucose concentration in crude semen differs by boar and the breeding conditions but the average is close to 43,85-53,56 ng/dl.
2. Glucose in the semen nozzles represents the source of energy for both the spermatozoon as for antibioresistent germs.
3. The level of glucose as well as the semen nozzles pH suffers a diminution related to the preservation period and the microbiological load. The microorganisms uses glucose for their metabolism and release acids wich change the pH value and leads finally to spermatozoon death.

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The significance of the "honeymoon" period in insulin-addicted diabetes mellitus in pets

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Within a variable period of time after the beginning of the insulin treatment, a remission of the diabetes is ascertained, due to the hyperfunction of the remnant beta-pancreatic cells in their tendency to produce sufficient insulin (through the amplification of the secretor function) necessary to the hyperglycemia compensation. This phenomenon is known in human medicine as the "honeymoon". Along time, the irreversibly destroyed pancreatic cells uses up its function and thus the illness settles and the patient becomes addicted to insulin-administration for the rest of its life.

Key words: diabetes mellitus, insulin-addicted, honeymoon, glycemia

Both in humans and in pets, the metabolic-nutritional pathology is represented mainly by the diabetes mellitus, obesity and dyslipemia. Lately they have become very aggressive populational diseases.

In humans, diabetes mellitus was signaled thousands of years ago **(B.C.)**, as the rate of diseases is very high.

In veterinary medicine, diabetes mellitus was signaled in all species (horses, oxen, sheep, pigs, poultry, even reptiles), but the higher frequency of this syndrome was registered in dogs and less in cats.

The clinical-evolutional particularities help identifying the diabetes mellitus, and in animals they create a well-structured and permanent active screening, and the diagnosis regards not only disease forms but as well death-leading interaction with other diseases and/or complications.

MATERIAL AND METHOD

Studies were performed between January 2005 and January 2007 on the cases diagnosed with diabetes mellitus within the subject Pathology and Medical Clinic of the Faculty of Veterinary Medicine of Iaşi and SC TEOVET SRL veterinary medical office of Iaşi.

The insulin-addicted diabetes diagnosis was set based on the classic clinical symptoms (polyuria, polydipsia and sometimes polyphagia), on the urine glucose determination by means of the "strip" bandelets of the DEKA-PHAN-LEUCO tests, and on the determination of glycemia with the help of the glycemia measuring device GLUCO TREND II.

OBTAINED RESULTS AND DISCUSSIONS

Table no. 1

Total number of cases	dogs	cats
Nr.	10	6
%	62.25	37.5

The diagnosis diabetes mellitus was set in 16 cases respectively 10 dogs and 6 cats, cases in which the anamnesis frequently revealed the annoyances generated by one or more characteristic manifestations, for which the animals were taken to see a doctor (ordered depending on frequency): **polyuria/ polydipsia, recent loss of weight, cataract, polyphagia, anorexia, vomit and an acetone smell of the breath.**

Normally the diuresis is of 25-50 ml/kg/day in dogs and of 20-30 ml/kg/day in cats, and the normal specific urine density is of 1.025 in dogs and 1.030 in cats.

In what concerns the animals brought for consultation, the water consume exceeded 100 ml/kg, and the urine quantity exceeded 50 ml/kg body weight a day.

Table no.2

Paraclinical exam.	Dogs		Cats		Total	
	Nr.	%	Nr.	%	Nr.	%
Glycosuria	10	62,5	6	37,5	16	100
Baruria(hyperstenuria)	6	37,5	5	31,25	11	68,75
Proteinurie	5	31,25	3	18,75	8	50,00
Cetonurie	2	12,5	1	6,25	3	18,75

In the urine test **glycosuria** was identified, which is normally absent, the urine had a sticky character, like a sugar solution, and in 11 cases (68.75%) meaning 6 dogs (37.5%) and 5 cats (31.25%) **baruria** (**hyperstenuria**) was identified (with the density between 1.005 and 1.012). In serious cases meaning 3 patients (18.75) ketonuria was also found, and in 8 (50%) of the patients had proteinuria as well.

"A jeun" hyperglycemia constituted one of the criteria for a positive diagnosis. It was not excessive in all cases, as the diabetic animals had **slightly increased** values **(150 – 180 mg/dl)** of **extreme concentrations (600 – 800 mg/dl)** in comparison to the **normal value of glycemia (70 – 110 mg/dl)**.

Table no. 3

Glycemia value	Dogs		cats		Total	
	No.	%	No.	%	No.	%
140 – 180	1	16.66	1	16.66	2	12.5
180 – 240	2	12.5	1	16.66	3	18.75
Beyond 240	7	43.75	4	25.00	11	68.75

For animals with values between 140-180 mg/dl a diabetes diet was recommended (Hill's dry food), for those with values between 180 – 240 mg/dl the diet was completed with an oral hypoglycemic treatment (**Meguan, Maninil, Novonorm, Fitodiab, tablets with cranberries etc.**), and those with the "a jeun" glycemia exceeding 240 mg/dl and the glycosuria higher than 500 mg/dl were recommended the insulin treatment. Most frequently **Mixtard-30 insulin**, with mixed action – 30% rapid and 70% retard – and **Insuman Comb 50 insulin,** with mixed action – 50% rapid and 50% retard were used.

The insulin dose was established by exploration, by gradually increasing the dose after daily glycemia tests. The real dose was established after 7-10 days, during which the body adapted itself to the administered type of insulin.

After establishing the real insulin dose, as a consequence to daily glycemia tests, after 2-3 weeks (during which glycemia was maintained at a satisfactory level) the animals showed an important hypoglycemia with values under 70 mg/dl. This generated a decrease in the necessary insulin quantity.

By means of repeated daily tests, it was ascertained that the glycemic remission lasted in most animals for a few weeks (5-6 weeks), during which the pancreas was capable of secreting insulin. The administered insulin quantity was much diminished and in 4 cases 25% (3 dogs and 1 cat) no insulin was needed any longer, depending on the secretive capacity of the pancreas.

Nevertheless, after the signaled period of time an increase in the glycemia up to alarming values was remarked, leading to the need of insulin administration.

The explanation is that the pancreatic cells are irreversibly destroyed, as they wasted their function and the animal becomes insulin addicted for the rest of its life.

The phenomenon called the “honeymoon” is due to the effort of the pancreatic cells to control the glycemia values (through the enhancement of the secretive function).

CONCLUSIONS

1. In certain situations, after setting the insulin-addicted diabetes diagnosis and imposing an appropriate insulin dose, a phenomenon of transitory remission appears, in which the glycemia level decreases, reaching normal values, called the “honeymoon”.
2. In the event of the “honeymoon”, a decrease in the insulin dose is necessary.
3. In order to avoid hypoglycemias as well as hyperglycemias, the glycemias must be monitored on a daily basis at the beginning, then on a weekly basis, and after the transitory remission every two weeks.

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Therapeutic aspects in the diabetic ketoacidosis in pets

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Diabetic ketoacidosis is a pathologic state of the body characterized by the accumulation of acetone and the decrease in the alkaline reserve of the blood. The symptomatology is represented by the lack of appetite, nausea, vomiting, abdominal pains and the increase in the urination frequency. If the treatment is not administered in time, it may lead to fainting and the risk of a diabetic coma.

Key words: diabetus mellitus, diabetic ketoacidosis, acetone corps

Ketoacidosis is a medical emergency which many times endangers the patient's life, as it appears when the metabolism of the cells that use glucose as an energetic layer is perturbed (especially nerve, muscular and hepatic cells). This complication usually appears in incorrectly or insufficiently treated diabetic patients, with a high level of glycemy.

Normally the body cannot use glucose as an energetic layer but in the presence of a sufficient quantity of insulin.

In the event of an insulin deficit, the energetic layer of the body is provided through the catabolism of lipids (the lipids are another source of energy that does not need insulin for metabolism. Apart from the necessary energy, the catabolism of lipids also produces certain metabolites toxic for the body (ketonic bodies) that penetrate the sanguine circuit and provokes the ketoacidosis.

MATERIAL AND METHOD

The studies were carried out on the animals brought to consultation to the Medical Clinic of the Faculty of Veterinary Medicine of Iaşi and on the animals brought to consultation to S.C. TEOVET SRL veterinary medical office between January 2006 and January 2007.

The diabetic ketoacidosis diagnosis was set based on the anamnesis, the clinical symptoms and on the results of the urinary paraclinical examinations (the determination of ketones and glucose in urine), by means of the DEKA PHAN LEUCO urinary and biochemical (glycemia, Na, K, arterial pH) tests by means of the pH-box, the GLUCO TREND II device and the EOS 880 plus biochemical semi-automatic device.

OBTAINED RESULTS AND DISCUSSIONS

The diabetic ketoacidosis diagnosis was set in 8 cases (5 dogs meaning 62.5% and 3 cats meaning 37.5%) brought to consultation between January 2006 and January 2007.

Table no. 1

Total no. of cases	dogs	cats
Nr.	5	3
%	62.5	37.5

Thus it was observed that many of the patients with diabetes are diagnosed with this disease, after they are diagnosed with diabetic ketoacidosis.

In order to support the diagnosis and the diabetic ketoacidosis treatment, the following investigations were carried out:

- Determining biochemical parameters (glycemia, Na, K, arterial pH), which are vitally important tests both in order to set a diagnosis and to chose the therapeutic strategy.
- Determining the ketones and glucose in the urine.

When determining the biochemical parameters the following were noticed: an increase in the glycemia values over 450 – 500 mg/dl, a decrease in the natremia values under 140.3 mEq/L (normal values is 120.8 – 131.2 mEq/L), a decrease in the potasemia under3.8 mEq/L (normal values is 2.1 – 2.8 mEq/L) and a decrease in the arterial pH down to values of 7.15 – 7.20 (normal values is 7,33 – 7,42), when the ketoacidosis becomes uncompensated.

Depending on the severity of the symptoms, the affected animals were subject to **intensive care**, which included:

*** Administering liquids intravenously against the supervened dehydration and electrolytic unbalances**

- To this purpose, a physiological serum (NaCl 9‰ 10 – 20 ml/kg, vitamin C mg/kg and vitamin B1 and B6 10 mg/kg were administered.
- Depending on the **glycemic evaluations,** the intravenously administration of insulin 0.1 UI/kg was necessary until reaching a normal glycemia value, after which it started to be administered subcutaneously. Actrapid insulin was used for intravenous administration, and the doses were adjusted depending on the glycemia values.

In order to fight against the electrolytic deficit K + 20 – 40 mmoles/h, and if the diuresis was repeated or normal, Mg++ and phosphates could be administered also.

* **The prevention of the cerebral edema** was carried out by administrating furosemid 10 mg/kg or, for **fighting against the cerebral edema** manitol (drastically diuretic) 0.25 – 0.50 mg/kg was administered when necessary.

* **In order to decrease the metabolic acidosis**, baking soda 1.3 – 1.4, 1 – 2 g/animal was administered when necessary.

* During the treatment the **cardiac frequency was monitored carefully, as well as the pulse, respiratory frequency and the consciousness state of the animal**.

* The frequent **biochemical evaluations** during treatment were the following: **glycemia, arterial pH and sanguine electrolytes**.

Table no. 2

Cases	Resolved		Unresolved	
	dogs	cats	dogs	cats
No.	4	1	1	2
%	50	12.5	12.5	25
TOTAL	5 cases 62.5%		3 cases 37.5%	

Of the 8 studied cases (5 dogs and 3 cats), 3 died meaning 37.5 %(1 dog and 2 cats), the rest of 5 (4 dogs and 1 cat) meaning 62.5% reached normal values of the arterial pH, K and Na, except for the glycemia, after 3 – 5 days of treatment. The glycemia reached values of 250 – 280 mg/dl. In order to adjust the values of glycemia to normal values, Mixtard 30, 70 semi retard insulin or Insuman Comb 50 was administered with repeated monitoring.

CONCLUSIONS

1. Diabetic ketoacidosis appears in all patients with diabetes mellitus type I (and in certain cases with diabetes mellitus type II), when glycemia values are very high and the glucose cannot be used as an energy source for the body, in exchange being catabolized the proteins and lipids, as ketonic bodies and fat acids are produced excessively.
2. The diabetic ketoacidosis treatment calls for the administration of liquids in perfusion for the control of dehydration and electrolytic balancing, as well as insulin intravenously in order to reduce hyperglycemia and stop excessive production of ketonic bodies.
3. Patients diagnosed with diabetic ketoacidosis must be carefully monitored from the electrolytic and glycemic point of view.

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Histopathological aspects revealed in multiple parasitic aggression on Black Goat species *(Rupicapra rupicapra)* and local reactivity

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The research has been conducted on cases of the Black Goat species (Rupicapra rupicapra). The hunt's support state was precarious, of pronounced weakening. The general necropsical examination was followed by the morphopatological examination of the cord, lungs, and liver, from which samples of tissue were drawn and processed through specific methods for the histopathological examination. The samples were sectioned at 5 µm, colored through the HEA, MGG, PAS methods, examined and microphotographed at MC5, with 10, ob. 10, 20, 40, and Imm. 63 oc.

The histopathological examination of the cardiac tissue revealed the aggression exercited by Sarcocystis spp. through intercellular or interstitial development of cysts with bradizoites, causing compression atrophy and affecting the autonom system of cord .

The histopathological examination of the pulmonary tissue revealed the brutal parasitary aggression on the pulmonary cells exercised by the nemathodes from the Protostrongylidae family (Muellerius genus) both in the larval stage, and as adults taking the form of "incubation nests". The local reactivity has been distinctively striking, characterized by the haemorrhages, micronecrosis, lymphohystiocitary infiltrations, "alveolarly epthelialization", hyperplasia, and hypersecretia on bronchioles, macrophagy with massive sincitia in alveoli, smooth muscle hyperplasia and fibrosis. Parasitic bronchopneumonia similar to sheep is signalized for the first time on this species.

In liver, the histopathological examination revealed recent aggressions on the hepatic tissue characterized by hemorrhagic, necrotic paths and acute angiocolitis also hemorrhagic-necrotic paths, a previous aggressions expressed by chronic angiocolitis and hepatitis.

These modifications are characteristic for the traumatic hepatitis produced by Cysticercus tenuicollis (cysticercosa hepatitis) during the hepatical migration, extremely aggressive, metacestod identified along with other parasitical structures disposed in the hepatical tissue.

Multiple and combineted parasitic aggression against body of Black Goat species (Rupicapra rupicapra) was complex , determining a local reactivity that was finally outnumber by the ter on action of parasitic species.

Key words: Hunt, Black goat (Rupicapra rupicapra), cord, lung, liver, Sarcocystis spp., Protostrongylidae, Cysticercus tenuicollis, multiple aggression, local reactivity

Digital radiography in perspective

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The study is concerned with the appearance, development, current state and perspectives of X-ray medical diagnosis in veterinary practice. It includes X-ray diagnosis by Conventional Film Screen Radiography, Computed Radiography, and Direct Digital Radiography.

Digital imaging provides many intangible benefits that are hard to quantify. Justifications for its use include increased veterinarian efficiency, better diagnostic analysis, and timely decisions about patient care. One major intangible benefit of CR is that it can help staff recognize that radiography is not a product but a service that provides diagnostic information, including the image and written report. Increasingly, veterinarians collect, store, and transmit medical information digitally, thus providing added convenience to veterinarians and clients.

Key Words: Direct Digital Radiography, Computed Radiography, Conventional Film Screen Radiography

Introduction

Imagine the excitement accompanying the first realization that noninvasive visualization of bones was possible. German physicist Wilhelm Conrad Roentgen was working in a darkened room on November 8, 1895, experimenting with electrical charges flowing through a vacuum tube, when a piece of paper coated with barium platinum cyanide glowed. Coincidentally, it had been left near a cardboard covered tube. Experimentation with the fluorescent paper and the charged tube followed. He discovered that if he held his hand between the tube and the paper, he could see the silhouette of hand bones on the paper (14, 16).

Roentgen took full advantage of the glowing paper and on December 22, 1895, produced the oldest existing radiographic record. It shows the bones of his wife's hand with a large signet ring on one finger (14).

Medical professionals quickly embraced this new technology. Since then many technological advances have improved radiological diagnostic capability. Keeping up with advancements can be difficult. A technician who stays abreast of the technology is a valuable clinic asset. This article will review film-screen radiography (FSR), discuss computed radiography (CR) in depth, introduce direct digital radiography (DDR), and contrast and compare the different systems. CR systems were introduced to the medical market in 1981. Although DDR equipment acquisition cost has been relatively steady, more economically feasible systems are now available through veterinary vendors. The cost of CR is financially impractical for most practices (5, 9, 11).

X-ray Generator

All three systems use an x-ray generator. The amount of radiation produced by this machine is regulated by adjusting controls for milliampere (mA), exposure time in seconds (s), and kilovolt peak (kVp). Single-phase (SP) 300-mA stationary and 100-mA mobile generators have been considered standard in veterinary practice (1, 3).

High frequency (HF) generators are becoming more popular primarily because they produce a more constant, concentrated, and consistent source of radiation than SP units. The HF unit offers shorter exposure times and decreased potential for motion blur.

Ideally, generators should produce x-rays traveling parallel to one another and striking the film plane at right angles. But x-ray production always results in some radiation scattering off in other directions. Filters (typically equivalent to 2.5 mm aluminum) positioned close to the x-ray source reduce this scatter and decrease patient and operator exposure. A lighted adjustable collimator is essential as it decreases scatter radiation even further (1, 2, 3).

Conventional Film Screen Radiography (FSR)

Conventional FSR relies on the use of film to record radiation after x-rays pass through the patient. Adjusting mA and exposure time influences the number of x-rays produced and the density (blackness) of the dark part of the image, but has no affect on the power or penetrating ability of the beam, hence no affect on contrast.

However, adjusting kVp influences the density and contrast of the resultant image on the film. When making technique adjustments to change contrast, while keeping the same density, it is important to maintain mAs-kVp balance. As kVp is increased, mAs must be decreased and vice versa. Many strategies have been developed to keep radiation “as low as readily achievable” (ALARA). Radiographic film has an emulsion layer of gelatin and crystals of light-sensitive silver halide bound to plastic. Exposure to light or radiation sensitizes the silver halide crystals, forming a latent image. Large crystals have a better chance of being struck by an x-ray than small crystals and require less exposure, but because of crystal size, the image is grainy when compared to images produced on slower films with smaller crystals (1, 2, 4, 7, 10).

Using the fastest film that will provide a diagnostic image not only decreases radiation, but also allows for faster exposures, resulting in less motion blur. Intensifying screens further decrease the amount of radiation needed. These screens have phosphor crystals that light up when struck by x-rays. This light triggers the silver halide crystals. Screens come in a variety of types (rare earth and calcium tungstate) and speeds (ultra- speed, high-speed and the relatively slower high-detail screens). There is a trade-off though, and the highest speed screens have the least resolution. It is important to match the film spectrum with the screen and use manufacturer-provided technique charts to determine exposure. A grid, which is a flat plate with lead foil strips separated by transparent spacers, can be positioned between the patient and the film to reduce the scatter radiation caused when the parallel traveling x-rays strike the patient’s body and deflect off at odd angles. Grids are classified by the ratio of lead strip height to distance between strips and by the number of lines per inch. A typical grid used in veterinary practice is 8:1 with 103 lines per inch. More expensive grids normally have higher ratio and more lines per inch, and absorb more scatter (2, 3, 16).

As an example, a 12:1 grid with 200 lines per inch which would be used to create a crisper image of higher quality, but also requires a higher dose of radiation. The use of a bucky, which is a moving grid, will prevent the appearance of grid lines. A grid is needed if the radiographed body part measures greater than 10 cm. After exposure, the film must be developed. The sensitized silver halide crystals are reduced to black particles of metallic silver while the film is in contact with developer solution. The unaltered silver halide is removed from the film by the fixer solution. Extensive rinsing of the fixed film is needed because remaining fixer solution will discolor the film. The development process can be carried out either manually with the chemical solutions in tubs or baths, or automatically with a processor applying the solutions and producing a ready-to-read film.

Computed Radiography (CR)

The term computed radiography (CR) refers to the process of creating a diagnostic digital image from data acquired with an imaging plate (IP) and reader. The CR process includes image acquisition, processing, and display. Commercial CR has been widely used in human medicine during the past decade. Recent introduction of reasonably priced CR have systems resulted in more veterinary interest. Equine practitioners led the way, taking advantage of mobile systems with the ability to acquire and view diagnostic radiographic images in the field (12, 13).

Direct Digital Radiography (DDR)

DDR is similar to CR except that the image is acquired directly, rather than by using an IP and reader. As technology advances, these units may become feasible for veterinary practices. Although the quality has been shown to be comparable to that of CR in some studies (10, 11, 12), a recent realistic head-to-head comparison in a human hospital setting resulted in a unanimous decision by the staff to use CR as their main radiological system (8, 11).

DDR is an indirect capture digital imaging technology, which means that plates are used to capture the image before it is transferred to a computer. The image is created on reusable storage phosphor imaging plates rather than film. The storage phosphor plates are similar to intensifying screens. When exposed to x-rays, intensifying screens emit light immediately, exposing the radiographic film. In contrast, when phosphor plates are exposed to x-rays, part of the radiation energy is absorbed by electrons, which store the image temporarily. The latent image is read by scanning the imaging plate with laser light. The electrons then return to their ground state by releasing visible light, which is detected and converted to a digital image (11). Exposure to a bright light (including sunlight) fully de-excites the trapped electrons, erasing the stored image.

In digital radiography, the x-ray beam is converted into an electronic form that is digitized and numerically encoded into discrete picture elements (pixels). The number of pixels per unit area determines the theoretical spatial resolution of the digital image. Actual spatial resolution of the digital image is determined by the efficiency of the imaging plate and the design of the plate reader. The spatial resolution of DDR images is lower than a high-quality film image. However, much of the increased film resolution is beyond the range detectable by the human eye, and as technology advances, this difference is becoming negligible. Many studies conducted in human medicine show that DDR images are equal to or better than traditional film for evaluating most body parts. Perceived image quality and the ability to visualize abnormalities depend more on software manipulation and processing of the image after it is taken than on spatial resolution. DDR has been clinically validated in human medicine for over 18 years in a variety of applications, including mammography, suggesting that its minimally lower spatial resolution is not a clinical limitation (4, 9, 11, 15).

The ability of the viewer to appreciate the image quality obtained with CR partially depends on the quality of the computer monitor.

Common Causes of Image Problems

The production of diagnostic images is hampered by technical errors resulting in image problem. They can be broken down into these categories: detail, film density, and other artifacts. Poor detail can be associated with too much or too little film density; motion of patient, machine head, or cassette; poor film-screen contact; or inappropriate film-screen combination. General causes of unacceptable film density include over and underexposure, over and underdevelopment, and film-based fogging.

Common causes of exposure problems include inadequate measuring of patient, improper use of technique chart, improper focal-film distance, incorrect machine settings, and inconsistent line voltage (1, 2, 4, 6, 7, 10, 13, 15, 16).

Contaminated developer can cause an overall film fog. Light fogging can occur from a light leak into the film bin, cassette, or darkroom. Safelight filter cracks can also be a source of light fogging. Scatter radiation can cause fogging if the loaded cassette is left in the x-ray room while other films are exposed. Film can get fogged during storage if it becomes outdated or is exposed to scatter radiation, high temperature or high humidity (13, 16).

Damaged or dirty cassettes often generate artifacts. Some common causes of nondiagnostic manually processed FSR images include air bubbles on the film, two films getting stuck together, fingerprints, rough film handling, static electricity, inadequate stirring or rinsing of chemicals, incomplete fixation, and evaporated developer. Causes of nondiagnostic automatically processed FSR images include dirty or damaged processor guide shoe, improper processor venting, developer rack problems, and inadequate developer recirculation (4, 10, 13).

Conclusion

Digital technologies can offer impressive benefits, but don't forget that traditional technologies still work. New equipment does not always provide a clear diagnostic advantage over properly exposed conventional radiographs.

Direct Digital Radiography system eliminate the need for a processing unit, film and processing chemicals, as well as the space required for a darkroom and film storage. Labor costs are reduced because there is no need for processor maintenance and retakes are minimized.

Another benefit of digital technology is the ability to send images electronically, all imaging equipment is DICOM (digital imaging and communications in medical standards) compatible. Disadvantages of using DDR include making the change to a new system, the need for training, and cost.

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Emphasis on drug resistant nematodes in horses

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Chimiorezistența față de antiparazitare, substanțe de sinteză, constituie o problemă de stringentă actualitate. În referat sunt prezentate aspecte ale chimioreziostenței, evidențiate la unele nematode parazite la cabaline (strongili mari şi mici, ascarizi, oxiuri), față de principalele grupe de antihelmintice utilizate în decursul timpului (fenotiazină, săruri de piperazină, organofosforice, benzimidazoli, pirimidine şi avermectine). În acest context, sunt prezentate principalele modalități, inclusiv cele de management, pentru evitarea sau reducerea fenomenului.

Key words: chemoresistance, nematodes, horse

Equidae is the family of horse-like animals, order *Perissodactyla*, and includes horses, donkeys, zebras, and onagers. All of these are in the genus *Equus.* According to Poynter (1970), parasitism of horses by nematodes has been known since 400 B.C. when Hippocrates mentioned the pinworm (*Oxyuris equi*). Further, Poynter (1970) states that a thousand years later (450-500 A.C.), Publius Vegetius Renatus, who was the first Roman writer to publish a document entirely on veterinary medicine, stated that ascarids were found in horses.

Equids can harbor over 100 species of internal parasites (Krecek et al, 1987). About one-half of these species are in the strongyle group (Lichtenfels et al, 1998). They are separated into two categories, large and small strongyles and have a direct life cycle; i.e., there is no intermediate host. These parasites live as adults in the lumen of the large intestine (cecum, ventral, and/or dorsal colon) of the horse.

The large strongyle group in horses historically is composed of three species in the genus *Strongylu*s (*S. vulgaris, S. edentatus,* and *S. equinus*). *Strongylus* spp., especially *S. vulgaris,* are the most pathogenic of the strongyles because they can cause colic and even death of horses. Detrimental effects of these parasites usually are most evident during migration of immature stages in organs outside the gastrointestinal tract. One important characteristic of larval stages, especially *S. vulgaris,* is that they migrate into blood vessels. This can result in occlusion of the blood vessels and periodic colics; even death of infected horses may occur.

The small strongyle group includes about 50 species worldwide (Lichtenfels et al, 1998). Virtually, 100% of horses are infected with at least some species of small strongyles (Reinemeyer et al, 1984). Small strongyles also are called cyathostomes. Beneficial references for identification of species of these parasites are Lichtenfels (1975) and Tolliver (2000). Numbers of these worms are usually lower in older horses that have had time to develop some immunity.

They are much less harmful than *Strongylus* spp. because the infective third stages (L_3) only migrate into the lining (mucosa or submucosa) of the large intestine where they encyst (Lyons et al, 2000). Here, they develop to the fourth and sometimes young fifth (adult) stage and then usually trickle out to the intestinal lumen and mature.

The location of small strongyles in the large intestine varies with species (Mfitilodze and Hutchinson, 1985) and larval and adult stages (Reinemeyer and Herd, 1986; Reinemeyer et al, 1988). In one study, about 10 % of adults were present in the cecum, and the others were evenly distributed in the ventral and dorsal colon (Ogbourne, 1978). In another study (Reinemeyer and Herd, 1986), almost 98% of encysted larvae were found in the cecum and proximal ventral colon. Encysted L_4 usually are present in low numbers in the dorsal colon except in individuals with

heavy infections. When encysted larvae emerge from the intestinal lining, they move distally in the lumen to a preferred site to mature.

Both adult and luminal L_4 are plug feeders; i.e., they suck a plug of mucosa into their buccal capsules. Secretions produced by the dorsal esophageal glands assist digestion of the plugs of mucosa. When the small strongyles attach to the intestinal lining, the corona radiata may prevent ingestion of luminal material. Historically, there is some evidence that species of small strongyles with small buccal capsules feed only on the glandular epithelium and species such as *G. capitatus* with bigger buccal capsules, can penetrate deeper into the intestinal lining.

Infections with *Parascaris equorum* is common throughout the world and is a major cause of unthriftiness in young foals. *Parascaris equorum* is a very large whitish nematode, up to 40 cm in length, and his life cycle is direct. Females lay large numbers of thick-shelled eggs which are quite resistant to environmental conditions. Eggs, because of the thick shell and lack of hatching until ingested, protect the embryo and allow survival for many years in contaminated areas such as stalls, paddocks, and pastures. Infection in equids perpetuates because of the abundant egg production of females and the longevity of the eggs in the environment. Ingestion of embryonated eggs results in hatching and then hepato-pulmonary migration of the larvae. After some development in the lungs, the larvae are swallowed and then mature in the small intestine. The prepatent period is about three months.

Infection with the horse pinworm, *Oxyuris equi*, is extremely common and, although, of limited pathogenic significans in the intestine, the female parasites may cause an intense anal pruritis during the process of egg laying. The adult worms are found in the lumen of the colon. The life cycle is direct: females lay the eggs in clumps, seen grossly as yellowish white gelatinous streaks on the perineal skin. Development is rapid and within 4-5 days the eggs contain the infective L_3. After ingestion, the larvae are released in the small intestine, move into the large intestine and migrate into the mucosal crypts of the caecum and colon where development to L_4 takes place within 10 days. These emerge and feed on the mucosa before maturing to adult stages which feed on intestinal contents.

Clinical signs of verminous enteritis may include weight loss, diarrhea, pyrexia, and subcutaneous edema, especially in the ventral abdominal area. The features of this disease in horses may result in poor appearance, delay in shedding the hair coat, diarrhea (acute or chronic), and colic. Large numbers of small strongyles may be shed in the feces. Other clinical indicators of larval cyathostomiasis include leukocytosis as a result of neutrophilia and blood serum changes such as hypoalbuminemia. Horses may die two or three weeks after the beginning of clinical signs. In many cases, the cause of the disease is not diagnosed antemortem. Overall, the small strongyles are not considered very pathogenic except under certain conditions, but they should not be overlooked as disease entities.

Some clinical signs of ascarid infection may include stunted growth, a pot-belly, rough haircoat, loss of appetite, and lethargy. Internal negative effects of ascarids can include reduced motility and hypermotility in different parts of the intestine and, in some cases, intussusception. A mass of worms, in addition to increased gut motility, may cause rupture of the small intestinal wall at the mesenteric attachment, causing peritonitis and death unless surgery is performed soon after perforation occurs.

In infection with the horse pinworm, intense pruritis around anus causes the animal to rub, resulting in broken hairs, bare patches and inflammation of the skin over the rump and tail head.

Control of internal parasites in horses has been attempted for several centuries by administration of various substances.

Most of the early so-called medications had tremendous toxic side-effects in the horse and were ineffective or effective only on a low number of parasite species. One of the first chemical

compounds used for internal parasite control in horses in the USA, and possibly other countries, was oil of chenopodium, distilled from the seeds or leafy part of the plant *Chenopodium anthelminticum*. Scientific testing of the efficacy of this product showed that it provided excellent control of horse strongyles when accompanied by linseed oil and after horses were fasted for 36 hours (Hall et al, 1918). However, for treated horses, there were reports of serious side-effects including lack of eating or drinking water for three or four days and weight loss.

Starting with phenothiazine in the 1940s until the 1980s, new classes of compounds have been marketed about every 10 years. These groups of compounds included piperazines in the 1950s, benzimidazoles in the 1960s and 1970s, the organophosphates in the 1960s (trichlorfon) and 1970s (dichlorvos), levamisole and pyrantels in the 1970s, and macrocyclic lactones in the 1980s (ivermectin) and 1990s (moxidectin). Thus, no new broad-spectrum compounds have become commercially available for control of internal parasites of horses for over 25 years. A few years ago, praziquantel, a pyrazinoisoquinoline, was marketed because of its activity on tapeworms in horses.

Phenothiazine, marketed in 1940, was active on strongyles and was the most commonly used compound for almost 20 years for control of these parasites in horses (Habermann et al, 1941; Gibson, 1953). Small strongyle resistance in horses to phenothiazine was reported at about the same time in England (Poynter and Hughes, 1958; Gibson, 1960) and the USA (Drudge and Elam, 1961). For about 10 years (1940-1950), a low-level administration regimen of phenothiazine was fed daily for the first 21 days of each month (Dimock, 1949; Todd et al, 1950). This dosage did not kill strongyles but it affected reproduction of female specimens; thus, reducing transmission of these parasites.

Piperazine salts were the first compounds active on more than one taxonomically different group of nematodes. They had excellent activity on ascarids, small strongyles, and pinworms. It also was found that piperazines had excellent activity against phenothiazine-resistant small strongyles. However, reduced activity of piperazine on small strongyles later was found.

The first **organophosphate** commercially available was trichlorfon which was active on bots, ascarids, and mature pinworms, but not strongyles. It was mixed with various other compounds for broader-spectrum activity. Activity on mouth stages of bots was not known until it was found for dichlorvos; trichlorfon had a narrow margin of safety.

Dichlorvos was the second organophosphate commercially produced. The pellet formulation, administered on the feed, was highly broad-spectrum. This formulation removed bots, ascarids, small strongyles (including benzimidazole-resistant species), large strongyles, and pinworms. Dichlorvos was the first compound found to be active on mouth stages of bots. Activity was based on the slow release of the drug from the pellets as they passed through the gastrointestinal tract; thus, affecting parasites located throughout the lumen. Some negative aspects of the pellet formulation were that it was not eaten always, water restriction was necessary for improved activity on bots and pellets, which contained residual drug when passed in the feces, could be toxic to birds that ate them. A gel formulation of dichlorvos was active on bots and, at a higher dose rate, also on ascarids and pinworms, but not strongyles.

The first single broad-spectrum anthelmintic (dewormer) was thiabendazole, a **benzimidazole** which became available in the early 1960s (Drudge et al, 1963)**;** it was effective on most species of gastrointestinal nematodes. However, soon after its commercial use, resistance of small strongyles was observed. This resistance of small strongyles to thiabendazole may have been related to earlier use of phenothiazine because of a similar mode of action of both drugs (Rew et al, 1986). Thiabendazole was mixed with other compounds, such as piperazine, for greater activity on ascarids. Initially, this combination was active on benzimidazole-resistant small strongyles. Later, however, resistance of small strongyles to the thiabendazole and piperazine combination was evident. Other similar benzimidazole products (cambendazole, fenbendazole,

mebendazole, oxfendazole, and oxibendazole) came on the market but side-resistance by the small strongyles also was evident.

Various other classes of dewormers were developed and marketed. Febantel is a probenzimidazole which is metabolized in horses to fenbendazole and oxfendazole. It had excellent activity against ascarids, large and small strongyles, and pinworms. After a period of time on the market, resistance of the small strongyles was found.

Two **pyrimidines** (pyrantel pamoate and pyrantel tartrate) were marketed. Pyrantel pamoate had excellent effectiveness on ascarids, small strongyles, *S. vulgaris* and *S. equinus*, but was less effective on *S. edentatus* and pinworms. Initially, this compound was active on small strongyles resistant to phenothiazine, benzimidazoles, and febantel. Later, resistance of small strongyles to pyrantel pamoate was evident.

The **avermectin class** of antiparasitic compounds or macrocyclic lactones (e.g., abamectin, ivermectin, and moxidectin) provides the broadest-spectrum antiparasitic activity even for a single compound. By themselves, they are effective on arthropods and nematodes.

In the U.S.A., only four chemical antiparasitic classes are currently on the market: *the macrocyclic lactones* (ivermectin and moxidectin), *benzimidazoles* (fenbendazole, oxfendazole, and oxibendazole), *piperazine* (piperazine), and *pyrimidines* (pyrantel pamoate and pyrantel tartrate).

To summarize activity of compounds on small strongyles:

1) resistance has been documented for all of the benzimidazoles, piperazine, and pyrantel pamoate and 2) macrocyclic lactones still seem to be active.

This phenomenon of drug-resistance of small strongyles has been observed in numerous countries, including Romania (Cernea et al, 2005), with usage of the afore mentioned currently inactive compounds in horses for varying periods of time (Lyons et al, 1999; Kaplan et al 2004; Matthews et al 2004; Meier and Hertzberg 2005; Nielsen et al, 2006).

In the last few years, discovery of drug-resistance for another horse parasite, the ascarid (*Parascaris equorum*), has been found. Ascarids are resistant to ivermectin and probably moxidectin (Boersema et al, 2002; Hearn and Peregrine, 2003; Lyons et al, 2006; Slocombe et al, 2006). This is the first documentation of horse ascarids resistant to a parasiticide.

It is a real dilemma to control internal parasites of horses with chemicals now because ivermectin and moxidectin are inactive on ascarids but remain active on small strongyles. On the other hand, the benzimidazoles and pyrantel pamoate are active on ascarids, but not on small strongyles. Historically, after a period of usage of drugs for parasite control, resistance occurs, especially for the small strongyle group (Lyons et al, 2006).

Opinions vary as to frequency of treatment and usage of compounds. Rotation of different classes of compounds is advocated. Fast rotation is the alternation of classes of drugs for each treatment. Slow rotation is using the same compound or class for several consecutive treatments before changing to another anthelmintic.

Various treatment schedules are used including every 6 to 8 weeks, strategic times such as spring and fall when parasites are usually present in greatest numbers, or only when fecal worm eggs counts (EPGs) are above a certain number. Even though there is resistance of small strongyles to all currently available compounds except ivermectin and moxidectin, it seems prudent not to use them exclusively. Therefore, especially in older horses, it is suggested that ivermectin or moxidectin be used sparingly, for instance in the spring and fall, and to give the other commercially available compounds in between which, as previously mentioned, are still active on non-small strongyle nematodes. Also, it appears that for ascarid control, ivermectin or

moxidectin should not be given to young foals, but horse owners need to give some other compound.

Several helpful management practices have been advocated for control of internal parasites of horses. These are: exposing larval stages on pastures to detrimental factors such as sunlight by clipping and chain harrowing, composting feces, removing feces from the environment, and rotating horses and cattle on pasture. The latter is effective because, except for one species, parasites of horses and cattle are host-specific and the parasites will not survive in the wrong host.

Research has been done on other methods of controlling internal parasites of animals besides chemotherapy (Lyons et al, 1999). This includes measures to kill or lessen environmental stages; i.e., eggs/larvae. Some of them are nematophagus fungi, microarthropods, protozoa, viruses, and bacteria. Although presently being researched, these features are premature on a practical basis. They and other similar control aspects are greatly needed because past experience has shown that giving chemicals to parasitized animals eventually may result in drug- resistance of the parasites.

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The study of horse strongyls resistance from Bihor county using egg hatch assay and larval development assay

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The studies accomplished in November, 2006 had as aim to point out in vitro the horse strongyls resistance to benzimidazole derivates and to macrocyclic lactones, using the egg hatch assay (EHA) and of the larval development assay (LDA). The study was done on horses from the Nojorid locality county Bihor, using a number of 30 horses, and performing 325 analyses.

The intensity of stongylsosis was of 180259 EPG and 2959.25 LPG, the extensity being of 96.67 % by quantifying LPG. The test EHA pointed out a very high efficacy of the benzimidazoles, the egg hatch assay percentage at 0.15 µl/ml being extremely low, similar to CL_{50}. Thus, the values of parameter Y and the regression line had negative tendencies being at maximum for molecule MBZ (-1276.32). At the larval development assay using IVM, the tendency of the regression line was negative, having the value of -211.83, MIC being of 0.0522 µl/ml. These studies have pointed out a good efficiency of both groups of medicines, this showing the fact that the population of strongyls didn't suffer any adaptive changes.

Key Words: stongylsosis, horse, drug resistence

Materials and methods

The research was done in November 2006, had as main aim to emphasize the horses strongyls resistence at benzimidazoles and macrocyclic lactones, using the two *in vitro* tests well-known worldwide: egg hatch assay (EHA) and larval development assay (LDA). The study was done on horses from the lacality of Nojorid (Bihor county), being tested a number of 30 horses, and performing a number of 325 analises.

In order to accomplish the two resistance tests, it was necessary to collect strongyls eggs, to perform tests with mebendazole (MBZ), febendazole (FBZ), albendazole (ABZ) and ivermectin (IVM) [Coles et al., 1992; Johansen, 1989; Craven et al., 1999; Taylor, 1990, Cernea et al., 2006]. All the obtained data were statistically analyzed by using the soft program -Anthelmintic Resistance Program (ARP) [Cernea et al., 2005].

Results

The intensity of strongylosis at horses from Nojorid (Bihor) was of 1802.59 OPG, and of 2959.25 LPG, the extensity being of 96.67 % by quantifying LPG.

The test EHA done in order to point out the phenomenon of resistence of strongyls at benzimidazole at the horses from Nojorid (Bihor), emphasized a very high efficacy of these anthelmitics. The egg hatching percentage at the reference concentration (0.15 µl/ml) was extremely low, having values between -14.20 at MBZ and 8.03 at ABZ. For all tested molecules, the lethal concentration 50 (CL_{50}) had presented negative values, this reflecting the high efficiency of the FBZ even at lower dilutions than 0.0049 µg/ml. Thus, the values of parameter Y and of the regression line had negative tendencies, having maximum values at the molecule of MBZ (-1276.32) (table 1).

Table 1.

Strongyls eggs hatching percentage (EHA) on MBZ, FBZ and ABZ solution, for the horses from Nojorid locality (November 2006)

Conc.	Mebendazole				Fenbendazole				Albendazole			
(µg/ml)	O	L1	T	%	O	L1	T	%	O	L1	T	%
5,0000	40	0	40	0,00	70	0	70	0,00	90	0	90	0,00
2,5000	70	0	70	0,00	70	0	70	0,00	60	0	60	0,00
1,2500	30	9	39	23,08	50	0	50	0,00	70	0	70	0,00
0,6250	60	0	60	0,00	60	0	60	0,00	50	0	50	0,00
0,3125	50	0	50	0,00	40	0	40	0,00	80	0	80	0,00
0,1563	80	0	80	0,00	50	0	50	0,00	60	0	60	0,00
0,0781	40	0	40	0,00	50	10	60	16,67	40	0	40	0,00
0,0391	40	0	40	0,00	60	20	80	25,00	40	20	60	33,33
0,0195	40	10	50	20,00	40	20	60	33,33	30	10	40	25,00
0,0098	30	20	50	40,00	40	20	60	33,33	20	40	60	66,67
0,0049	40	30	70	42,86	50	30	80	37,50	20	40	60	66,67
H_2O	10	60	70	85,71	10	70	80	87,50	10	70	80	87,50
DMSO	70	10	80	12,50	50	10	60	16,67	80	10	90	11,11
General mean of hatching percentage at control samples = 50,16												
Obtained parameter by ARP	**a**		**b**		**a**		**b**		**a**		**b**	
	-260.23		24.83		-184.99		28.20		-189.97		36.52	
	Hatching % at 0,15µg/ml concentration											
	-14.20				0.45				8.03			
	CL_{50}											
	-0.0967				-0.1178				-0.0709			
	Y maxim											
	-1276.32				-896.75				-913.33			

O = eggs; L1 = hatching eggs and larva; % = hatching percentage;
T = total eggs, hatching eggs and larva

At the larval development assay done with IVM, on the same population of strongyls drawn from those 30 horses, we could notice the appearance of the first larvae in third stage at 0.025 µg/ml concentration. This phenomenon has determined a regression line with negative tendency, the maximum value of parameter Y being -211.83 (table 2). Corroborating these data with the value of the minimum inhibitory concentration (MIC) which was of 0.0522µl/ml, we can conclude that the use of anthelmintic based on IVM will have a high efficiency in the treatment of horses strongylidosis.

Tabelul 2

Development of the third stage larva (LDA) on IVM solution, for the horses from Nojorid locality (November 2006)

Conc. (µg/ml)	IVM			
	O	**L3**	**T**	**%**
0,4000	70	0	70	0,00
0,2000	90	0	90	0,00
0,1000	90	0	90	0,00
0,0500	60	0	60	0,00
0,0250	70	10	80	12,50
0,0125	80	20	100	20,00
H_2O	20	80	100	80,00
Larval development percentage at control samples = 80				
Obtained parameter by ARP	**MIC**			
	0,0522			
	a		**b**	
	-609,12		31,82	
	Y maxim			
	-211,83			

O = eggs; L3 = third stage larva; T = total eggs and third stage larva; %= larva development percentage; MIC = minimum inhibitory concentration (µg/ml);

Conclusion

1. The intensity of the strongylidosis at the horses from Nojorid locality was of 1802.59 EPG, and of 2959.25 LPG, the extensity being of 96.67% by quantifying LPG.
2. The test EHA with benzimidazoles pointed out a good efficacy of all tested molecules, the maximum value being at the molecule MBZ (Y= -1.276.32)
3. The test LDA done with IVM pointed out a high efficacy of this molecule, the value of parameter y being of -211.83, and the MIC of 0.0522 µg/ml.

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In vivo study of horse strongyls resistance at Febendazole and Ivermectin

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During February-March, 2007, in order to point out the phenomenon of horse strongyls resistance at benzimidazole and macrocyclic lactones, there have been made in vivo testing using the FECRT tests (Faecal Egg Count Reduction Test), in village Nojorid, from Bihor county region [Coles et al., 1992; Cernea, 2005b; Albonico, 2003; McKenna, 1990). The assays have been done on a number of 30 horses that were divided into two equal groups. Each group was treated by using a product based on fenbendazol (FBZ), and the other one with a product based on ivermectin (IVM). The tests were done before treatment (AT) and post-therapeutically (PT) at an interval of 7 days, for4 time (7^{th}, 14^{th}, 21^{st}, 28^{th}).

The mean AT intensity of the stongylidosis in the 30 horses was of 1777.980 EPG. The mean level of extensivity, which was counted, using McMaster method, was of 91%. After the administration of FBZ, at 7 days, 21 days, 28 days PT, we can notice zero values of the intensivity and extensivity, meaning that this anthelmintic drug has a good efficacy.

The lot of 15 horses treated with ivermectin (IVM) pointed out an important lowering of the EPG at 7 days post therapy, the values being of 6.6. This decreasing tendency reaches the phase of stage, the values of EPG being zero, this meaning an absence of the adaptive phenomenon of the stongyls at the medication used.

Key Words: horse, strongyls, resistance, Febendazole, Ivermectin

Materials and methods

The tests have been done on 30 horses grown up in a farming system in Nojorid, Bihor. The horses were divided into two equal groups, the first one being treated with a product based on fenbendazole (FBZ), the second one with a product based on a product with ivermectin. The samples were gathered before treatment (AT) and after (PT) at the interval of 7 days, thus being realized 4 collections of samples (days: 7^{th}, 14^{th}, 21^{st}, 28^{th}) [Cernea 2004; 2005a]. The tests were analyzed using the MacMaster method, in order to determine the number of eggs/g - EPG), by analyzing faecal cultures and identifying the infested larvae [Ueno and Gutierres, 1983 quoted in by Madeira De Carvalho, 1991; Madeira De Carvalho, 2001]. The intensivity and extensivity AT and PT were calculated, the data obtained being statistically interpreted as concerning: the determination, the standard deviation, the percentage of reduction, the superior and inferior confidence interval limit 95% and the probability index "p" compared to obtained AT values [Waller, 1989; Coles et al., 1992; Motulsky et al., 1999; Motulsky, 2004].

Results

The mean AT intensivity of stongylosis at the 30 horses tested was of 1777.98 EPG. The mean level of extensivity, according to the McMaster method, had the value of 91%. As to group (n=15) treated with FBZ, the AT intensivity was of 1341.67 EPG, the extensivity of the stongylosis being of 86.67% (table 1). After the administration of FBZ at 7, 21, 28 days post-therapeutically, we can notice zero value of the intensivity and extensivity, meaning a good efficacy of this anthelmintic molecule.

Table I

AT and PT results obtained through McMaster method at horses treated with FBZ (Nojorid locality-February-March 2007)

Sex	Age	AT	7 days	14 days	21 days	28 days
		EPG				
M	14	300	0	0	0	0
F	9	1000	0	0	0	0
F	11	800	0	0	0	0
F	3	100	0	0	0	0
F	6	0	0	0	0	0
F	2	100	0	0	0	0
F	7	400	0	100	0	0
F	12	1500	0	0	0	0
F	17	1000	0	0	0	0
F	7	5800	0	0	0	0
M	3	1400	0	0	0	0
F	4	2800	0	0	0	0
F	6	0	0	0	0	0
F	12	500	0	0	0	0
F	5	400	0	100	0	0
Mean		**1073.33**	**0**	**13.33**	**0**	**0**
Standard deviation		**1505.45**	**0**	**35.19**	**0**	**0**

At 14 days post therapy, the value of EPC was of 13.13, this easy increasing being due to, probably, the adults which return from migration in the intestinal lumen [Monahan, 2000].

The efficacy of the FBZ was also emphasized by quantifying the statistical data expressed at the level of EPG (table 2). During the whole PT period, the percentage of reduction was 100%, exception being the percentage of reduction at the interval of 14 days, when it had the value of 98.75% (EPG), value which is over the limit of 95%. At the interval of 14 days we can notice a lower level of confidence limit of the accepted level of 95%, but this is over the minimum accepted value of 90% (94% at EPG) (table 2). The analysis of the "p" index shows significant statistically differences, during the whole experiment by quantification EPG (table 2).

Table 2

Value of statistic data used to quantify the in vivo resistance at horses treated with FBZ (Nojorid locality, February-March 2007)

	PT 7 days	PT 14 days	PT 21 days	PT 28 days
reduction %	100	98.75	100	100
Lower confidence limit 95%		94		
Upper confidence limit 95%		100		
"p" index (compared with AT values)	0.01530	0.01676	0.01530	0.01530

The second horse group (n=15) treated with ivermectin (IVM) had a therapeutic intensity of 2214.29 EPG and an intensity of 93.33% (table 3)

Table 3.

AT and PT results obtained through McMaster method at horses treated with IVM (Nojorid locality - February-March 2007)

Sex	Age	AT	7 days	14 days	21 days	28 days
		EPG				
F	2	450	0	0	0	0
F	8	250	0	0	0	0
F	15	600	0	0	0	0
F	11	1100	0	0	0	0
F	13	11300	100	0	0	0
M	2	1950	0	0	0	0
F	14	4500	0	0	0	0
F	3	7000	0	0	0	0
F	7	800	0	0	0	0
F	8	1200	0	0	0	0
F	5	200	0	0	0	0
F	4	600	0	0	0	0
F	18	150	0	0	0	0
M	14	0	0	0	0	0
F	10	900	0	0	0	0
Mean		**2066.67**	**6.67**	**0**	**0**	**0**
Standard deviation		**3182.80**	**25.82**	**0**	**0**	**0**

At 7 days PT, we can notice an outstanding decreasing of OPG, mean value being of 6.6. This decreasing tendency attains the phase of stage at 21 and 28 days, values of EPG being zero. The statistical interpretation of the data proves the high efficacy of IVM, the reduction percentage being of 100% at 14, 21 and 28 days PT (table 4). Following this, the limits of the accepted level of 95% confidence interval at this horses group treated with IVM has values over 90% during the whole experiment. The "p" index has significant statistically differences, at any experimental phase.

Tabelul 2.13.

Value of statistic data used to quantify the in vivo resistance at the horses treated with IVM (Nojorid locality February-March 2007)

	PT 7 days	PT 14 days	PT 21 days	PT 28 days
reduction %	99.67	100	100	100
Lower confidence limit 95%	97			
Upper confidence limit 95%	100			
"p" index (compared with AT values)	0.02435	0.02475	0.02475	0.02475

Analyzing the resistance phenomenon of horse strongyls from village Nojorid (Bihor) using the FECRT, we can conclude that the two drugs can be used without any risks at the following treatments. The lack of adaptable phenomena at strongyles can be explained because of the

almost total lack of use of anthelmintic medication. However, the high level of intensivity and extensivity of strongylosis imposes the implementation of periodical programs of prevention, in order to decrease the contamination degree of pastures.

The determination of the structure of population of strongyles at the horses from Nojorid (Bihor) displayed for the a hole period of this study, led to the identification of species belonging to the *Strongylinae* subfamily in the percentage of 8.8% (*Strongylus vulgaris* 4%, *Strongylus edentatus* 1% and Oesophagodontus robustus 3,8%). *Cyathostominae* subfamily was represented by *Cyathostomum spp*. (89%) and *Poteriostomum spp*. (2.2%) this reflecting the higher resistance of this subfamily at the used medication.

Conclusions

1. The AT mean intensivity of the strongylidosis at the group of 30 horses was of 1777.98 EPG and extensivity being of 91%.
2. At the horses group treated with FBZ, the percentage of reduction was 100%, the values of the confidence interval limit being over 90% and the analysis of the "p" index shows out significant statistical differences.
3. At the horses group treated with IVM the percentage of reduction was of 100%, the value of the confidence interval limit 95% was over 90%. The “p” index shows out statistical significant differences during all experimental phase.
4. The determination strongyls population structure at the horses from Nojorid locality led to the identification of species belonging to the S*trongylinae* subfamily in percentage of 8.8%, the rest belonging to the *Cyathostominae* subfamily.

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The therapeutical protocol for a German Shepherd dog with tetraplegia

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A 10 months old German shepherd dog was presented to the Internal Diseases Clinic of the Veterinary Medicine Faculty in Iasi with clinical signs of paralysis of all limbs, installed progressively within 48 hours. After a general physical, neurological and radiological exam, an inflammatory nerve root and peripheral nerve disease was suspected. Specific therapy and nursing care were instituted immediately. All through its hospitalisation, the dog underwent physical therapy, including massages twice a day and laser therapy.

Within two months, the patient recovered completely.

Key words: tetraplegia, dog, laser therapy, kinetotherapy, acute idiopatic poliradiculoneuritis

Tetraplegia is a disorder of the nervous system that consists of complete loss of motor functions in the four limbs. It can have causes of various natures: congenital, inflammatory, traumatic, degenerative, metabolic, nutritional or neoplastic.

The severity of its manifestation demands for urgent intervention with an adequate therapy, and at the same time trying to establish an exact diagnosis through the folowing methods of investigation: radiography, cerebrospinal fluid analysis, magnetic resonance imaging, computer tomography and electromyography.

Materials and methods

A 10 months old female German Shepherd dog was presented to the Internal Diseases Clinic of the Veterinary Medicine Faculty in Iasi, in a state of tetraplegia installed progressively within three days. The dog's owner did not report any previous disorder or trauma. Clinical signs began with tremor and ataxia of the pelvic limbs, progressing to paralysis. Later, within approximately 24 hours, the condition advanced similarly in the thoracic limbs.

The physical and neurological exam showed flaccid paralysis of all limbs and desensitization. Further diagnostic tests included a hematological exam, cerebrospinal fluid analysis and a radiological exam.

Symptomatic and maintenance medication were instituted using fluids: 5% glucose solution and isotonic saline solution, vitamin B group injection (Multiject B), vitamin C, multi-mineral complex (Calcium Borogluconate) and antibiotics (CTP12). To prevent complications following prolonged lateral recumbency (hypostatic pneumonia and decubital ulcers), the animal was turned every 6-8 hours and groomed daily. At the same time, massages were applied twice a day and laser therapy was used (Roland IR-27). In order to reverse the muscle atrophy, anabolic steroids (Anabolin Forte) were administered, sustained by a high protein diet.

Results and discussions

The physical and neurological exams showed: normal behaviour, the animal being conscious, presence of appetite, permanent lateral recumbency, inability to raise its head and dysphony. During the first week of observation, the dog had a body temperature of around 39.2º C,

discordant breathing with an average of 90 breaths / min.,a heart rate of 124 beats / min with a heart rhythm disorder. The functional exam of the nervous system revealed atony and akinesy of the neck, trunk and limb muscles, loss of superficial pain, absent superficial and deep spinal reflexes, except for the anal reflex. Urination and defecation remained normal. The cerebrospinal fluid analysis revealed a remarcable increase in protein (1g/l) and the blood exam showed granulocytosis. The myelography proved the integrity of the spine.

The information collected from the initial clinical assessment, clinicopathologic tests and diagnostic imaging could not be synthetised in one of the nervous syndromes described in literature. However, flaccid paralysis and desensitization from the cervical region to the phylum terminale, could be considered characteristic for acute idiopatic poliradiculoneuritis, as the clinical tableau matches the description of this disease.

Based on the symptomatic diagnosis of tetraplegia and anaestesia, specific therapy was instituted in order to maintain the body's main functions, to prevent infections (antibiotics) and at the same time laser therapy and kinetotherapy were applied.

The laser was used at a frequency of 6000 Hz, in the spinal region. The 10 sessions were programmed during three weeks and a half, i.e. tree sessions per week. Anti-inflammatory gels (Diclofenac gel, Nifluril gel) were used while applying the laser, to facilitate the drug's penetration into the tissues.

Every day, all through the dog's hospitalization period, she underwent specific kinetotherapeutical procedures aimed at delaying severe muscle atrophy, the shortening of ligaments and articular degeneration due to total lack of movement. Massages were applied to all muscle groups of the neck, trunk and limbs, along with manual flexion, extension, abduction and adduction of the joints. After a period of motor and sensitive recovery, when the animal was able to maintain normal posture, both in station and motion, sustained exercice was encouraged.

The evolution of the animal's recovery was impressive, as the first signs appeared during her second week of hospitalization i.e. movements of the neck and the ability to support her head in an upright position. Gradually, the animal was able to move its trunk and tail, and she showed signs of sensitive recovery (the dog reacted to subcutaneous injections) in the thoracic region. Shortly afterwards, the senses of touch and pain returned in the pelvic limbs, followed by the thoracic limbs a few days later. Day by day, the superficial and deep reflexes intensified, as did the dog's perception of pain, and she tried and succeded to position herself in sterno-abdominal decubit, then to stand on her hind and fore limbs. Within approximately 5 weeks of hospitalization the dog managed to adopt a quadrupedal position in standing and in motion, with help and support. Within the next two weeks, the animal recovered completely.

The complex therapy applied to this case prevented possible complications of an inflammatory nature, contributed to stimulating lymph and blood circulation, to alleviate muscle and joint pain, as well as to the recovery of sensitive and motor functions.

CONCLUSIONS

1. The therapeutical protocol in tetraplegia consists of antibiotherapy, vitamin therapy, anabolic and maintenance medication, as well as laser therapy and kinetotherapy.
2. Laser therapy was applied to the spine for its anti-inflammatory, antalgic and biostimulating properties.
3. Anabolic medication and support of the main body functions, along with kinetotherapy, played a major role in maintaining the physiological status of the musculoskeletal system and led to the complete recovery of the pacient.
4. The clinical tableau, onset and evolution, along with the therapeutical results in the case studied are suggestive of acute idiopatic poliradiculoneuritis.

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Methods and techniques of radiography of the thorax

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The radiographical diagnosis is an extremely valuable "weapon" in diagnosing cardiac and thoracic diseases. The study performed in the Radiological Diagnosis Laboratory of the Veterinary Medicine Faculty in Iasi, on dogs of different ages and breeds showed that the radiological examination helps confirm or exclude a clinical suspicion, or even establish a diagnosis. The radiography of the thorax offers information on: the conformation and pathology of the rib cage, cardiac size and contour and its report to the other organs, the aspect of pulmonary and vascular tissue, pleural, mediastinal and diaphragmatic changes.

Key words: thoracic radiography, radiographic film, cardiac disease diagnosis, thoracic disease,dog

The thoracic radiograph allows the general examination of the rib cage and the interpretation of cardiac anomalies, the evaluation of the size, shape and position of the heart, and the observation of the consequences of heart failure (pulmonary edema, pleural effusions, ascites, changes of the pulmonary vessels), identification of some extracardiac affections that might cause cardiac changes (chronic pulmonary disease, tracheobronchic lesions) (4).

Materials and methods

From the total of patients presented to consultation, twelve cases were chosen to represent the study material. Five of these cases showed cardiac pathology and seven cases had various pulmonary disorders. From the patients with cardiac affections, three were diagnosed with heart failure and two with cardiomegaly. In the case of patients with pulmonary diseases, three dogs were diagnosed radiologically with bronchitis, two with diffuse bronchopneumonia and the other two with pleuropulmonary affections.

After the physical examination the dogs were radiographed in the roentgendiagnosis laboratory of the Faculty of Veterinary Medicine in Iasi, with the ELTEX 400 X-ray machine, which has a maximum power of 125 kV.

On all patients, radiographical images of the thorax were taken in two projections minimum: in dorsoventral or ventrodorsal projection and in lateral projection. The radiological parameters applied were according to the animal's size and the type of suspected condition. For example in a dog weighing between 25-30 kg, to examine the heart, the parameters applied were: a tension of 62 kV and exposure of 0.4 s. In order to examine the lungs in a dog of the same weight the parameters change as follows: the tension rises to 65 kV and exposure decreases to 0.2 s.

Results and discussions

The results of the radiological exam are described according to the clinically diagnosed condition.

In left-sided heart failure we noticed, in the lateral view, the enlargement of the left atrium, the dorsal deviation of the trachea and pulmonary hylum, the enlargement of the contact area between the heart and the diaphragm. In the dorsoventral view we noticed the curved shape of the left ventricle and of the cardiac apex, as well as its deviation to the right and the enlargement of the left atrium.

In right-sided heart failure we noticed, in the profile view, the curved shape of the heart's cranial margin and the enlargement of the contact area between the heart and sternum. In the dorsoventral view we observed the curved shape of the right ventricle and/or the right atrium, the deviation of the apex to the left and the enlargement of the pulmonary artery.

In the profile view cardiomegaly was identified through: the enlargement of the longitudinal (base to apex) and craniocaudal diameters, dorsal elevation of the trachea, compression of the bronchies by the enlarged left atrium, the curved shape of the cranial margin of the heart, the enlargement of the contact area with the sternum, the dorsal deviation of the caudal vena cava. In the dorsoventral view we noticed the round cardiac silhouette, and the deviation of the heart apex caudal, slightly to the left.

The patients who were diagnosed with bronchopneumonia showed areas of relative opacity of small dimensions, focal, poorly margined or well shaped, with diverse locations, especially in the cardiac and diaphragmatic lobes. Radiological transparency areas correspondent to compensatory emphysema delimit these areas of radiological opacity.

In bronchitis, we observed the opacity of the vessels associated to inflammated bronchies and sometimes modifications of bronchiectasis.

In the case of pleuro-pulmonary complications, the lateral view showed large areas of radiographical opacity, situation confirmed especially in dorsal decubit – in this position areas of lateral radiographical opacity are noticeable, delimitating the thoracic wall and compressing the pulmonary tissue.

For the radiological examination of the thorax, numerous methods or techniques can be used, each of specific importance and results, such as: radioscopy, classical radiography, teleradiography, ortodiagraphy, kimography, tomography, and vasography (7). Classical radiography, which consists of capturing the image on radiographic film, is the most frequently used method in the diagnosis laboratory of the Veterinary Medicine Faculty in Iasi, representing a standard procedure for the radiographical exam of animals.

The evaluation of the heart and major vessels must include two radiographic projections: dorsoventral or ventrodorsal and lateral, and the film should be exposed at peak inspiration. In lateral view, the heart is oriented slightly slanting, at an angle of approximately 45 degrees, situated between the 3^{rd}- 8^{th} thoracic vertebrae and occupies about 3 intercostal spaces. On the radiological film, in this projection, you can measure the longitudinal axis (the long axis) and the transversal one (the short axis). The long axis represents the distance between the base and the apex of the heart, and the short axis, perpendicular on the long one, is located craniocaudaly in the broadest area of the heart. The long axis is equal to the distance between vertebrae T4-T8 and the short axis to T4-T7 (4).

In the dorsoventral or ventrodorsal view the heart has a roughly elliptical shape with a curved right ventricular and relatively straight left ventricular border. Anatomical structures include (clockwise): aortic arch extending from 11 to 1 o' clock, main pulmonary artery segment from 1 to 2 o' clock, left auricular appendage from 2 to 3 o' clock, left ventricle from 2 to 6 o' clock and right heart from 6 to 12 o' clock. This synthetic representation of the anatomical structures of the heart on a clock's dial, help the examiner exactly identify changes of their shape and volume (5).

The radiographical evaluation of the pulmonary tissue must also include two projections: the lateral one which represents the summed images of the right and left lung, and the dorsoventral or ventrodorsal projection, which helps the examiner locate the changes on the right or left pulmonary area. The aspect of pulmonary tissue on radiological film is expressed by an alternation of radiologically opaque and transparent vertical strips (7). In the lateral projection, we can make observations regarding the distances between a modified area and the lung's tip and base. Vertically, we can measure the distance to the vertebrae and ventrally to the sternum. In the

dorsoventral and ventrodorsal projection, we can measure the distances to the ribs and to the mediastinum.

In the radiological exam of the lungs, the right hemithorax is preferred, as it is normally free of parasite images (of other thoracic organs), offering a wide area of examination, as compared to the left hemithorax (easy to recognise from the orientation of the heart's apex towards the thoracic wall) which is mostly radiographically opaque because three quarters of it are occupied by the heart, placed almost totally in the medio-ventral area of the radiological image (7).

The radiologically opaque modifications on the pulmonary area are due to accumulation of liquids or to processes of pulmonary densification or organization. This kind of changes can be localised or generalised. Discrete generalised radiological opacities are generally of a pleural origin as a result of acute conditions. Clinical pleural conditions (a thickening of the serouses) are translated into intense, uniform radiological opacity of irregular intensity. Radiological transparency can manifest locally, in case of an accumulation of air in newly formed cavities or in unilateral pneumothorax and partially in localised pulmonary emphysema.

The radiological evaluation of the thorax must take into account: breed conformation, relative state of hydration, state of respiration, obesity (fat deposits may accumulate in the sternal region and can mimic pleural effusions, subpericardical fat simulates cardiomegaly and in the mediastinum it can look like some kind of mass) and the animal's position (1).

In evaluating a radiograph, the following systematic approach should be used: evaluate technical factors (exposure technique, respiratory phase and positioning), evaluate extrathoracic structures, evaluate intrathoracic structures, correlate radiological findings with clinical presentation (3).

Conclusions

1. The techniques and conditions of radiographical examination of the heart must be strictly followed, because frequent errors of interpretation of shape and size can appear.
2. Heart failure (left-sided, right-sided or general) can be radiologically diagnosed only when changes of shape and size of the heart are visible, or it can be suspected when its consequences (pulmonary edema, pleural effusions) appear.
3. Pleuropulmonary affections accompanied by pain and intense dyspnea call for the administration of analgesic-anaestethic substances before the radiography can be taken.
4. Changes of the thoracic vessels and intracardiac structures noticed radiographically should be examined in detail through the echographic technique.

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Researches concerning reproduction parameters of cows with clinical mammitis

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Researches made on 140 cows from Holstein-friza type, with different forms of clinical mammitis, were focused, comparing with healthy cows, the following index of reproduction: the period of birth – the first insemination (L.A.); service - period (S.P.) and the insemination index (no. L.A./ gestation)

Comparing with healthy cows it was noticed that the period of birth - the first insemination (L.A.); was longer with 6 days at cows with serous mammitis, with 17 days at cows with cataral mammitis (P < 0,05) and with 47 days at those with purulent mammitis, (P < 0,001); service - period (S.P.) presented distinst significant increases (P < 0,01) at cows with cataral mammitis and very significant (P < 0,001) at cows with purulent mammitis; the insemination index was with 0,2 higher than at cows with cataral mammitis and with 0,8 at those with purulent mammitis.

Key words: cow, mammitis, index of reproduction

Mammitis, at dairy cows, are the most important affections of mammary gland with real zoo-economical implications due to looses in milk production, early reformation of sick animals as well as the risk that the infected milk presents for public health (1,3,4,6,).

Some authors saw at mammitis cows also the affection of reproductive function manifested by the decrease of the conception rate and the increase of abortion cases(5,6,).

Another auhtors say that in important cases of mammitis the ciclical ovarian activity may be interrupted when mammitis appears between 15-28 days after birth when the first estru postpartum is expected (2,5,7,).

In this context, the approaching of the problem concerning the mammitis represents an opportunity being necessary suplementary studies which should have all their implications on milk production and reproduction.

MATERIALS AND METHODS

There have been studied 1750 cows from Holstein – Frize type breed in an intensive system, cows with different ages and different levels of milk production. Among those a number of 140 cows (8%) presented different types of clinical mammitis. The following index of reproduction, comparing with healthy cows, were watched: the period birth – the first insemination (I.A.), service-period (S.P.) and index of insemination (No. I.A./gestation).

RESULTS AND DISCUSIONS

After analysing the data from Table 1, we notice that the period birth – first I.A. registers medium values between 84 and 125 days at cows with different forms of mammitis, while at cows without mammitis that parameter presents a medium value of 78 days. We also notice that the difference of only 6 days, registered by the medium values

Tabel no. 1

Parameters of reproduction at cows with or without mammitis

Specification	No. of Animals	UM	Parameters of reproduction		
			Period - days		No. IA/ gestation
			Birth-I-aIA	IA gestant (SP)	
Cows without mammitis	1610	Average	78	109	1,4
		Minimum.	55	55	1,2
		Maximum	110	140	1,6
Cows with serous mammitis	45	Average	84	119	1,4
		Minimum	60	60	1,2
		Maximum	125	155	1,6
Cows with cataral mammitis	79	Average	95 *	130 **	1,6
		Minimum	70	88	1,4
		Maximum	135	170	2,0
Cows with purulent mammitis	16	Average	125 ***	150 ***	2,2
		Minimum	95	125	1,8
		Maximum	165	220	2,4
Total of cows with mammitis	140				
Total of cows	1750				

**** significant difference, P<0,05; ** distinct significant difference, P<0,01; *** very significant difference, P<0,001),***

of this parameter, between healthy cows and those with serous mammitis (78 days and repectivelly 84) is statistically insignificant, but it becomes statistically significant (P≤0,05) between healthy cows and those with cataral mammitis (78 days, respectivelly 95 days) and very significant statistically (P≤0,001) between healthy cows and those with purulent mammitis (78 days, respectivelly 125 days).

Concerning the evolution of service-period (S.P.) we also notice higher medium values (119 – 150 days) at cows with mammitis comparing with those registered at healthy cows (109 days). Those were with 10 days longer at cows with serous mammitis (statistically insignificant difference), with 21 days in the case of cows with cataral mammitis (distinct significant difference, P≤0,01) and with 41 days longer in the case of cows with purulent mammitis (very significant difference, P≤0,001).

The same increasing tendency can be noticed also in the case of insemination index (No. I.A./gestation) which showed medium values between 1,4 and 2,2 values that were higher with 0,2 at cows with cataral mammitis and with 0,8 at cows with purulent mammitis, comparing with those with no mammitis.

On the whole, we notice that the advanced forms of mammitis affect more profound the reproductive function, an aspect illustrated by increasing of the medium values of analysed parameters.

The increase of those values leads, on the one hand, to the non-achieving of the goal concerning the getting of one calf in every year for each cow, and on the other hand, leads to suplementary costs with the higher number of doses of insemination material in order to obtain a pregnant cow. All those shortcomings came together with the ones that are more important and that are represented by the registered looses in milk production and by the suplementary costs imposed by the treatment of mammitis.

CONCLUSIONS

1. The period birth – first insemination (I.A.) presented, in the case of the cows with mammitis, medium values between 84 and 125 days, being higher than at the cows without mammitis (78 days) with 6 days at cows with serous mammitis, with 17 days at cows with cataral mammitis (statistically significant difference, $P \leq 0,05$), and with 47 days at cows with purulent mammitis (very significant difference $P \leq 0,001$).
2. Service-period presented distinct significant differences ($P \leq 0,01$) between the medium values registered in the case of the cows with cataral mammitis (130 days) and at those without mammitis (109 days) and very significant differences ($P \leq 0,001$) between the values from the cows with purulent mammitis (150 days) and those from cows without mammitis (109 days).
3. The insemination index at cows with mammitis registered medium values between 1,4 and 2,2, being higher with 0,2 at cows with cataral mammitis and with 0,8 at those with purulent mammitis, comparing with cows without mammitis (1,4).

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Use of ultrasonography for diagnostic of some prostate gland disorders in dogs and therapy principles

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In 16 out of a 200 male dog population (8%), of different ages and breeds, various prostate disorders were found. Benign prostate hyperplasia was the most common disease, representing 12/16 cases (75%). In 4 from 12 cases (33%) the main clinical sign was urinary retention and in other 2/12 cases (16.5%) perineal hernia and constipation was noticed. In other cases dysuria, cystitis, orchitis or hematuria was observed. Ultrasound exam of the prostate shows diffuse, relatively symmetric hyperplasia, with homogenous hyperechogenity. Small multiple cystic structures were seen in 3 form 12 dogs (25%)

Prostatic abscess in 3 cases was confirmed by ultrasonography, on the basis of asymmetric prostatomegaly, with intraparenchymal fluid-filled spaces, slightly echogenic, with numerous particles in suspension, suggestive for the abscess.

Chronic prostatitis was associated with chronic urinary tract infection (cystitis), urolythiasis and urinary retention. Ultrasonography showed an asymmetric prostate, with irregular echogenity, containing some cysts or micro abscesses. Urinary blader was enlarged, with small calculi

Key words: dog, benign prostate hyperplasia, prostatic abscess, chronic prostatitis

The prostate gland disorders are very common in dogs including: benign hyperplasia, squamous metaplasia, prostatitis and prostate abscess, intra and paraprostatic cysts and prostatic neoplasia. Clinical signs are very similar because of prostatic enlargement or inflammation, including: dysuria, tenesmus, constipation, blood dripping from the urethra independent from the urination or discrete hematuria and recurrent urinary tract infection, locomotion disorders. Urethral obstruction or urinary incontinence are less common (1, 3, 5, 6, 7).

Abdominal or rectal palpation may give some dates about the size, shape, symmetry, consistency and local pain, but can not differentiate among various prostatic disorders. Abdominal radiography help define the size, shape and position of the prostate (4, 6, 7). We used the ultrasonography to obtain the additional dates about the homogenenity of the parenchyma, size and shape of the gland, urethral diameter and to evaluate focal or diffuse nature of disease.

MATERIALS AND METHODS

A male dog population of 200 animals was examined. In 16 dogs of different ages and breeds we diagnosed various prostate disorders (Table 1). To confirm the diagnostic we used the Aquila Vet ultrasound machine (Pie Medical), with sectorial probes of 5 – 7.5 MHz. Additional paraclinical exams were made in some cases: urine biochemistry, bacteriology, and haematology.

RESULTS AND DISCUSSIONS

Benign prostate hyperplasia was the most common disease, representing 12/16 cases (75%). In 4 from 12 cases (33%) the main clinical sign was urinary retention and in 2/12 cases (16.5%) perineal hernia and constipation were the main sign. Some authors shows that constipation dominate the clinical picture and urinary troubles are secondary (7). In other 4 cases (33%) dysuria, cystitis, orchitis or hematuria was observed. In 2 of the cases no clinical signs related to prostatic disorders were observed, hyperplasia being noted at ultrasonographic exam.

Table 1

Type of prostate gland disorders encountered in dogs

Nr. Crt.	Disease	Patient (breed, age in years)	Associated clinical signs
1.	**Benign prostatic hypertrophy**	German Shepherd 14 yr	Hematuria
2.		Amstaff 3 yr	-
3.		German Shepherd 9 yr	Dysuria
4.		Scottish Terrier 8 yr	Orchitis, hematuria
5.		German Shepherd 9 yr	Perineal hernia, constipation
6.		Pekinese 12 yr	Perineal hernia, constipation
7.		Pekinese 6 yr	Urinary retention
8.		Akita Innu 7 yr	Urinary retention
9.		Crossbred 8 yr	Urinary retention
10.		German Shepherd 8 yr	Cystitis
11.		Bichon M. 9 yr	Urinary retention
12.		Doberman 11 yr	Endocarditis
13.	**Intraprostatic abscess**	Boxer 7 yr	Hematuria, fever, locomotion disorders
14.		German Shepherd 7 yr	Hematuria , fever, locomotion disorders
15.		Caniche 10 years	Pyuria, dysuria, locomotion disorders
16.	**Chronic prostatitis**	Dutchshund 12 yr	Cystitis, urolithyasis, locomotion disorders

homogenous hyperechogenity. Small multiple cystic structures were seen in 3 form 12 dogs (25%) (fig. 1, 2,3). In a case, secondary prostatitis and cystitis was noticed (fig.4).

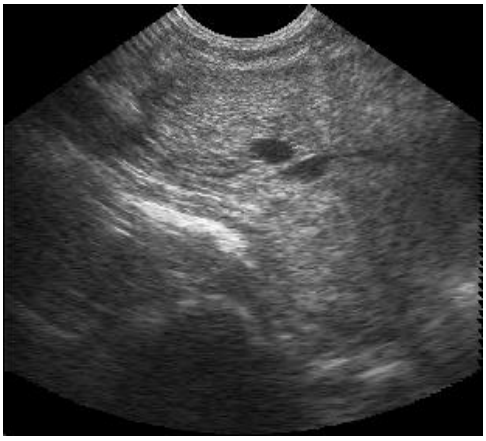

Fig.1 Benign prostate hyperplasia with two small intraprostatic cysts

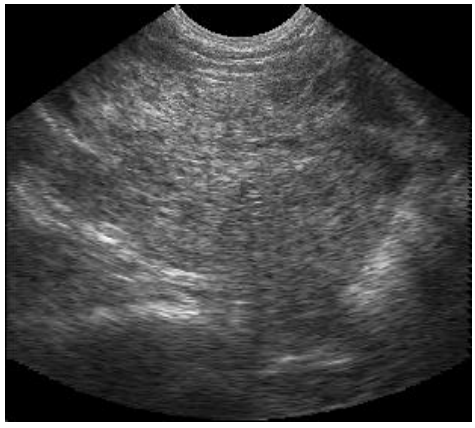

Fig.2 Benign prostate hyperplasia

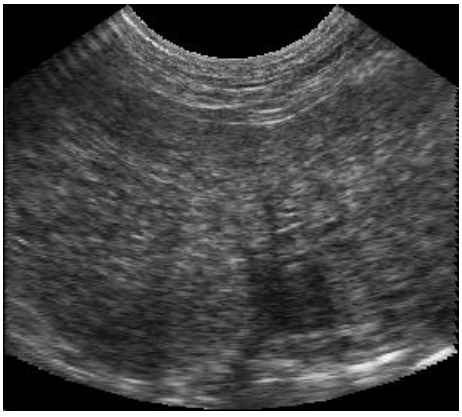

Fig.3 Benign prostate hyperplasia

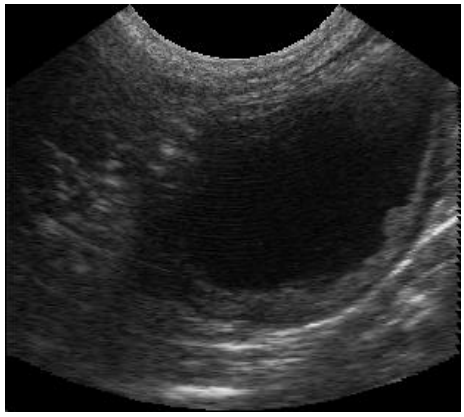

Fig.4 Chronic cystitis secondary to prostatitis

Initially prostatic enlargement is due primarily to glandular hyperplasia. This progresses to cystic hyperplasia (6).

As therapy we recommended castration in all the cases showing clinical signs of benign prostatic hyperplasia, especially for those with perineal hernia, urinary retention, dyisuria or hematuria. Prostatic involution was evident in 4 – 6 weeks.

In 4 cases, conservative treatment with Prostamol Uno (purified alcoholic extract of *Serenoa repens* fruits) gived transitory clinical improvement.

Estrogen therapy to reduce prostatic hyperplasia, although initially effective, is not recommended because repeated low doses as well as overdosage of estrogens can induce squamous metaplasia of the gland and worsen clinical signs (6).

Prostatic abscess was suspected on the basis of general, functional and physical signs (fever, hematuria, pyuria) and was confirmed by ultrasonography, on the basis of asymmetric prostatomegaly, with intraparenchymal fluid-filled spaces, slightly echogenic, with ***numerous particles in suspension*** (fig. 5, 6,7), suggestive for the abscess (2). Locomotion disorders were observed in all the cases.

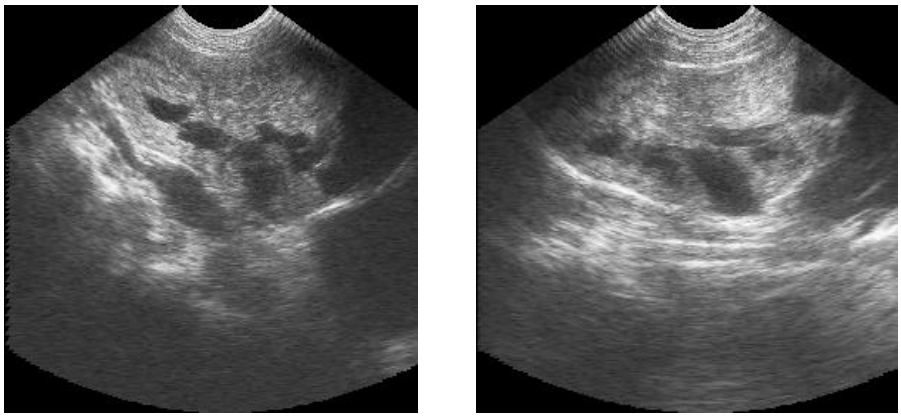

Fig. 5. Prostatic abcess. Small multilocolar hypoechogenic cavities with particles in suspension

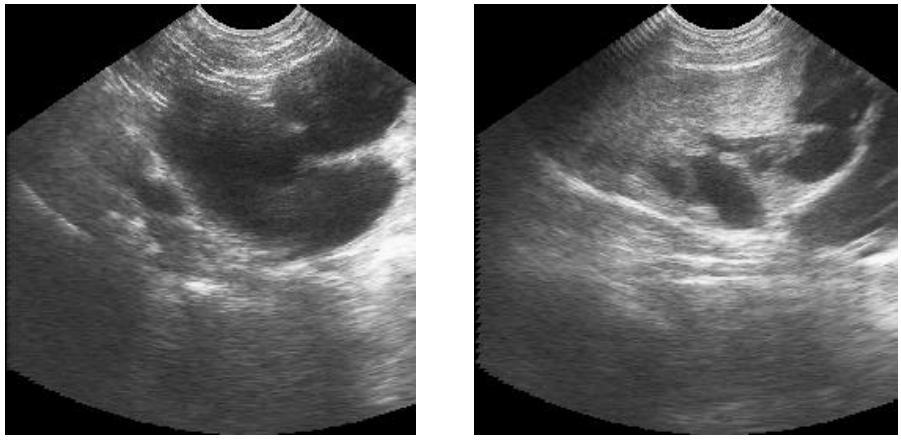

Fig. 6. Prostatic abcess. Large multilocolar intra and paraprostatic hypoechogenic cavities with particles in suspension

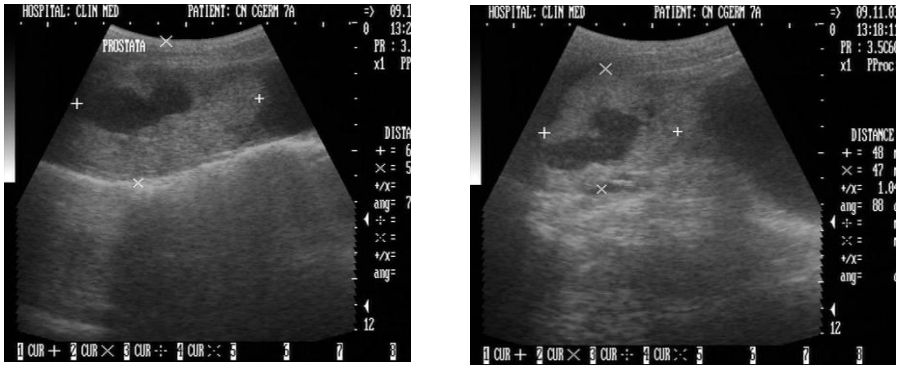

Fig. 7. Prostatic abcess. Large unique intraprostatic hypoechogenic cavity, with particles in suspension

Prostatic abscess may be drained by fine needle aspiration under ultrasound guidance. Large abscess are treated by surgical drainage and marsupialisation followed by antibiotic daily lavage of the cavity of the abscess.

General antibiotic treatment should be continued for 2 – 3 weeks. Acute prostatitis and prostatic abscesses could be life treating disorders. The blood prostate barrier is quite effective in preventing drug penetration into the prostatic parenchyma. Erytromycin, clindamycin, oleandomycin, trimethoprim-sulphonamide, chloramphenicol, carbenicillin, enrofloxacin and ciprofloxacin are the agents most capable to reach therapeutic concentration in the prostate (6).

Castration is very helpful in all cases of intraprostatic abscess, but not very useful in paraprostatic abscess.

Chronic prostatitis was found in a Dutchshund 12 years dog with chronic urinary tract infection (cystitis) and urinary retention.

Ultrasonography showed an asymmetric prostate, with irregular echogenity, containing some cysts or micro abscesses. Urinary blader was enlarged, with small calculi (Fig. 7, 8). Castration and long term (3 weeks) enrofloxacin therapy was recommended.

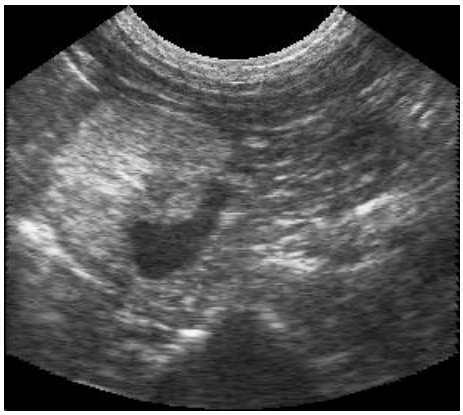

Fig 7. Chronic prostatitis. Irregular unechogenic cavity

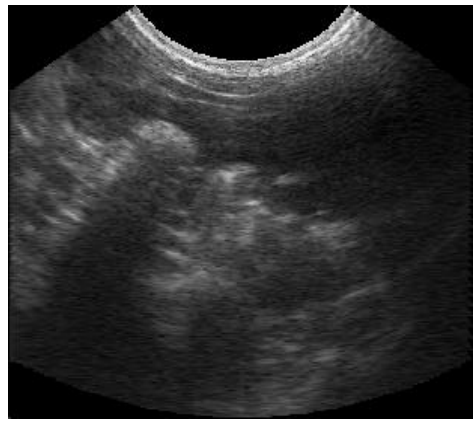

Fig.8. Urolythiasis in dog with chronic prostatitis

CONCLUSIONS

1. In 16 out of a 200 male dog population (8%), of different ages and breeds various prostate disorders were found. *Benign prostate hyperplasia* was the most common disease, representing 12/16 cases (75%). In 4 from 12 cases (33%) the main clinical sign was urinary retention and in other 2/12 cases (16.5%) perineal hernia and constipation was noticed. Ultrasound exam of the prostate shows diffuse, relatively symmetric hyperplasia, with homogenous hyperechogenity. Small multiple cystic structures were seen in 3 form 12 dogs (25%)
2. *Prostatic abscess* in 3 cases was confirmed by ultrasonography, on the basis of asymmetric prostatomegaly, with intraparenchymal fluid-filled spaces, slightly echogenic, with numerous particles in suspension.
3. *Chronic prostatitis* was associated with chronic urinary tract infection (cystitis), urolythiasis and urinary retention. Ultrasonography showed an asymmetric prostate, with irregular echogenity, containing some cysts or micro abscesses.

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Full-thickness and split-thickness grafts

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Over the last one hundred years skin grafting became an important method for burns and other wounds management, because the restoration of skin as an intact barrier is essential for the organism homeostasis. The purpose of this study is to describe indication, surgical techniques, donor site considerations and aftercare for full-thickness and split-thickness grafts, and to present advantages and disadvantages for these two types of skin graft.

Key Words: skin graft, wounds management

Introduction

Skin, the largest organ of the body, covers this entire external surface and is the main site of interaction with the surrounding world. As such, it performs a multitude of specialized functions and the possible most important is that it serves as a protective barrier, preventing internal tissues from exposure to mechanics, physics, chemical and biotical factors. Other important functions include thermoregulation, control of fluid loss, sensitive and immunological functions. For this reasons, restoration of an intact barrier is critical after wounding and burning and may be achieved in numerous ways, including skin grafting.

The history of skin grafts begins in ancient India, where Sanskrit texts confirm skin transplants performed by Hindus in 3000-2500 BC (7,9). After this early attempt at plastic and reconstructive surgery, hundred of years passed until further work improved the practice of skin transplantation. In Italy, in 1442, Brancas developed a novel technique of nasal reconstruction, but did not receive recognition for his technique. His fellow countryman, Gaspare Tagliacozzi, who is considered to be the pioneer of modern plastic surgery, was credited for that, over a hundred years later (9). Modern interest in skin grafting began at the end of the 18th century, and over the last one hundred years skin grafting has evolved into an essential component of the surgeon`s armamentarium (7,9).

Generally, skin grafting is used when other methods of reconstruction such as primary closure, second-intention healing, or local skin flaps are inappropriate, are unavailable, or would produce a suboptimal result (8). The multitudes of skin graft usage include accelerated healing of burns and other wounds (like ulcer wound, defects with extensive skin loss), reduction and prevention of scar contracture, reduction of fluid loss, and protection from bacterial invasion.

Skin grafts are divided in two major categories: full-thickness skin grafts and split-thickness skin grafts (6,8,10,13,16).

Full-thickness grafts

A full-thickness graft is composed of epidermis and the entire dermis (6,10,13,14,16), and contains the full amount of elastic, vascular and connective tissue, and cells of the skin from donor site (10). The tissue can be used either *per se* (as full-thickness unmeshed graft), or after numerous slits have been cut in parallel, staggered rows, to allow the graft to expand in two directions (full-thickness meshed graft) (6,13).

Full-thickness meshed grafts

There are three indications for this type of graft: to cover a wound that is less than ideal (e.g., one with exudates, blood), to cover a large skin defect when the donor site is inadequate, such as

in extensively burned animal and to reconstruct irregular surfaces (concave or convex) that are difficult to immobilize. In first case, grafts allow drainage from the wound (6).

Contraindications. These aspects do apply to all types of skin grafts. Skin grafts will not survive on tissue without blood supply, e.g. bone devascularized by removal of the periosteum, cartilage without the perichondrium, tendon without peritenon, and nerve without perineurium (10,13,14). Other wounds that lack sufficient vascularity to support a graft, like wounds with tissue denuded of its connective tissues covering, irradiated tissue and avascular fat are reconstructed with flaps rather than graft (6).

Instruments: scalpel, tissue forceps, scissors, needle holder, surgical needles, surgical marker (or sterile cotton-tipped applicator sticks and methylene blue solution), sterile thick cardboard and pushpins (6).

Anesthesia: NLA or N-NLA (depend on species of animal), local analgesia through subcutaneous infiltration or intercostals nerves block (1-3% lidocain or procain) (3,4); for adequate haemostasis in donor site 1‰ adrenaline is added at 20-30 drips for 100 ml anesthetic solution (4).

Surgical technique. The defect is prepared for accepting the graft. In old wound, if chronic granulation tissue is present, is completely excised, and grafting is delayed until a healthy granulation bed forms, usually within 4 to 5 days (6,14,15). After that, the epithelium at the wound edges is removed, and the top of the healthy granulation tissues is scrapped, or the superficial 0,5-2 mm layer may be excised with a sharp blade. The defect is covered with a surgical sponge soaked in antiseptic or antibiotic solution while the graft is harvested. A fresh wound is debrided and lavaged with an antiseptic or antibiotic solution and an adherent bandage is initially applied until healthy granulation tissue appears. After this moment a non adherent dressing is indicated to prevent granulation tissue damage (6,14).

Graft may be harvested from the cranial lower lateral thoracic area where the skin is thin and hairy in dogs (6,19) or from the lower abdominal area in horses (5,12). Preparation in donor site consists in cutting and shaving the hair and antiseptization of skin (in an area which exceeds the edges of donor site with 5 to 10 cm) (6).

The graft bed is covered by a piece of sterile cloth to obtain a blood imprint of the wound. Scissors are used to cut the pattern which is laid on the donor site, considering the direction of hair growth. The pattern is traced on the skin with a surgical marker or a cotton-tipped applicator stick dipped in methylene blue. The traced line is cut with a scalpel blade and the skin graft is removed from the donor site and placed with dermal side up on a piece of sterile cardboard. Around the edge of the graft, long segments of suture material are placed and are pulled through slits cut in the edge of the cardboard to stretch the graft (few sterile pushpins can be also used) (6,17,19). To remove all subcutaneous tissue, scissors and tissue forceps are used. After that, a scalpel blade is used to cut slits in parallel, staggered rows, with the incision being approximately 1 to 1,5 cm long and 0,5 cm apart (6,17).

The graft is removed from the cardboard and placed on the wound with the direction of hair growth properly aligned. To fix the edge of the graft to the edge of the wound, simple interrupted or simple continuous sutures (with non absorbable material) are used. To ensure an open mesh on the graft for wound drainage, 2-3 mm of skin is excised from the graft edge before it is sutured. The graft is additionally immobilized by placing simple interrupted sutures between the slits in strategic point (6).

Simple interrupted or simple continuous sutures are used to fix the edge of the wound from donor site (6).

Aftercare. Over the surface of the graft, a dressing which is constituted by multiple layers (first layer, in contact with skin, is non adherent, absorbent and coated of antibiotic ointment, the second is absorbent, and the last is represented by a porous adhesive tape or elastic bandage

material). If the graft is over a joint, a splint is included in limb bandages, but only during the first 10 to 21 days after grafting, while the dressing is maintained for 21 days. Bandages are usually changed daily during the first week, and as the healing takes place, the change is less frequently (6). In other opinion, the first bandage change is performed on day 3 post grafting in dogs and on day 6 in cats, and a second change an day 6 or 7 (9 in cats) (14). Siegfried et al. (15) recommend no bandage change before the fourth day after grafting in cats.

Advantages and disadvantages. Advantages of the full-thickness mesh graft, in comparison with unmeshed graft and split-thickness grafts are numerous and significant: these grafts can be adapted to a graft bed with irregular edge (10); the slits in the graft provide flexibility and allow conforming to irregular surfaces (6,11); retain more of the characteristics of normal skin, including color, texture, elasticity, thickness and hair grow (in comparison with split-thickness grafts) (1,11,13); the graft is stable because of additional simple interrupted suture placed between the slits (6,11), and growth of granulation tissue into mesh holes provide further immobilization and a source of additional vascularization as vessel grow from these "plugs" into adjacent dermis (1,10,13); blood, serum and exudates can drain from wound surface through the slits, allowing a good contact between the graft and the recipient (6,10); if the infection develop on the surface of the graft, only a small pieces of tissue will be destroyed (10); the postoperative contraction is minimal, an aspect very important especially if the graft is located over a joint (1,6,15); no expensive equipment is required (6).

A possible disadvantage is that excess granulation tissue may grow up to the slits and over the top of the graft (1,6).

Full-thickness unmeshed graft

A full-thickness unmeshed graft is prepared just as a full-thickness meshed graft, but no slits are cut in the tissue. Before placing the graft on to the bed, a closed suction drain may be placed for drainage from beneath the graft. For this can be used a butterfly catheter in which are cut holes. Two incisions, a little smaller than the diameter of the drainage tube are made in the skin, approximately at 1 cm below the distal edge of the wound, respectively at 1 cm above the dorsal edge, and two subcutaneous tunnels are made direct to graft bed. The fenestrated catheter is passed through distal incision across the graft bed to the dorsal incision. The tube is adjusted to lie flat against the graft bed, and his dorsal end is secured with a suture placed through the skin and around the tube; similarly is fixed ventrally. The graft is placed on bed over the drainage tube and is fixed at the wound edge with simple interrupted or simple continuous sutures (with non absorbable material). An evacuation tube is placed immediately on the needle of the butterfly (to provide drainage and to prevent clots from forming in the catheter). On a large graft it may be necessary to place a second drainage apparatus. Simple interrupted or simple continuous sutures are used to fix the edge of the wound from donor site (6).

Aftercare. Antibiotic ointment may be placed around the graft edges, and bandaging of the graft is performed as described for full-thickness meshed graft. The first bandage is usually changed 48 hours after surgery. If haematoma or fluids are noted beneath a graft, it can be removed by making a small incision in the graft and using a gentle pressure over the graft, or by using a sterile cotton-tipped applicator. The evacuation tube should be incorporated into the dressing in a way that it can be replaced without changing the entire bandage. When no further fluid is drained from under the graft, usually after 48 to 72 hours, the drainage apparatus is removed (6).

Advantages and disadvantages. Full-thickness unmeshed graft has the same advantages like full-thickness meshed graft, excepting those related to presence of the slits. This type of graft has certain disadvantages: are limited to relatively small, uncontaminated, well-vascularized wounds (1,10,13), do not survive as well as full-thickness meshed graft and split-thickness grafts, unless

drainage is provided (6) and a large portion of the graft is destroyed in the presence of infection (1,6,10); in pad graft, intervening wound areas did not become covered with the heavier keratinized epithelium of the pads, and this tissue is more sensitive than the original (18).

Split-thickness grafts

A split-thickness skin graft is composed of epidermis and a variable quantity of dermis. Depending of the amount of dermis included, this type of grafts are categorized as thin (0,15-0,25 mm), intermediate (0,30-0,45 mm) and thick (0,60-0,75 mm) (6,10,13). Also, the grafts can be used either *per se* (as split-thickness unmeshed grafts), or after meshed by hand, as described earlier, or with an mechanical meshed instrument (as split-thickness meshed grafts) (6,10).

The main *indication* for this type of graft is for reconstruction of defects with extensive skin loss, but is not applicable in cats because the skin of this species is too thin (6). Second-intention healing can be combined with a split-thickness graft to correct significant discrepancies in the depth of the surgical defect and surrounding unaffected skin surface (8).

Contraindications. These types of graft can not be used in areas where good cosmetic is essential (like area of the head) or where significant wound contraction could compromise function (like area of the joints) (8).

Split-thickness meshed grafts

With meshing technique the area of the graft can expand up to nine times the surface of the donor site and this makes the technique very effective, especially in situations where there is not enough donor skin to work in a large wound, or when the recipient site is irregularly contoured and uniform adherence of a solid sheet is a concern (2).

Instruments: freehand dermatomes (scalpel blades, double-edged blades and free-hand knives) or powered dermatomes (air powered or electric), mechanical meshed instrument or aluminum block with small cutting blades and Teflon roller, tongue depressor, tissue forceps, towel clamps, needle holder, surgical needle (6,8,10). Free hand dermatomes are useful for harvesting small pieces of split-thickness graft (8).

Anesthesia: NLA or N-NLA (depend on species of animal), local analgesia through subcutaneous infiltration or intercostals nerves block (1-3% lidocain or procain) (3,4); for adequate haemostasis in donor site 1‰ adrenaline is added at 20-30 drips for 100 ml anesthetic solution (4).

Surgical technique. Preparation of the recipient and the donor site follows the same steps as in full-thickness skin graft. In addition, aliquots of sterile physiological saline are injected subcutaneous in donor site to elevate the skin from the ribs. The skin is lubricated with sterile mineral oil or a water-soluble gel (6,10).

Sampling of split-thickness skin grafts is different, depending on the instrument used.

In sampling with a free hand knife, a sterile tongue depressor or the fingertips are used by an assistant surgeon to pull the skin in one direction, while the surgeon pulls in opposite direction (6). The skin can be additionally stretched with towel clamps attached lateral to harvest site (12).

The surgeon uses his remaining free hand to cut the graft. The knife blade, lubricated with sterile mineral oil or water-soluble gel, is placed at about 30^{o} angle against the skin, and a few back/and forth strokes are made to begin cutting the skin. The angle of the blade is then changed about 15^{o} as it moves back and forth about 3-5 cm behind the surgeon hand that is pulling on the skin. Two forceps tissue or 2 to 4 stay sutures provide tension on the skin as the knife cuts the remaining graft (6,12). With this technique, the skin is cut at widths varying between 4 and 8 cm and at a thickness of approximately 0,60 cm (12).

Split-thickness graft can be also cut with a scalpel blade or a double-edged blade (ordinary razor blade). A partial thickness incision is made in the donor site using a scalpel blade held perpendicular to the skin surface. After that, the scalpel blade or the razor blade is held parallel to the skin surface, and cutting is started. After a 3-4 mm of partial graft has been cut, 2-4 stay sutures are placed in the graft to apply traction on it as the blade cuts the remainder of the graft. The blades are difficult to control when cutting grafts larger than 1 cm and the resulting graft do not have regular thickness (6).

The easier method for harvesting split-thickness skin graft uses air powered or electric dermatome. The dermatome blade is lubricated with sterile mineral oil or water-soluble gel and is placed in the dermatome. The thickness of the graft is determined by adjusting a knob. It is usually set to 0,35 to 0,75 mm (6). The dermatome is held in the dominant hand of the operator at a 30-45° angle from the donor skin surface. With the nonoperating hand providing traction behind the dermatome and an assistant providing traction in front of the dermatome (with a sterile tongue depressor or the fingertips). The dermatome is activated and advanced in a smooth, continuous motion over the skin using gentle downward pressure (13). Once cutting has started, the dermatome is continuously advanced or the graft may be severed (6). After the appropriate length has been harvested, the dermatome is tilted away from the skin and lifted off the skin to cut the distal edge of the graft and complete the harvesting (13).

The graft then may be gently washed to remove the lubricant and wrapped in a moistened saline sponge until it is ready to be used (13). At this point, the split-thickness graft can be meshed. A scalpel is often used to create slits or fenestrations to allow the drainage of serosanguineous fluid and accommodate minor expansion of the graft. A graft-meshing machine may be used if further expansion of the overall surface area of the graft is required; the machine allows for an expansion ratio of 3:1 to 9:1 (8,13). The graft is placed with dermal side down on an aluminum block that contains parallel rows with small cutting blades, and a Teflon roller is passed over the graft in the direction of the blades, to cut slits in the tissue (6,12).

The graft is placed on the bed with the direction of hair growth the same as the hair on the surrounding area. It is placed so it overlaps the wound edge by 1-2 cm, and the overlapped portion can be excised after fixation of the graft (6). Interrupted sutures, skin staples or tissue adhesive fix the graft to the surrounding skin, and additional sutures or skin staples are used between mesh holes to immobilize it into the wound (6,8,13).

The donor site can be excised completely and the edges sutured to give a cosmetic appearance. Alternatively, it can be covered with a sterile non adherent dressing and antibiotic ointment, a petrolatum impregnated gauze or a Scarlet Red gauze, to manage it as an open wound (6).

The *aftercare* of these grafts is like that for full-thickness mesh graft (6).

Advantages and disadvantages. This type of graft have several important advantages: it has a better viability than full-thickness grafts (6); the mesh holes in the graft provide flexibility and allow conforming to irregular surfaces (10,13,16); fluids can drain from wound surface through the slits, allowing a good contact between the graft and the recipient (6,8,10,13); the graft is stable because of additional simple interrupted suture placed between the mesh holes (6); growth of granulation tissue into a mesh holes provides further immobilization and a source of additional vascularization (6,10,13); because is thin, revascularization is produced rapidly (6,10); if the infection develops on the surface of the graft, only a small pieces of tissue will be destroy (8).

Split-thickness grafts has also several disadvantages: it is less durable and more subject to trauma than full-thickness graft (6,8,13) and that makes them of questionable use on limbs (6); this grafts can not be used over a joint (16); the hair growth may be absent or sparse (6,10,13,16); the number of perspiratory and sebaceous glands is lower than in the full-thickness graft, color of the skin is modified (hiperpigmentation or hipopigmentation) 6,10,13,16); graft harvesting requires special and sometimes expensive equipment, but always skill and practice (6,8,13).

Split-thickness unmeshed graft

A split-thickness unmeshed graft is prepared just as a split-thickness meshed graft, but no mesh holes are cut in the tissue. Application of the graft is like that for full-thickness unmeshed graft, excepting the drainage apparatus. The aftercare is similar with all types of grafts. Split-thickness unmeshed grafts have almost all the advantages of the split-thickness meshed graft (excepting the ones that are given by mesh holes) and all the disadvantages (1,6,8,13).

When comparing the functional and aesthetic results of full-thickness skin grafts and split-thickness skin grafts in terms of morbidity, skin elasticity, sensitivity, matching, and scar recurrence, full-thickness skin grafting seems to be the most adequate technique.

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A study on the ascorbinemia levels in dogs

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The scope of this study is to emphasize the maximum levels of ascorbinemia, when a dosage of 60 mg/kc per dog are administered while considering the fact that vitamin C administration in therapeutics focuses on achieving a maximum level of ascorbinemia in a short interval, and on maintaining a high level of ascorbinemia for long periods.

The maximum level of ascorbinemia was reached after 30 minutes from administration and was maintained at higher levels as compared to the initial ones even seven hours later.

Key words: ascorbinemia, plasma

Introduction

If ascorbic acid is administered by injection or by feeding bones to the dog, the blood plasma level increases temporarily as well as that from organs (tissues, cells). This fact determines a stimulation of the leucocytes activity and an inhibition of the glucocorticoid secretions.

Hepatocytes transfer ascorbic acid directly into blood plasma from where it is directed towards other organs (tissues, cells), with the aid of some transportation systems. A large quantity is transported into hypophisis, suprarenal glands and leucocytes. This is eighty times bigger than the level from macrophage cells and 20 to 40 times the level contained by blood plasma. [13].

The level of ascorbic acid in blood plasma is an indicator of the saturation level for this vitamin. From the blood plasma the ascorbic level is transported into the cells through a system of transportation. The larger quantities are transported into the hypophisis, brain, suprarenal glands, testicols, yellow bodies and leucocytes [4, 7].

The use of vitamin C concoctions has the following effects:

- stimulates body growth either through collagen formation or through stimulating the transformation of the non-specialized mezenchimal cells into specialized cells (mioblasts, condrocytes, osteoblasts)[11].
- improves the reactions of the immunitary system and thus speeds up infection combating
- speeds up the removal of the O_2 radicals (super-oxide anions, hydroxyl radicals) which results in a protective effect on cells. The ascorbic acid is the most important anti-oxidating cellular [8].
- results in an increase of the ascorbic acid in the suprarenal glands thus inhibiting the cortisol secretion (anti-stress effect) [10].

Ascorbic acid is also necessary for the maturization of the sexual cells (ovules, spermatozoids).

Also the ascorbic acid is also important for the carnitine synthesis, billiard acids, collagen, adrenaline, noradrenaline, and other hormones (the oxytocine melanocystostimulating hormone) [12].

Material and method

When the level of ascorbic acid in blood is determined, this must be done from the blood plasma, as soon as possible after centrifugation, because the leucocytes which are still retained by the plasma are intense consumers of ascorbic acid. It is because of this that the blood serum is

not suitable for determining the ascorbic acid (due to the fact that when coagulation takes place at room temperature, blood cells consume more ascorbic acid)[6].

For determining the level of ascorbinemia in dogs, three dogs were used in our experiment (two females and one male).Their characteristics are presented in table 1.

Tabel 1

The characteristic of animal used in experiment

No	Kcorp	Sex	Breed	Age	Administrated doses (mg)
1	20	female	commune	3,5	1200
2	22	masculine	commune	7	1320
3	33	female	Mioritic shepherd	5	1980

(original)

After weighting the animals which were previously subjected to a diet of 24 hours, blood samples were collected from them at time 0, and then "Vitamic C 200" tablets were administered to them in a 60 mg/kc dosage, in their feeding bowls. The blood was harvested from their ante-branchial vein, with a vacutainer in a 5 ml container with anticoagulant (EDTA).

After administering ascorbic acid, blood samples were taken at regular intervals of 30, 60, 120, 240, 360, and 480 minutes. The samples were processed immediately, in our clinical laboratory from the Faculty of Veterinary Medicine, according to the method described in the subchapter "Material and method".

The principle of the method

After precipitating the proteins with tricloracetic acid 15%, the filtration is oxidited, and the color reaction is obtained with 2,4 –dinitrophenilhydrazine in a high saturated acid environment.

Sample processing.

2ml of blood harvested through EDTA, are treated with 8 ml of tricloracetic, strongly agitated and centrifugated for 15 minutes, 3000 spins per minute.

This produces the plasma to be further processed:

6 ml of plasma are treated with 1 g of coal activate for retaining impurities, strongly agitated and kept still for 48 hours in the fridge.

It is centrifugated resulting in a supernatant which is processed according to the standards, 4 m supernatant are treated with 2 ml dinitrophenilhydrazine solution (2:1) and 3 droplets of alcoholic tiorea, in a test tube.

The mixture is boiled for 5 minutes in hot water, and then cooled in iced water while H_2SO_4 is continuously poured in a concentration of 5 ml – drop by drop.

After cooling the Screen Master Plus analyzer is used for reading the samples λ = 505 mm, in a tank of 1 cm.

Observations: it is very important that the 2: 1 ratio between the supernatant and dinitrophenilhydrazine is maintained irrespective of the sampling quantity which is processed.

The samples were centrifugated immediately and kept in the fridge until processing. (< 8 hours).

Results and discussion

The results following the ascorbinemia determination are presented in table 2.

Tabel 2

Acid ascorbic level

Animal	Time of blood sample						
	0'	30'	60'	120'	240'	360'	420'
1	1,4 mg/dl	4,2 mg/dl	2,3 mg/dl	2 mg/dl	1,9 mg/dl	1,81 mg/dl	1,74 mg/dl
2	0,66 mg/dl	2,9 mg/dl	2,04 mg/dl	1,96 mg/dl	1,53 mg/dl	1,6 mg/dl	1,2 mg/dl
3	1,99 mg/dl	6,1 mg/dl	4,3 mg/dl	3,2 mg/dl	3,03 mg/dl	2,93 mg/dl	2,1 mg/dl

(original)

The table indicates normal values of ascorbinemia in animals 1 and 3, while in the case of the second animal, the initial level of ascorbinemia was low due to the fact that the animal suffered from untreated otitis.

It was noticed that the level of maximum ascorbinemia wass registered after 30 minutes from the vitamin administration, at a level of 6,1 mg/dl in the 3rd animal and of 4,2 mg/dl in the second animal and of only 2,9 mg/dl in the case of the second dog (the one with untreated otitis).

The consulted literature showed maximum values of ascorbinemia after a longer interval (only after 2 hours from administration), but in such case it should be said that the animals were not subjected to such a long diet as in our case. [6].

It was also noticed that the level of ascorbinemia was maintained for a long period in all three animals as the ascorbinemia level was still superior to the initial values even 7 hours from administration.

The levels of ascorbinemia obtained in our study are comparable with those of other researchers [5,6].

After operation (for plague cicatrisation), in pyometer and painful states the recommended dose is of 30-50 mg/kc which can lead to a good saturation of the body (per bone and intravenously) [5].

During reproductive stages a daily oral administration is recommended: 100-200 mg in large dogs and 50 mg in small dogs.

When administered intravenously to dogs with Carre's disease, in a dose of 1,5 – 2,5 g/day for 3 days, a clinical amelioration was followed by a recovering of health, especially when the treatment was done in due time. [1] In acute hepatitis in dogs the administration of daily doses of vitamin C and B complex are recommended.

In starvation cases an intravenous administration is recommended 10 -20 mg/kc, several times, at an interval of 2-3 days.

For stress prevention and reduction caused by exhibitions, training, and change of owner situations, similar doses with those indicated in the reproductive period are indicated.

In pulmonary cancer prophylaxis in dogs which live together with their smoking owner and for any other passive smoking animals (cats, dogs, and cage birds) a supplementation of vitamin C is recommended.

In the case of German shepherds vitamin C can be administered to pregnant females (3-4 g/day/ bone) and then continued in puppies in the following dosage 50-100/mg/day/bone in the first weeks of life, 500 mg/day until the 4th month and 1-2 g /day up to the age of 2 years in hip displesia prophylaxis. These high doses are justified because the digestive absorption of vitamin C in dogs is limited. For a good development in artificially milk fed puppies the milk replacement must contain 150mg vitamin C/liter.

Conclusions

The used dosage in this experiment (60mg/kc), could be use in plague cicatrisation therapy, in pyometer and certain painful states, considering that in this way a good body saturation with this vitamin can be attained.

The maximum level of ascorbinemia was recorded in a short interval (30 minutes) and was maintained at a higher level for more than seven hours, as compared to the initial values.

The experimental dose is one that it is very easy to administer orally and thus physiologically suited for dog species.

In cases of low vitamin C levels we consider the administration of vitamin C beneficial for sick dogs as there is no peril of overdose because the excess vitamin C can be eliminated renally.

A high level of ascorbinemia above the physiological limits is an indicator of renal deficiency or sugar diabetes.

We can thus conclude that the blood level of vitamin C, although subject to multiple physiological variations, can have a diagnosticated and therapeutical value.

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Results concerning the freezing pretability of buck semen and fecundity after artificial insemination of goat

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The aim of this study was to valuate the freezing pretability of semen collected from 38 bucks Alpine and Saanen breeds.For this reason semen was frozen in Tris and natrium citrate medium using the rapid freezing method. The results of freezing pretability of semen were valuated by monitoring cryobiological indicators : fresh semen motility versus frozen semen motility at 24 h after freezing process, motility after eosine-nigrosine colouring and finally , directly testing of fecundante capacity by artificial insemination of goat.The mean values of fresh semen motility were 86.81±0.88% for Alpine and 85±2,24% for Saanen, while after defreezing were 46.34±2.66% for Alpine semen and 56.12±4,02% for Saanen semen.The results were appreciated by „in vivo"testing of frozen semen , using artificial insemination of goats and monitoring the fecundity and prolificacy.All the results showed that buck semen has high pretability to freezing and can be used for artificial insemination.This study followed to find a possibility to preserve the good quality semen and to be distributed in time and space.

Key words: frozen semen, pretability, motility, fecundity, prolificacy

INTRODUCTION

The advantages of frozen semen are well-known: zootehnically, economically and veterinary. Preservation of genes from goats and its dissemination in time and space, make easy the international genes exchanges. Artificial insemination with frozen semen contributes to improvement of local goats populations. All this presume the technologies improvement for semen freezing process. The frozen buck semen at high quality is used in artificial insemination of goats from farms in natural mate season and against season.

MATERIALS AND METHODS

The studies were made on 38 bucks from Alpine and Saanen breed imported from Germany, from which semen was collected in view to freezing. The semen was collected with artificially vagina after bucks training for 7-10 days , in the presence of one goat with induced oestrus .After semen collecting it was made the first dilution 1:1 with Tris and natrium citrate medium , at pH 6,8-6,9.

The fresh semen valuation referred to: spermatic volume directly readed in collection glass; spermatozoon concentration valuated with Spermaque photometer; valuation of semen motility on 1 to 100 scale in microscope. The ejaculates with more than 70% motility were washed in washing medium in view to remove the seminal plasma.

The semen dilutions were made in laboratory as follows: a dilution at 37°C until the semen concentration reached to $1x10^9$/ml;the last dilution at 4°C with dilution medium containing the glycerol as cryoprotector. Then, the semen was frozen IN Tris and sodium citrate medium with a glycerinic level 8% and 10% yolk of egg, using the rapidly freezing method. The packing of semen prepared for freezing was done in plastique paillettes with 0,25 ml volume, in the freezing room.

This method presume to place for 8 minutes the paillettes on a support at 4 cm distance from nitrogen level (nitrogen vapours) and then by immersing in the nitrogen liquid at -196°C.

The frozen-defrozen semen valuation was execute after 24 hours from freezing process by monitoring the general motility, individual motility ,the percent of alives or deaded spermatozoons (viability) by eosine-nigrosine colouring of semen smear.In our experiment were frozen 2800 paillettes.

To „in vivo" testing of frozen semen , the goats were prepaired for artificial insemination .The oestrus was induced using Chronogest sponges with 45 mg fluorogestone acetate for 11 days, then it were administrated 125 µg prostaglandine PGF2α and Folligon 400 UI on day 8.Artificial inseminations were done at 43 hours after sponges removal, using a vaginoscope and insemination pistolet ,in 1 or 2 intracervically insemination.

RESULTS

The results valuation was made by: monitoring of **cryobiological indicators** compairing with spermatic indicators (fresh semen motility versus frozen semen motility at 24 h after freezing process), motility after eosine-nigrosine colouring and finally , directly testing of fecundante capacity by artificial insemination of goat.The it was calculated the **reproduction indicators**: the fecundity and the prolificacy of inseminated goat.

The mean motility of Alpine fresh semen was 86.81±0.88% and the coefficient of variance 8% .The mean motility for Saanen fresh semen was 85±2,24% and the coefficient of variance 14%.After defreezing , the viability mean expressed by by eosine-nigrosine colouring of semen smear, was 46.34±2.66% for Alpine semen and 56.12±4,02% for Saanen semen. The coefficient of variance were 48% and 39%,respectively, which indicated a high individual variability concerning the semen pretability to freezing (table 1)

Table 1

The cryobiological indicators of frozen semen (Alpine and Saanen)

Tabel 1

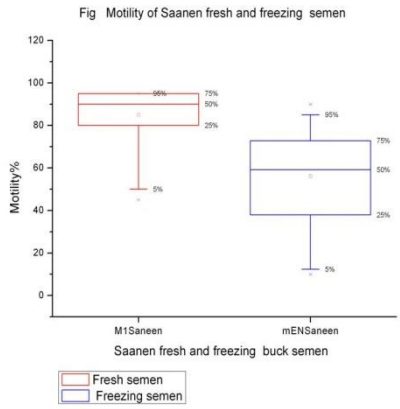
Indicii criobiologici ai spermei congelate de la tapi de rasa Alpina si Saanen importati din Germania

Statistics	Alpine			Saanen		
	Volume (ml)	Motility of fresh semen (%)	Motility of freezing-thawaing semen (%)	Volume (ml)	Motility of fresh semen (%)	Motility of freezing-thawaing semen (%)
N	10	10	10	10	10	10
Mean	1,07391	86,81159	46,34884	1,15933	85	56,12828
Sd(yEr±)	0,33195	7,32497	22,17687	0,36174	12,31764	21,66022
Se(yEr±)	0,03996	0,88182	2,66978	0,06604	2,24888	4,0222
Min	0,3	70	0	0,3	45	10
Max	2	95	80	1,8	95	90
Range	1,7	25	80	1,5	50	80
Median	1	90	47,6	1,15	90	59,15
Coefficient of Variance	31%	8%	48%	31%	14%	39%
Kurtosis	-6,12E-04	0,01381	-0,58762	-0,137	4,4527	-0,65434

The figure 1 shows the comparative diagram of minimum and maximum values of fresh and frozen semen:45% and 95% motility for fresh semen versus 10% and 90% for frozen semen (Saanen breed).

Figure 1

Motility of Saanen fresh and frozen semen



The individual variability concerning the semen pretability to freezing is show in figure 2 and 3, where it notice the motility dynamic of the two types of semen (fresh and frozen) from Alpine and Saanen breed.

Figure 2

Dynamics of fresh and frozen semen of Saanen bucks

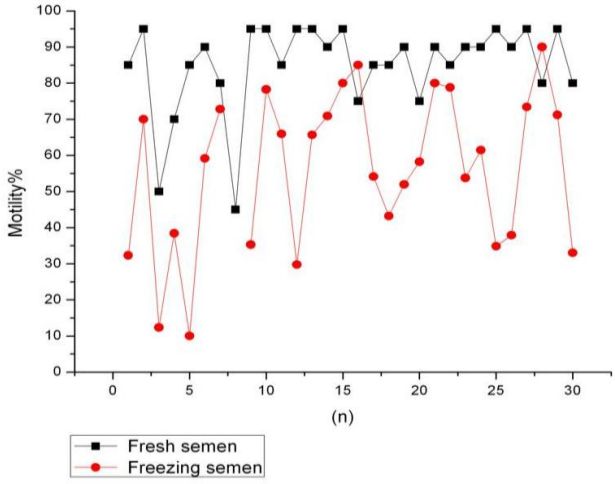
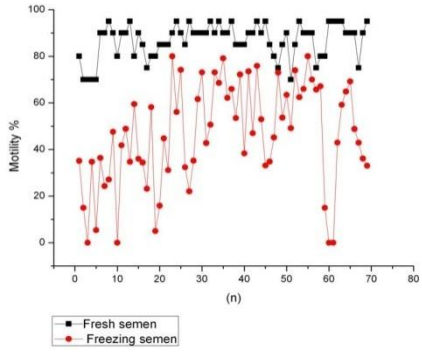


Figure 3

Dynamics of fresh and frozen semen of Alpine bucks



The fecundity was tested "in vivo" on 255 goats from private farms. It was appreciated the fecundity and prolificacy percent.

The artificial insemination in single dose with frozen Saanen semen was done on 195 goats from Avram farm. The fecundity percent was 28,2%, the number of dropping goats being 55.The prolificacy was 160% ,the number of kids being 88.

On 60 goat from Boitan farm were done artificial inseminations in double doses with Alpine buck semen .The fecundity percent was 46,6 % (n=28) and the prolificacy 132% (n=37).

CONCLUSIONS

1. The buck semen is pretable to freezing in view to preserve the genetic material.
2. The motility after freezing was 40-60%, which make possible the artificial insemination
3. A high individual variability of semen concerning pretability to freezing it was notice.
4. Fecundity and prolificacy had high values on condition that the technologies of freezing and artificial insemination are respected.

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Results concerning the freezing pretability evaluation of ovulation rate in goats after different type of gonadotrophins

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In the present study were performed superovulatory treatment with 3 types of hypophisary gonadotrophines (FSH-o, FSH-p , Stimufol and eCG) on 168 adults goats from local Carpatine breed. , in two consecutive years (2004 and 2005) , in 48 females series . The results obtained demonstrated the best ovulation rate is obtained after FSH-o treatment in group 1 (CL=6,21 ± 4,69) ,followed by FSH-p treatment in group 2 (CL=4,42 ± 3,58).

The lower ovulatory response showed the Stimufol treatment, ovulation rate being 6,85 ± 2,00. Ovulation rate in goats with eCG treated 4,28 ± 2,89. It was recorded high individual variability in superovulatory response and generals averges were smaller than those reported by other teams researchers using the same products (Vallet, 1992; Cognie Y 1997; RO 7-11) . The average of nonovulated follicles was increased in donor goats with eCG treated (2,07 ± 1,49) and more decreased in the other groups 1,21; 2 and 0,92 , respectively.

Key words: goat, superovulation corpus luteum, nonovulate follicles, FSH

INTRODUCTION

Embryo transfer is a technique of assisted reproduction which increase the reproductive capacity of female in terms of the number of offspring per female per year; decreases the generation interval; allow faster evaluation and selection of the best specimens; and easier shipment of genetic material , with no risk of transmitting diseases . At the present the embryo transfer in goat are limited in the practice , many embryos transfer teams development the method due the many mentioned advantages and for the scientific reasons also (Baril,1995;Cognie Y, 2000, Pereira R,1998, Greve T, 2002). The mean of ovulation rate in goat are usually range 4-10 embryos after superovulatory treatment and the pregnancy rate are 50-60 % . Also, the freezing goat embryos are presently developed with very good results after transfer (Rittar 1998, Fieni F, 1995) The objective of superovulatory in goats is to achieve a great number of embryos with a great competencies to develops into viable kidds . In goats there are several team in ET , specially in France, Canada, USA, Italy, but also are several team in a very few East European countries like Romania ,Cech Republick and Poland. Many bovine ET teams from Europe for superovulatory treatment ,using the different type of FSH in proportion of 80% and only 20 % using the eCG (Becker F,2002) .

Therefore, the objective of the present study was to verfy the ovulation rate after 4 type of gonadotrophins :FSH-o(Ovagen), FSH-p(Folltropin), Stimufol and eCG(Folligon)

MATERIALS AND METHODS

The experiments were achieved on 4 groups of goats, each group numbering 14 animals.The females were keeped in Department of Reproduction and Biotechnologies bio-base from Research and Developmental Institute for Sheep and goat Palas Constanta,.The treatments were

done in 2004 (84 goats) and 2005 (84 goats), against season (march-april) , on adult Carpatine goats. With 40 days before treatments application the goats received concentrates supplements. To induce oestrus and superovulation in females, in view to embryos collection ,it was implemented 4 treatment protocols .The oestrus synchronization was made with Chronogest sponges which were maintained into vagina for 11 days.The fluorogeston acetate doses from one sponge it was 45 mg .

Supraovulation treatments respected the follow scheme:

1.In group 1 was administrated ovine-FSH (Ovagen) ,while in group 2 was administrated p-FSH (porcine Follicle Stimulating Hormone),the gonadotrophin concentration being the same (18 mg, administrated in 6 injections with increasing doses of FSH).

2. In group 3 was administrated eCG (Folligon) in a single dosis 1200 UI. In group 4 was administrated Stimufol with a 250 μg concentration. **Stimufol** is a lyophilised product who contain 500 μg pFSH (porcine Follicle Stimulating Hormone) and 100μ pLH (porcine Luteinizing Hormone).This product is used to induce the superovulation in ruminants and aplied for the first time in Romania.Stimufol administration was made once at 12 hours, for 3-4 days,in decreasing doses and the last injection after Chronogest sponges removal.

All the donor goats were natural reproduced 2 times/day , after clinical manifestation of oestrus, with males able to reproduction. The goats were submitted to laparatomy examination at 5-6 days after sexual contact in view to observ ovulatory response as a result of the 4 differents treatments .Only the goats with a ovulatory response higher than 5 ,were prepared , anaesthetized for embryos collection and embryos freezingThe results of embryos collection , embryos quality before and after freezing , and the results of „in vivo" testing of embryos viability was the subject of another research object(II).

The results evaluation used the ovulation rate values (OR), the percent of anovulated follicles(NF) and follicular population (FP) existing on ovaries at moment of control . The results were statistic processed by Student test.

RESULTS AND DISCUSSIONS

It was calculated the ovulation rate in each group and being followed follicular population: corpus luteus counts and anovulated follicles counts.

The results obtained as consequences of the two types of gonadotrophins administration o-FSH (ovine Follicle Stimulating Hormone) and p-FSH (porcine Follicle Stimulating Hormone), in the same concentration (18 mg) are presented in tabel 1:

Table 1

Ovulatory rate using two types hypophisary gonadotrophins treatments (FSH-o şi FSH-p)

	FSH-o 18mg			FSH-p 18 mg		
	1-CL	2-FN	3-PF	4-CL	5-FN	6PF
N	14	14	14	14	14	14
Mean	6,21429	1,21429	3,57143	4,42857	0,92857	3,21429
Sd(yEr±)	4,69334	1,3114	2,06488	3,58875	1,20667	2,51698
Se(yEr±)	1,25435	0,35049	0,55186	0,95913	0,3225	0,67269
Min	0	0	1	0	0	0
Max	15	3	8	13	4	8
Range	15	3	7	13	4	8
Median	4,5	1	3	4	1	3
Coefficient of Variance	76%	108%	58%	81%	130%	78%
Kurtosis	0,00978	-1,60221	-0,22552	1,11147	2,61641	0,01783

Legend; CL – corpus luteus ; FN- follicles nonovulated; PF- follicular population

As consequences of statistics calculation the mean of corpus luteus after FSH-o treatment was 6,21429 and after FSH-p treatment was 4,42857.Difference between the two means is 1,7857, being higher than 0,01 which demonstrated the significant differences of this two groups. Coefficient of

variance in this groups (FSH-o group and FSH-p group) is higher than 35% and therefore it had to use the median values .Thus ,the median of corpus luteus count was 4,5 in group 1 and 4 in group 2, while the median follicular population in both group had the same value ,3 respectively.

Coefficient Kurtosis has normal value 1,6. Is possible to observe that this value maintain only in case of corpus luteus and follicular population of the two groups, but not in case of nonovulated follicles when his value is close to limit in the group 1 (1,60221) and even exceeded it in the group 2 (2,61641).

Table 2

Follicular response after two types of gonadotrophines treatment (PMSG and Stimufol)

Statistics	PMSG 1200 UI			Stimufol 250 g		
	7CL	8-FN	9-PF	10-CL	11-FN	12-PF
N	14	14	14	14	14	14
Mean	4,28571	2,07143	2	6,85714	2	1,71429
Sd(yEr±)	2,89372	1,49174	2	2,0702	1,56893	1,32599
Se(yEr±)	0,77338	0,39868	0,53452	0,55328	0,41931	0,35438
Min	0	0	0	4	0	0
Max	9	5	6	12	5	5
Range	9	5	6	8	5	5
Median	4	2	2	7	2	1,5
Coefficient of Variance	68%	72%	100%	30%	78%	77%
Kurtosis	-0,72098	-0,31321	-0,50612	1,78998	-0,52859	1,72564

Legend; CL – corpus luteus ; FN- follicles nonovulated; PF- follicular population

In table 2 are presented the results of statistic processing data of group 3 and 4 treated with PMSG and Stimufol, respectively.The mean of total corpus luteus after PMSG administration was 4,28 and after Stimufol administration was 6,85.The difference between the means of corpus luteus in this two group was 2,57143 ,so higher than 0,01. Coefficient of Variance is also higher than 35% , except the mean of corpus luteus after Stimufol treatment(group 4).Therefore more relevant is median whose values is approached between the two groups for the follicular population (2 for group3 and 1,5 group 4) and nonovulated follicles (2- groups 3 and 4).But in case of corpus luteus , the median values are differents (4-group 3 and 7- group 4). Coefficient Kurtosis has normal value,except group 4 where is 1,78998 for corpus luteus and 1,72564 for follicular population .

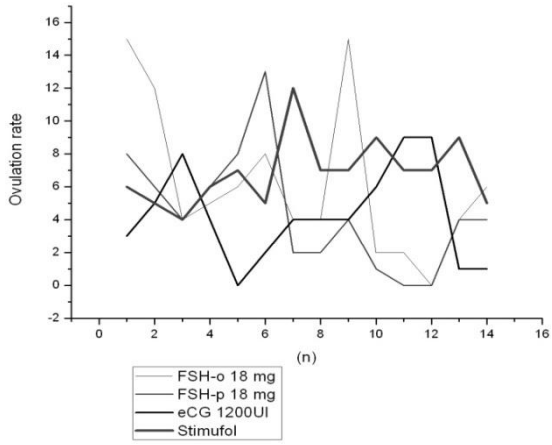

Figure 1

Ovulatory response in donor goats after different superovulatory treatments with gonadotrophines

In conclusion, the higher rate of ovulation was obtained after FSH-o treatment at group 1, followed by FSH-p treatment (group 2).In the other way, the lower reponse it was observed after using Stimufol (group 4)(figure 1).

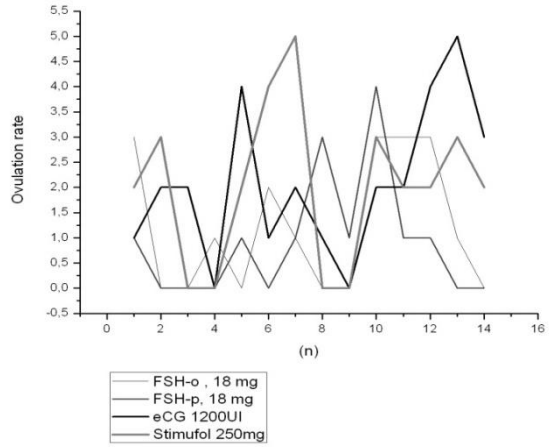

Figure 2

Anovulated follicles in superovulated goats depending on type and doses of gonadotropines

As regards the average of anovulated follicles,it was higher in group 3 (average 2,07143) and group 4 (average 2), compared with group 1 (1,21429) and group 2 (0,92857),respectively, - figure 2.

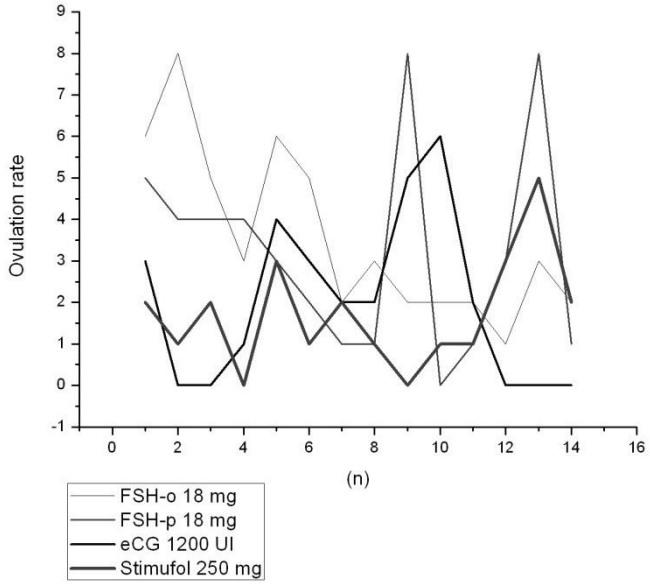

Figure 3
The evolution of follicular population in donor goats after different treatments with gonadotrophines

The average of follicular population registered increased values at the same groups, 1 and 2 (3,57143 respectively 3,21429), while in groups 3 and 4 were 2 and 1,71429,respectively(figure3).

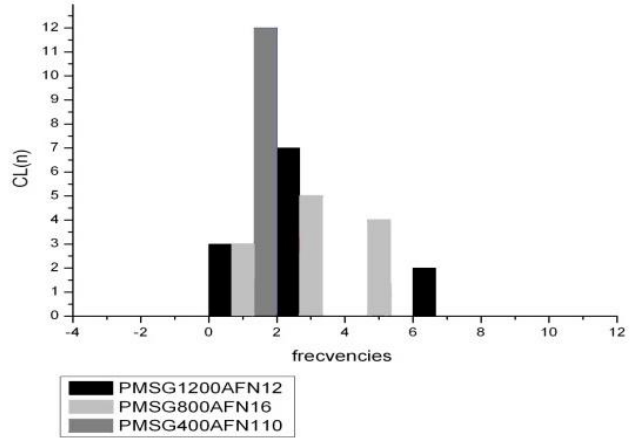

Figure 4
Distribution frequence of CL (corpus luteus) after superovulatory treatment with gonadotrophine (eCG).

CONCLUSIONS

The obtained results showed :

1. A better ovulation rate is obtained after FSH-o treatment in group 1 (CL=6,21 ± 4,69) , followed by FSH-p treatment in group 2(CL=4,42 ± 3,58)
2. The highest ovulatory response was after the Stimufol treatment ,who recorded a ovulation rate 6,85 ± 2,00.
3. Ovulation rate after eCG treatment was 4,28 ± 2,89.
4. It was recorded high individual variability in superovulatory response , medium to high after FSH treatment and very high after eCG treatment..

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Observations concerning hematological profile in goat

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This study followed the hematological profile of goats (females and males) and the influence of sex on the hematological values. On the red blood cell (RBC) counts of goats , sex had no influence.The hemoglobin (Hb) and hematocrit (Ht) values were smaller in both sexes (8.18±0.27 in females and 8,2±0,17 in males) as well as the packed cell volume (PCV)values(14,37±0,36 in females and 15,5±1,6 in males) .Mean corpuscular hemoglobin concentration (MCHC) was higher in male than female goats (43,65±1,01in male and 44,09±1,21 in female).Leucocytes mean was high in both sexes which may be interpreted as a potential infection. Lymphocytes represented more than 50% of the total white blood cell (WBC) counts in male and females goats.

Monocyte and basophil mean was not influenced by sex. But, in the case of eosinophil average, the males had smaller values than females and both were smaller than normal.Mean of neutrophils was lower than normal

Key words: goat, hematologic profile, sex

INTRODUCTION

Despite the social and economic values of goats as source of meat, milk and hides, with a great production potential, the research effected on goats in our country were neglected for long time.The goats revaluation depends on various factors, including the great prevalence of diseases, poor management practices and extensive production systems .The diseases action is the most aggressive on animals.From this point view, clinic and paraclinic exams are essential to sanitary strategies (control, prevention or treatment).

The hematological tests served as information base for animal health assistance. It has been reported that regardless of age, sex and climate, goats reared under traditional husbandry system have low hematological values compared to those reared under modern husbandry (Coles, 1980; Schalm *et al,* 1975). Low nutritional grassland pasture, stress, parturition and climatic factors greatly alter the blood values of goats (Anosa and Isoun, 1978, Radostits *et al* 1994).

Blood is an important and reliable medium for assessing the health status of individual animals (Oduye, 1976). Much work has not been done on hematological profiles of goats. Therefore, this paper focused on the hematological values of apparently healthy goats as influenced by sex in The Department of Reproduction and Biotechnologies from The Research and Developmental Institute for Sheep and Goat Palas Constanta,

The RBC, Hb, PCV, MCV, MCHC and WBC values obtained in this study in both sexes in goats were comparable to those previosly reported (Sarror and Schil, 1977; Anosa and Todd et al, 1952);

MATERIALS AND METHODS

The goats used in this study were kept in The Department of Reproduction and Biotechnologies bio-base. The animals were apparently healthy. The study was made on 14 goats , divided by sex (14 females and 4 males).Two ml of blood was collected from each animal from the external jugular vein following proper restraint by the attendants and with minimal excitement. The blood were collected in ethylenediamine tetracetate (EDTA) vacutainer tubes and transported to the

laboratory for analysis. The samples were analyzed within two hours from collection with the hematological analyzer MS 4-5 Meled Schloesing,Germany.

The red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), hemoglobin concentration (Hb), differential leukocyte counts (DLC) mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined as described by Schaim et al (1975).All of them were statisticcally processed with the purpose of constituting the hematological profile..It is know that goats and the sheep have smallest erythrocytes as volume, but in highest number 14-15 mil/mm)

The statistical analysis was carried out using statistical Student test.

RESULTS

The hematological (mean ± Se) profiles of the goats are presented in tables 1 to 6. The RBC mean on female was, 13.06±0.45 and 12,57±0,28. The coefficients of variance permits the use of mean as statistic interpretation.This means are closed to the normal mean of RBC 14-15 mil/mm(Table 1 and 2).

Table1

Erythocyte paramters (mean ± S.e.) of female goats

female	Statistics							
	N	Mean	Sd (yEr±)	Se(yEr±)	Min	Max	Median	Coefficient of Variance
RBC (m/mm)	14	13.06071	1.71986	0.45965	9.11	15.8	13.37	13%
MCV	14	14.37857	1.36505	0.36482	12.6	18.3	14.35	9%
HCT (%)	14	18.77857	3.30947	0.88449	12.4	23.8	20.05	18%
MCH (pg)	14	6.22857	0.33611	0.08983	5.7	6.7	6.25	5%
MCHC (g/dl)	14	44.09286	4.54761	1.2154	35.7	53.9	43.85	10%

Table 2

Erythrocyte parameters (mean ± S.e.) of male goats

Statistics	male				
	RBC (m/mm)	MCV	HCT (%)	MCH (pg)	MCHC (g/dl)
N	4	4	4	4	4
Mean	12.57	15.5	19.55	6.5	43.65
Sd(yEr±)	0.5658	3.23316	4.90748	0	9.17987
Se(yEr±)	0.2829	1.61658	2.45374	0	4.58993
Min	12.08	12.7	15.3	6.5	35.7
Max	13.06	18.3	23.8	6.5	51.6
Range	0.98	5.6	8.5	0	15.9
Median	12.57	15.5	19.55	6.5	43.65
Coefficient of Variance	5%	21%	25%	0%	21%
Kurtosis	-6	-6	-6	0	-6

In both, males and females the coefficient of variance is less than 30% which revealed that the mean of erytrocytes and erytrocitar constants are representative for this category of goats. The erythrocyte parameters HCT, MCH and MCHC were analyzed in both sexes. HCT mean was 18,77 ±0,88% in females and 19,55±2,45 % in males. MCH had the following values: 6,22±0,08 in females and 6,5±0 pg in males. MCHC was 44,09 ±1,21 in females compared with 43,65±1,01 in males.

Coefficient of variance did not exceed the limit of 35%, which can by used in statistically interpretation .Except MCHC , HCT and MCH were higher in males than females.

The mean of hemoglobin concentration was lower than normal :8,18 ±0,27 g/100 ml in females with a minimum of 5,5 and a maximum of 9,3 and 8,2±0,17 in males with a minimum of 7,9 and a maximumof 8,5.These parameters translated to a potential presence of anemic disease which can explaine the biological reduction of the hemoglobin. The hemoglobin mean is smaller than the normal values 10-15 g/100ml. In corroboration , the hemoglobin with the hematocrit can establish the anemic status of animal(table 3 and 4).

Table 3

The hematocrit and hemoglobin means in female goats

Statistics	female	
	HCT (%)	Hb (g/dl)
N	14	14
Mean	18.77857	8.18571
Sd(yEr±)	3.30947	1.01364
Se(yEr±)	0.88449	0.27091
Min	12.4	5.5
Max	23.8	9.3
Range	11.4	3.8
Median	20.05	8.55
Coefficient of Variance	18%	12%
Kurtosis	-0.4819	2.7667

Table 4

The hematocrit and hemoglobin means in male goats

Statistics	male	
	HCT (%)	Hb (g/dl)
N	4	4
Mean	19.55	8.2
Sd(yEr±)	4.90748	0.34641
Se(yEr±)	2.45374	0.17321
Min	15.3	7.9
Max	23.8	8.5
Range	8.5	0.6
Median	19.55	8.2
Coefficient of Variance	25%	4%

The RBC values in the ruminants in this study may ,among other things, be due to excitement or strenuous exercise during handling (Gartner et al., 1969). This leads to the release of adrenaline and hence spleen contracts and this causes the release of more RBC into circulation.The MCV and MCHC values in both sexes fluctuated and their values are dependent upon RBC, Hb and PCV values.

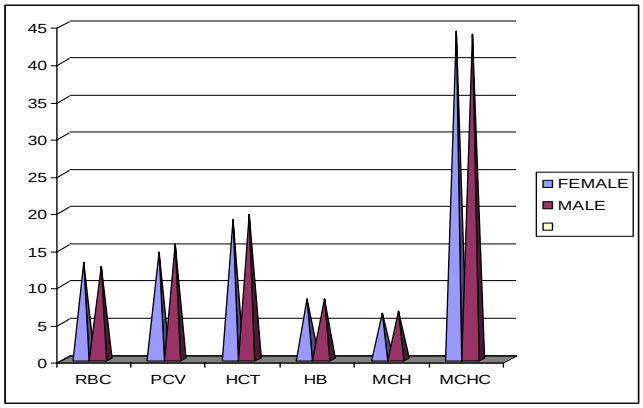

Figure 1 .Representation of erythrocytic parameters in male and female goat

The fluctuation of this values are represented in figure 1., where we observed the differences between females and males.

The total WBS mean in males and females is between 15,41± 1,43 with a minimum of 10,21 and a maximum of23,75 and 12,77±1,47 with a minimum of 10,21 and a maximum of 15,33, respectively. Both categories of goats had higher values than normal (5-14 mil/mm) and can be attributed to immune response to different environmental factors and physiological status (table 5-6)

Table 5

Leucocyte values (mean ± S.E.) of female goats

	Statistics							
females	N	Mean	Sd(γEr±)	Se(γEr±)	Min	Max	Median	Coefficient of Variance
WBC (m/mm)	14	15.41429	4.52373	1.2090	10.21	23.75	14.89	29%
Lym(%)	14	54.53571	5.35919	1.4323	49.3	69.4	52.2	10%
Mon(%)	14	4.82857	0.75695	0.2023	3.6	6.4	4.9	16%
NEU(%)	14	35.5	4.92888	1.3173	22.6	41.8	35.7	14%
EO(%)	14	4.16429	2.54486	0.6801	0	8.8	3.35	61%
BA(%)	14	0.47143	0.26726	0.0714	0.1	1	0.45	57%

Table 6

Leucocyte values (mean ± S.E.) of male goats

Statistics	male					
	WBC (m/mm)	Lym (%)	Mon (%)	NEU (%)	EO (%)	BA (%)
N	4	4	4	4	4	4
Mean	12.77	53.8	4.85	38.1	2.85	0.4
Sd(yEr±)	2.95603	2.65581	0.28868	2.42487	0.40415	0.11547
Se(yEr±)	1.47802	1.32791	0.14434	1.21244	0.20207	0.05774
Min	10.21	51.5	4.6	36	2.5	0.3
Max	15.33	56.1	5.1	40.2	3.2	0.5
Range	5.12	4.6	0.5	4.2	0.7	0.2
Median	12.77	53.8	4.85	38.1	2.85	0.4
Coefficient of Variance	23%	5%	6%	6%	14%	29%

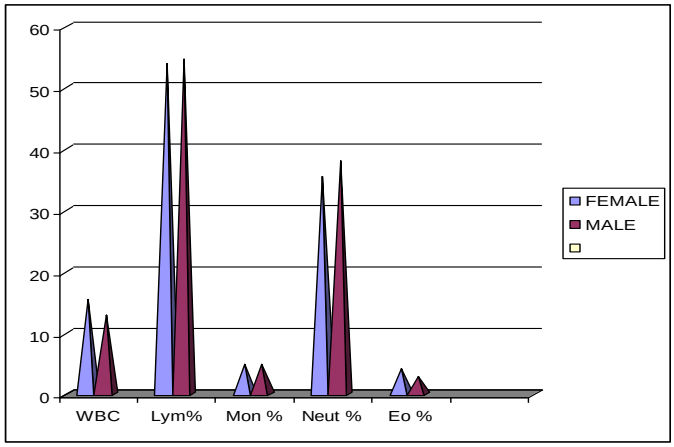

Figure 2. Representation of leucocytar parameters in male and female goats

In leukocytary series , the mean of lymphocytes was 54,53 ±1,43% in female and 53,8±1,32%in males, respecting the normal rapport between 50-55%. The monocytes are in the same normal limit(3-5%) and their means are 4,82± 0,20% for females and 4,85±0,28 %in male. Neutrophils average was smaller than normal (40-45%) as follows: 35,5±1,31% in females and 38,1±1,21% in males.The 4,16±0,68% value of eosinophils in females indicated a potential helmintic or infectious aggression compared with the males value 2,85±0,20% (figure 2).This fact can be explained by the isolation of females that graze on the field , from the males which remain in the stable.

The white blood cells (WBC) are the soldiers of the body and their high counts may also be due to the increase of the complement in the immune systems o the animals. It may also be attributed to physiological phenomena i.e. excitement or strenuous exercise during handling.

CONCLUSIONS

1. The MCV and MCHC values in both sexes fluctuated and their values are dependent upon RBC, Hb and PCV values. Hemoglobin has low level, indicating an anemic status of female and male goats.
2. The total WBC mean had very high values in both sexes and can be attributed to the immune response to different factors.
3. The high lymphocyte counts in the animals in this study might be attributed to stress and immune response to the environment which harbours various detectable and undetectable parasitic and/or bacterial organisms. The eosinophil values can translate to an infection or helmintic aggression.
4. Since the animals are apparently healthy, any value beyond the upper limit in one or both sexes may be regarded as leucocytosis and any value below the lower limit may be termed leucopaenia.
5. Sex showed relatively influence on the haematological values of the goat studied , existing fluctuations in all the hematological parameters of both sexes
6. What caused the fluctuation in various parameters may be undetected minor infections, weather extremities and poor management

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The consequences of the global heating upon the seasonal dynamics of Babesiosis at dogs

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Babesiosis, a parasitic disease, common both to men and animals, is produced by the sporozoar Babesia psp., after it was transmitted by the vector mites (Dermacentor reticulates, Ripicephalus sanguineus, Haemaphysalis spp. etc).

300 dogs were investigated, in the period January – February 2007, in order to determine the prevalence of babesiosis. The examination was made through a clinical and microscopical exam and the evaluation of the epidemiological indicators was made after the statistical interpretation of the data obtained within the epidemiological inquiry.

The modification of the seasonal dynamics of babesiosis, as a consequence of the phenomenon of global heating, was determined after monitoring the values of the temperature for the period 1 January – 28 February 2006/2007, in Bucharest.

Key words: babesiosis, prevalence, seasonal dynamics, temperature

Introduction

Babesiosis, as well as zoonose and dogs parasitic disease, has a geographical distribution which is not regular because it is characteristic to the European areas and to the south-eastern Africa (1). The evolution of the disease is seasonal and the maximum values of incidence are registered from April until October, except for the Mediterranean areas where the disease is present during winter too (3). In our country, the disease was diagnosed for the first time in 1984, in the Eastern area of Romania and there is a risk regarding its apparition in other areas of the country due to the presence of the transmitter mites. The spreading of the disease is conditioned by the presence of the mites biotopes and it is correlated to the active phases of these arachnids. (5).

The consequences of the global heating have been felt more and more acutely, during the last few years, both by men and by animals. In Romania, this phenomenon became even more serious in January, February a.c., when the average temperature for this period (- 20ºC) in Romania grew and it reached values of -4º-16º C (January, a.c.) and -8º- 17º C (February a.c) (****).*

The registration of the temperature values was made in order to determine the action of the environment factors upon the evolution of a seasonal disease with characteristic manifestations for our country during spring – autumn months.

Material and methods

The researches were made in a private veterinary clinic from Bucharest, on a number of 300 dogs, males and females, from different breeds, during two months (January – February) in 2007.

In order to put the diagnostic of Babesiosis, a series of tests were performed, as follows:

After the clinical examination of the dogs the following manifestations were seen: itchiness, bad mood, microabscesses, remittent fever, nervous and respiratory semeiology, diarrhea, vomit. A macroscopical examination was performed in parallel in order to determine the presence of mites

in different areas of the body: interdigitally, on the legs, on the internal side of the thigh, on the concha, on the eyelids, around the mouth, on the lips and in the abdominal area.

The validation of the babesiosis diagnostic was made after performing the smear from the peripherical blood, coloured through the MGG method in which the parasitary forms are relieved. The microscopical examination dignified the babesia which appeared inside the red-blood cells, coloured in violet-blue, in piriform and amiboid forms. The connection between the data obtained in the epidemiological inquiry and those obtained in the laboratory and the clinical exams led to the conclusion that the diagnosis was of babesiosis.

Results and discussions

In the year 2007, after the clinical examination of 300 dogs, 200 cases of temporary ectoparasitism with mites were discovered, out of which 195 dogs were diagnosed with babesiosis.

The clinical exam relieved acute manifestations of babesiosis in correlation with the massive infestations with mites. Characteristic for the 195 dogs infested with Babesia sp. was the remittent fever (40-42,8º C) and the fulminant evolution of the disease. The characteristic symptoms were: apathy (80%), fever (50%), icterus (30%). The mortality percentage that was registered is of 41% (80 mortality cases).

The diagnosis of babesiosis was determined by the microscopical examination of the smears, which relieved the babesia located inside the red-blood cell, coloured in violet-blue and piriform.

The prevalence of the cases of babesiosis for the investigation period (2007), was established using the standard calculation formula for this epidemiological indicator (7) and it was ascertained that for January – February, the registered prevalence had values of 65%.

In order to determine the way in which temperature influences the values of the prevalence, an epidemiological analytical retrospective inquiry was made, aiming to determine the prevalence of babesiosis for the period January – February 2006. In this respect, all the cases registered at the same veterinary clinic in the period mentioned above were assessed

For the same number of dogs (300), a prevalence of 31% and a mortality percentage of 41% were determined. On the other hand, the number of cases of ectoparasitism with mites was significantly higher in the year 2007 (200 cases), in comparison with the year 2006, when for the same number of examined dogs (300), the temporary ectoparasitism with mites was present at 115 dogs (chart 1).

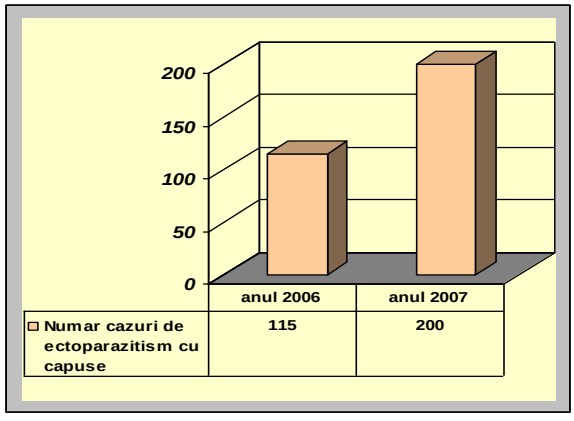

Chart 1 – The number of cases of temporary ectoparasitism with mites at dogs for the year 2006-2007

The significant difference of the prevalence of babesiosis, registered between the two years of investigation, can be a consequence of the high and constant temperatures, in January – February 2007. Consequently, the determination of a connection between – *the phenomenon of global heating and the high values of the prevalence* – was made by registering the minimum and the maximum temperatures, for January – February 2006 – 2007 and by the evaluation of their effects upon the biotopes of mites.

The evolution in dynamics of the temperature for the two years that were investigated is presented in charts 2, 3.

After analyzing the charts, significant differences of the temperature values registered in January – February 2006, in comparison with 2007.

For the year 2006, January registered minimum values of -17ºC and maximum values of 6ºC (3 January) and February 2006 registers maximum values, both positive and negative, and only 2 days are mentioned with values of over 10ºC (Chart 2,3) ;

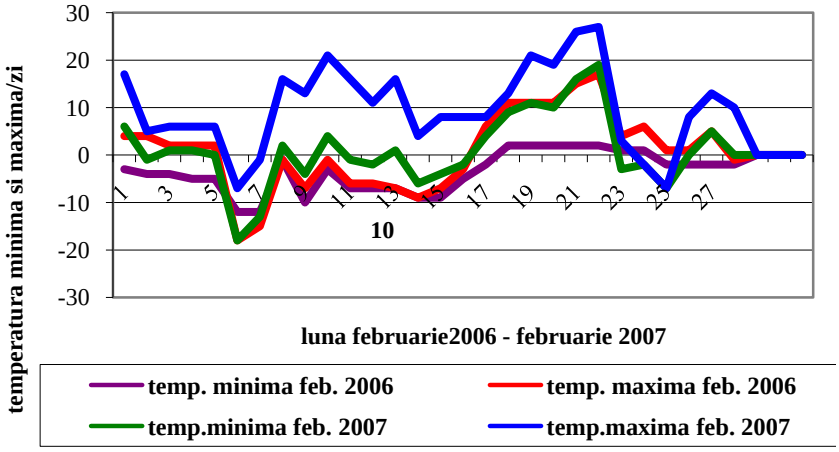

Chart 2 – The dynamics of temperature in February - 2006/2007

In January 2007, all the maximum registered values were positive (16ºC - on 23 January) and in February 2007 all the maximum values were positive, over 10ºC were registered during 8 days; the minimum values of the month are negative, the value of -8ºC, was not past during 9 days.

The significant difference for the temperature values is obvious for January (chart 3,5).

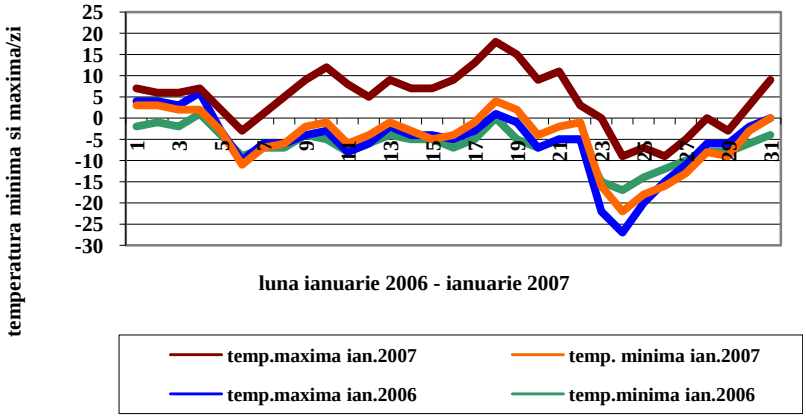

Chart 3 – The Dynamics of Temperature in January - 2006/2007

It can be easily noticed that both the maximum and the minimum values are significantly higher in January 2007 in comparison with January 2006 ; also the ascendent tendency of the maximum temperature is obvious and constant in 2007 (chart 3).

Conclusions

Due to the global heating phenomenon, in Romania, some animal diseases have the tendency to change their seasonal character, as shown in the present study. In the case of babesiosis, the seasonal evolution (spring – autumn) tends to modify in the direction in which the prevalence grows in the cold season (January – February), in a period of the year which is not proper for an active maintenance of the mites biotopes.

The advance of the minimum value of the temperature determined for our country (-20 ºC), led to the duplication of the prevalence value for the year 2007 (P- 65%) in comparison with 2006 (31%), and it was noticed, at the same time, the increment of the number of cases of external ectoparasitism with mites at dogs in the cold season.

Since it is known the fact that the mites biotopes are active in spring/summer at minimum temperatures of 5-10ºC – maximum of 15-25ºC and precipitations of „0,37% (R^2 = 0,0037)(4), the increment of the prevalence in the winter months, may be a consequence of the temperature modifications, especially because the size of the populations of ixodidis from the spring and summer, depends on the wintering conditions and more precisely, on the conditions registered at the level of the vegetal carpet (6).

The optimum conditions for the activation of the mites biotopes in the winter period, are sustained by the maximum value of the temperature which is positive (0-17ºC) during the whole month of February 2007 (chart 3). The modification of the temperature values, thus getting higher, during the months of January – February 2007 (with minimum values of -8ºC and maximum of 17ºC), in comparison with the year 2006, led to the activation of the mites biotopes, to the increment of the degree of ectoparasitism at dogs, as well as to the modification of the seasonal evolution of babesiosis at dogs in Romania.

The limitation of the harmful effects of the phenomenum of global heating upon animals state of health can be achieved by improving the prophylactic methods and by introducing some deparasitation schemes which should be correctly applied to pets, in order to prevent ectoparasitism with mites and to diminish the risk of contamination with *Babesia* sp.

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* ****Agenţia Naţională de Meteorologie**. **http://www.inmh.ro/**

Predisposing factors of mammary carcinogenesis at bitch

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This study was conducted to observe the evolution of mammary injuries, as oncoinductor factors. We noticed at 15 bitches with mammary traumas, that the lack of medical management stimulate autotreatment by licking, that produce a continuous irritation of repairing tissues. In the end, healing modifications are produced and in 20% of cases, mammary tumours appear or in another 20% excessive cicatrization that can turn to evolutive tumours.

The results demonstrate the importance of an early and accurately treatment of mammary traumas to annihilate the oncoinductor factors of mammary carcinogenesis.

Key Words: dog, mammary carcinogenesis, predisposing factors

Epidemiological studies reveals that mammary carcinogenesis is influenced by some oncoinductor factors that increase the frequency of mammary cancer (3,4,5,10).

Among this factors are untreated mammary traumas that can induce mammary cancer (1,2,6,7,8,9).

In this paper we presented the evolutive aspects of traumas of the nipple or mammary tissue. These traumas represent factors that can induce the moment when a cell capable to multiply for regeneration deviate from normal activity to uncontroled multiplication.

MATHERIAL AND METHOD

The research was performed in Surgical Clinic of Veterinary Medicine Faculty, Iasi at 15 bitches with accidental traumas of nipples or mammary tissue. The wounds, with different etiology, were untreated by surgery stimulating the animal to autotreatment by licking that produce a continuous irritation of the wound disturbing the healing process.

RESULTS AND DISCUSSIONS

The destruction of the tissues are followed by healing process through normal cicatrization.

The healing process is deviated if factors that disturb the normal evolution of regeneration intercede. Noticing this process at 15 bitches with mammary wounds, we ascertain the normal time of healing or deviate the normal healing to excessive cicatrization, lack of cicatrization or canceration.

The results are presented in table 1 and centralized in table 2.

Table 1

Irritation influence on the healing of the mammary wounds at female dogs

Wound clinical aspect	*The result of tissue healing*
1.Open wound at nipple 3 left	*Healing by second intention after 26 days*
2.Open wound at mammary tissue between nipple 3 and 4 right	*Aton wound – surgical treatment after 4 weeks*
3.Open wound at mammary tissue of nipple 4 left	*Healing by second intention after 29 days*
4. Open wound at nipple 5 left	*Healing by second intention after 25 days*
5. Open wound at nipple 4 left	*Excessive cicatrization after 39 days*
6.Closed wound at nipple 1 right	*Healing by second intention after 26 days*
7. Open wound at nipple 5 left	*Canceration of mammary tissue*
8.Open wound at nipple 5 left	*Excessive cicatrization after 41 days*
9. Open wound at nipple 3 right	*Canceration of mammary tissue*
10.Open wound at nipple 4 right	*Canceration of the nipple*
11. Closed wound at nipple 1 left	*Healing by second intention after 31 days*
12.Open wound at nipple 5 left	*Healing by second intention after 35 days*
13.Open wound at nipple 2 right	*Healing by second intention after 25 days*
14.Open wound at nipple 3 right	*Aton wound – surgical treatment after 32 days*
15.Open wound at nipple 4 right	*Excessive cicatrization after 44 days*

Table 2

Wounds no.	*Autotreatment results*							
	Healing		*Aton wounds*		*Excessive cicatrization*		*Canceration*	
	No	*%*	*No*	*%*	*No*	*%*	*No*	*%*
15	*7*	*46,7*	*2*	*13,3*	*3*	*20*	*3*	*20*

Centralized data regarding the irritation influence on the healing of the mammary wounds at feamale dogs

Dates from table 1 reveal the seriousness of the lack of medical and surgical treatment in mammary wounds. The irritation produced by the animal and the factors of environment disturb normal evolution of healing process, therefore only 46,7% of cases are normally healing. For all that, the regeneration begin after 25-35 days, comparing with an surgical treated wound that is healed in 10-12 days.

In 2 cases (13,3%) the licking of the regenerated tissue determined the aplatization of this, turning the wound into an aton tissue. The treatment in this cases is surgical ablation and primary suture.

If the balance healing stimulated and inhibiting is disturb, the regenerated tissue develops excessively.

Therefore in 20% of cases the normal cicatrization turns to excessive cicatriyation or canceration. In 3 cases (20%) excessive cicatrization was notice at mammary tissue, too. The base of nipple was grown as dimensions and firmness, and the skin resemble with a hypertrophic scar. This accidentally wounds were 65-75 old.

In mammary cancer the prevention is equally important as the treatment, therefore, this study establish those moments when proper intervention can prevent cancerization.

The results of this research establish that untreated mammary tumours represent an disturbing moment in normal healing, when clone head appears, as other author noticed (1,3,5,10).

CONCLUSIONS

1. UNTREATED MAMMARY TRAUMAS REPRESENT AN ONCOINDUCTOR FACTORS.
2. LACK OF MEDICAL INTERVENTION STIMULATE THE ANIMAL TO AUTOTREATMENT, DISTURBING THE HEALING PROCESS
3. DISTURBING THE HEALING PROCESS HAS AS CONSEQUENCES CANCERIZATION AT 20% OF CASES OR EXCESSIVE CICATRIZATION AT 20% OF CASES.

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Early management of limbs degloving injuries

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Traumatic injuries of the limbs resulting in avulsion of skin and variable damage to the underlying hard and soft tissues are common in small animals. They are most frequently due to motor vehicle trauma but may also be caused by another injuries in moving machines, traps, and entanglement in fances. Pure degloving tissues and the blood vessels supplying them are avulsed from the underlying muscle and fascia, are occasionally seen, but combined degloving and crush injuries or abrasive injuries are much more common. Besides the avulsion of skin, there may be extensive damage of the muscle, blood vessels, nerves, tendons, ligaments, and bones.

The purpose of this paper is to review techniques and materials used in management of degloving wounds.

Key Words: limbs degloving injuries, management

MATHERIAL AND METHODS

The research was performed in Surgical Clinic of Veterinary Medicine Faculty, Iasi, on 21 dogs presented to consult with several limbs traumas selected from the casuistry attended in 5 years.

After the clinic examination we establish the stage of medical management:

-Initial assessment

-Anesthesia/analgesia

-Initial preparation, lavage and debridement

-Bandaging and medication

RESULTS AND DISCUTIONS

Initial assessment

In the critically injuried animal, resuscitation and stabilization must be performed before considering the management of superficial injuries. Life-threatening internal injuries, are treated before evaluating any traumatic injuries of the extremities. Those should be evaluated for vascular, neural and musculoskeletal injuries after surrounding skin has been clipped and prepared.

Anesthesia, sedation/analgesia

The general physical condition of the animal will be a governing factor as to whether the animal can be anesthetized and what type of anesthesia or sedation\analgesia can be used for wound assessment, lavage and debridement. Some tissues may be so obviously devitalized that they can be removed without anesthesia. However, the minimum anesthetic is necessary to allow complete assessment and surgical procedure performed. Sedation and local analgesia, tranquilizers or neuroleptanalgesics are commonly used with local analgesic agents. I prefer sedation and local analgesia. A sedatif-analgesic is used to reduce fight, calm the animal and control pain.

The local analgesia further controls pain.

There are several combinations of drugs that may be used as neuroleptanalgesics but in most cases we prefer Xylazine 2% administrated at 1ml/10-15Kg intramuscularly (i.m.) or 1ml/15-30Kg intravenously (i.v.).

The most commonly used local analgesic drug is lidocaine hydrochloride.

An effective and simple means of rendering analgesia in a limb to allow through debridement of a degloving injury is the ring block

The tissues proximal to the wound are infiltrated with analgesic agent from skin to bone by encircling the limb. The technique requires no precise anatomical knowledge of the limb, however, several injection sites are necessary which increases the risk of introducing bacteria.

Initial preparation, lavage and debridement

The initial management of degloving injuries centers on eliminating or reducing contamination of the wound and debridement of any devitalized tissues. The wound surface may be filled with sterile gauze or sterile sponges to prevent further contamination. The hair should be clipped from entire circumference of the limb and any remaining loose hair or debris is removed.

The sponge or gauze are removed and wound is lavaged with sterile saline or lactated Ringer`s solution to remove any trapped debris. A new gauze or sponge is applied and the surrounding skin is aseptically prepared. Local analgesics are usually infected at this time.

After gauze or sponge are removed, the wound is assessed and lavaged with copious quantities of irrigating solution to remove foreign bodies and to reduce the bacterial contamination.

The addition of an antiseptic to the lavage solution is recomanded. A 0,05% of clorhexidine diacetat made with sterile water has been effective and has not caused any clinical adverse effects. Application of the lavage solution under moderate pressure obtained using a 35ml syringe and an 18-20 gauge hypodermic needle, is much more effective in removing foreign bodies and reducing bacterial counts. Lavage should be performed in presence of lavage. The objective are:

-to remove all devitalized tissue

-to enlarge the wound to allow complete inspection

-to remove any remaining foreign material and

-to control bleeding

Indentifying devitalized tissues by visual inspection, especially of skin and muscle, may be difficult. Muscle that is not viable should be completely excised.

Exposed but unsevered tendons, nerves and ligaments associated with degloving injuries should be provided either temporally (bandage) or permanent (tissue) cover. Severed tendons and nerves may be anastomosed if the wound is clean, there has been little tissue trauma, and the repaired structures can be covered with tissue. If these factors are not present, definitive repair should be delayed until there has been healing of the surrounding tissues.

During the delay, the leg should be immobilized to reduce separation of the segments. In addition, temporary anastomoses may be performed to help prevent separation of tendon and nerve segments.

Assessing the viability of skin is often very difficult with the goal being to retain as much viable skin as possible for reconstruction. Obviously devitalized skin should be excised. Skin of questionable vitality should be observed at each bandage change, removing nonviable skin as it appears – staged debridement.

Surgical debridement of all devitalized tissue is preferred. However, in instance where might geopardize vital structures, where tissue of questionable vitality is to be left in the wound needs to be cleared of small amounts of necrotic tissue and debris following surgical debridement, enzymatic or adherent bandage debridement may be used.

Enzymatic, adherent bandage and staged debridement are methods that help conserve as much viable tissue as possible for reconstruction of degloving injures.

Bandaging and medication

Early bandages

Bandages as well as topical and systemic medication are considered after surgical debridement and lavage. If there remain some nonviable and questionable tissue and foreign debris on the wound along with a copious low viscosity exudate, a dry-to-dry adherent dressing help finish debridement. A wide mesh gauze without cotton filler is used for contact layers. The bandage becomes dry and loose debris, foreign bodies and necrotic materials are incorporated in the large interstices of the gauze. These are removed at the time of daily bandage removal. If a wound is producing a viscous exudate, a wet-to-dry dressing is indicated. The wide mesh gauze contact layer is saturated with physiologic saline or an antiseptic solution (0,05% chlorhexidine diacetate).

Systemic and Topical Medication

Before manipulation of the wound and the surownding area , it is admisable to obtain a swab of the wound for bacterial culture and sensitivity. Administration of systemic antibiotics is most beneficial within the first hours after wound infection.

Topical medication are often used to treat open wounds. Effective antibacterial agents include silver sulfadiazine as 1% water miscible cream (Dermazin) witch is effective against most gram-positive and gram-negative bacteria. Topical medication that have been found effective is Salvaderm ointment (foto 1). This helps debridement and stimulate tissue regeneration (foto 2).

Late bandage

When wound has reached the reparative stage of healing the contact layer should be nonadherent. Wound is free of nonviable tissue and still is producing some exudates. An nonadherent contact dressing with an open mesh is used to allow transport of exudates to the overlying intermediate layer. When a wound has healthy granulation tissue, a serosanguineous drainage, is starting to epithelialize, a occlusive dressing amnion prevents fluid, protein and electrolyte losses from wounded tissue, decreases pain at the wound site and promote early return of mobility (foto 3).

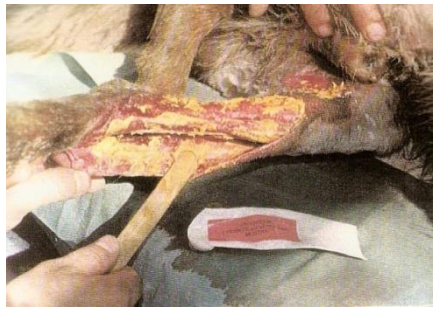

Foto 1. Degloving injuries to pelvic limb with extensive damage to underlying tissues. Salvaderm application on wound

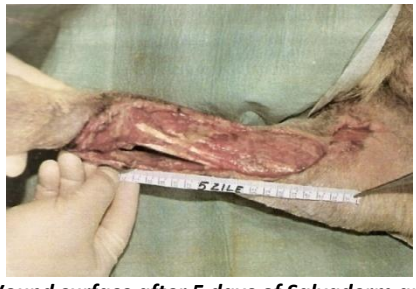

Foto 2. Wound surface after 5 days of Salvaderm application

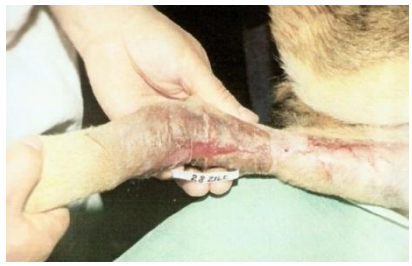

Foto 3. Wound surface in late stage covered with equine amnion

CONCLUSIONS

1. In critically injuries animal resuscitation and stabilization must be performed before considering the management of superficial injuries.
2. Anesthesia has necessary for further debridement. In most instance we used xilazyna and local anesthesia.
3. The initial management of degloving injuries centers on eliminating or reducing contamination of the wound and the debridement of any devitalized tissues.
4. Early bandages, a dry-to-dry or wet-to-dry adherent dressing are used after surgical debridement to help finish debridement
5. Salvaderm is a good debriding agent which promotes accelerated wound healing
6. In the late repair stage of healing, the contact bandage layer should be nonadherent. In this stage Amnion has most of the characteristics.

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Study of some hematological parameters in non-steroidal anti-inflammatory therapy in dog

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Non-steroidal anti-inflammatory drugs (NSAIDs) associate in various proportions the analgesic, anti-inflammatory and antipyretic effects, being used symptomatic, for the analgesic and antipyretic actions in different pains and feverish states or for the anti-inflammatory action in different osteo-articular diseases. NSAIDs act upon cyclooxygenase, blocking the transformation of arachidonic acid in cyclic endoperoxides.

The purpose of this study was to evaluate curative effects and eventual adverse reactions consecutive the usage of some NSAIDs in the therapy of osteo-articular afflictions.

The biological material of this study was represented by 15 dogs from both sexes, with ages between 4 and 9 years and weights between 4 and 15 kg. These dogs were diagnosed with osteo-articular diseases (arthrosis, arthritis, different degrees of hip dysplasia, discopathy, etc.) and they were treated with drugs containing three non-steroidal anti-inflammatory substances: flunixin meglumine, meloxicam and carprofen.

Consecutive to the usage of these NSAIDs in therapy of some osteo-articular afflictions in dogs, there were not recorded significant adverse reactions that could limit their indications.

Statistic and comparative analysis of the investigated parameters consecutive to the usage of these three NSAIDs showed the fact that the adverse effects are focused preponderantly upon the factors implicated in blood coagulation, with a tendency to hypocoagulability.

The modifications of the hematological parameters were more significant in case of flunixin meglumine, meloxicam and carprofen being well tolerated.

Key Words: dog, non-steroidal anti-inflammatories, adverse reactions

Non-steroidal anti-inflammatory drugs (NSAIDs) associate in various proportions the analgesic, anti-inflammatory and antipyretic effects, being used symptomatic, for the analgesic and antipyretic actions in different pains and feverish states or for the anti-inflammatory action in different osteo-articular diseases. [3]

During inflammation, under the action of phospholipase A, the phospholipids from cellular membranes are transformed in arachidonic acid, which follows two pathways: lipooxygenase pathway, with the synthesis of hydroperoxides (hydroxides and leucotrienes), and cyclooxygenase (prostaglandin-sintetase) pathway, with the synthesis of cyclic endoperoxides (prostacyclins, prostaglandins and tromboxans).

NSAIDs act upon cyclooxygenase, blocking the transformation of arachidonic acid in cyclic endoperoxides. [1, 2]

Recent data showed that cyclooxygenase (Cox), the enzyme implicated in the biosynthesis of prostanoids, exists in two isoforms: Cox_1, present in normal tissues, and Cox_2, present only in the inflamed tissues. [5]

Classic NSAIDs inhibit both types of cyclooxygenases; the inhibition of Cox_2 justifies the pharmacodynamic effects, while the inhibition of Cox_1 induces the main adverse reactions. [4]

Recently there were synthesized NSAIDs that selectively inhibit Cox_2; these substances (celecoxib, parecoxib) induce proper pharmacodynamic effects with minimum adverse reactions. [1]

The purpose of this study was to evaluate curative effects and eventual adverse reactions consecutive the usage of some NSAIDs in the therapy of osteo-articular afflictions.

MATERIALS AND METHODS

The biological material of this study was represented by 15 dogs from both sexes, with ages between 4 and 9 years and weights between 4 and 15 kg. These dogs were diagnosed with osteo-articular diseases (arthrosis, arthritis, different degrees of hip dysplasia, discopathy, etc.) and they were treated with drugs containing non-steroidal anti-inflammatory substances.

Each of the three non-steroidal anti-inflammatory substances used in this experiment was administered to a lot consisting in 5 dogs, as follows:

- flunixin meglumine – was administered subcutaneously, in dose of 1,1 mg/kg, for 7 days;
- meloxicam – was administered orally, in dose of 0,1 mg/kg, for 30 days;
- carprofen – was administered subcutaneously, in dose of 2 mg/kg, for 30 days.

In all cases, NSAIDs were administered alone, without any association with other drugs.

At the start of the experiment, 7 days after the start of flunixin meglumine administration and 15 and 30 days after the start of meloxicam and carprofen administration there were sampled blood samples from all animals in order to study the following hematological parameters: hemoglobin, hematocrit, red blood cells, leukocytes, trombocytes, reticulocytes, leukocytes formula, Quick coagulation time.

RESULTS AND DISCUSSIONS

The average values of hematological parameters after the administration of flunixin meglumine in dogs are presented in Table no. 1.

Table no. 1

Average values of hematological parameters after the administration of flunixin meglumine in dogs

Blood parameter	Initially	After 7 days
Hemoglobin g%	15,1	13,05
Hematocrit %	47,6	40,1
Red blood cells (nr./ml)	4.729.000	4.370.000
Trombocytes (nr./ml)	185.000	378.000
Leukocytes (nr./ml)	9.870	14.670
Neutrophils %	64,3	47,7
Eosinophils %	3,2	4,5
Basophils %	0	0
Lymphocytes %	27,2	38,5
Monocytes %	6,3	8,9
Reticulocytes %	16,3	44,8
Quick coagulation time (sec.)	9,2	10,1

In case of flunixin meglumine administration in dogs it can be noticed the decrease of hematocrit and red blood cells values, the increase of leukocytes, the decrease of neutrophils ratio and the increase of lymphocytes ratio, as well as a significant increase of trombocytes number and coagulation time.

The average values of hematological parameters after the administration of meloxicam in dogs are presented in Table no. 2.

Table no. 2

Average values of hematological parameters after the administration of meloxicam in dogs

Blood parameter	Initially	After 2 weeks	After 4 weeks
Hemoglobin g%	14,34	13,54	13,25
Hematocrit %	48,9	42,8	45,7
Red blood cells (nr./ml)	4.689.000	4.516.000	4.702.000
Trombocytes (nr./ml)	159.000	210.000	250.000
Leukocytes (nr./ml)	11.020	11.930	12.700
Neutrophils %	61,2	58,1	53,4
Eosinophils %	6,7	6,8	7,1
Basophils %	0	0	0
Lymphocytes %	27	31,1	32,7
Monocytes %	4,7	3,5	6,2
Reticulocytes %	24,9	37,5	40,7
Quick coagulation time (sec.)	10,9	12,8	12,4

Meloxicam produces a much lower hematocrit decrease comparatively to flunixin meglumine, and also the increase of trombocytes and leukocytes number, the decrease of neutrophils and the increase of lymphocytes ratio.

The average values of hematological parameters after the administration of carprofen in dogs are presented in Table no. 3.

Table no. 3

Average values of hematological parameters after the administration of carprofen in dogs

Blood parameter	Initially	After 2 weeks	After 4 weeks
Hemoglobin g%	15,35	14,42	13,85
Hematocrit %	47,2	46,8	45,3
Red blood cells (nr./ml)	4.772.000	4.810.000	4.820.000
Trombocytes (nr./ml)	175.000	252.000	295.000
Leukocytes (nr./ml)	8.780	7.420	9.970
Neutrophils %	50,7	52,8	62,7
Eosinophils %	5,6	6,1	5,1
Basophils %	0	0	0
Lymphocytes %	36,8	36,2	28,5
Monocytes %	5,1	4,6	3,5
Reticulocytes %	11,35	17,9	28
Quick coagulation time (sec.)	11,22	12,3	12,4

The administration of carprofen in dogs induced a slightly decrease of hematocrit and red blood cells number, a significant increase of trombocytes, an increase of leukocytes and neutrophils number and a decrease of lymphocytes ratio.

This study showed that, in dog, the secondary effects induced by the used NSAIDs are minimal.

Flunixin meglumine determined the increase of Quick coagulation time, trombocytes and leukocytes number and the decrease of hematocrit and red blood cells number (Figures no. 1, 2, 3). Therefore, it is recommendable that the treatment with this substance must not exceed one week, being chosen especially in acute or subacute osteo-articular afflictions.

The experiments effectuated on dogs with chronic osteo-arthropathies shown a good efficacy and tolerance of meloxicam, the recorded secondary effects being minimal after 4 weeks of treatment. The administration of meloxicam in acute articular inflammatory processes determined slight modifications of hematological parameters (Figures no. 1, 2, 3).

Meloxicam's clinical efficacy regarding analgesic and antipyretic effects, and also the tolerance of the product were investigated in dogs after surgical interventions at the osteo-articular level. After the treatment with meloxicam, the functional incapacity of the effected member(s) was reduced in a visible and progressive manner. Nevertheless, it was noticed an adverse reaction, consisting in the delay of wound healing, this aspect suggesting that meloxicam affects the proliferative phase of the healing process. Therefore, it is recommendable that post-operator treatment with meloxicam must not exceed 4-5 days.

Coagulability disorders pursuant to the treatment with carprofen were studied in dogs which were sterilized. Bleeding time was similar with the one recorded at the control lot, which indicates the fact that carprofen does not induce significant secondary effects in this species (Figures no. 1, 2, 3).

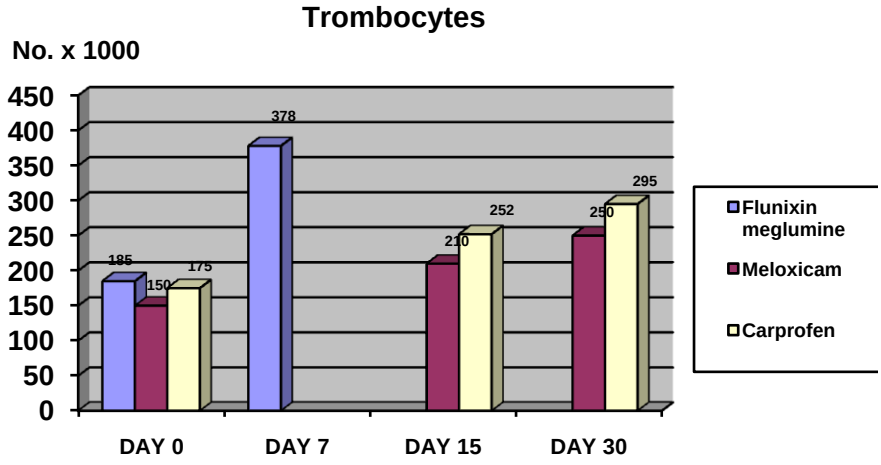

Figure no. 1
Comparative variation of trombocytes number/ml. blood after the administration of flunixin meglumine, meloxicam and carprofen in dogs

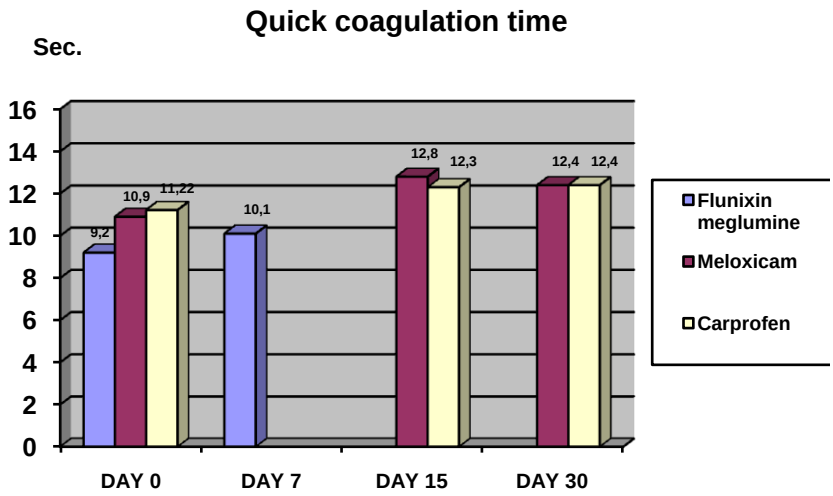

Figure no. 2
Comparative variation of Quick coagulation time after the administration of flunixin meglumine, meloxicam and carprofen in dogs

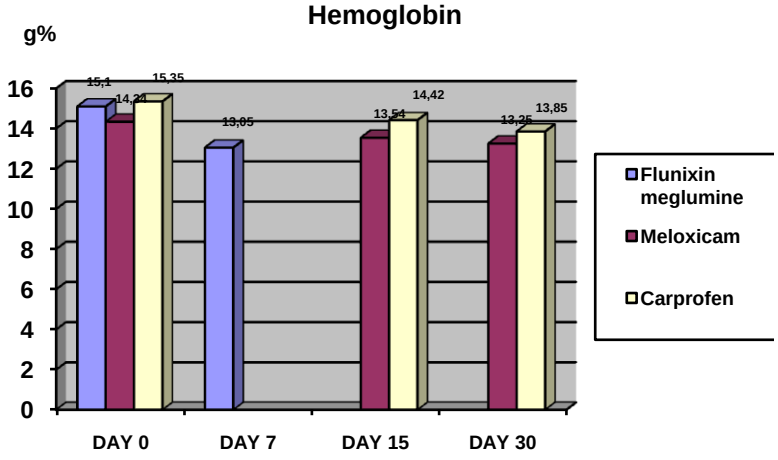

Figure no. 3
Comparative variation of hemoglobin after the administration of flunixin meglumine, meloxicam and carprofen in dogs

CONCLUSIONS

1. The experiment effectuated on the 3 lots of dogs had as purpose a comparative study on secondary effects of three NSAIDs with different molecular structures used in veterinary therapy: flunixin meglumine, meloxicam and carprofen.
2. It was noticed a good tolerance from the organism, as well as the easiness and safety of these NSAIDs usage in veterinary therapy.
3. Consecutive to the usage of these NSAIDs in therapy of some osteo-articular afflictions in dogs, there were not recorded significant adverse reactions that could limit their indications.
4. Statistic and comparative analysis of the investigated parameters consecutive to the usage of these three NSAIDs showed the fact that the adverse effects are focused preponderantly upon the factors implicated in blood coagulation, with a tendency to hypocoagulability.
5. The modifications of the hematological parameters were more significant in case of flunixin meglumine, meloxicam and carprofen being well tolerated.

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Comparative acaricide activity of *Euphorbia cyparissias L.* on ixodides

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The study is the object of a CNCSIS research grant and it proposes the comparison of Euphorbia cyparissias tincture acaricide effect in decreasing concentrations in vitro and in vivo, on Dermacentor marginatus and Haemaphysalis punctata ticks, at sheep.

The identification of genders has been made after the morphological characters and after the determination keys known in parasitology.

The tincture, obtained after the prescriptions of Romanian Pharmacopoeia was used in mother solution (10%) and in dilutions: 5, 2, 1, 0.5, respectively 0.25%.

Ixodides viability after in vitro applications was followed at 30, 60, 90 and 120 min. The treatment effect after 30 and 60 minute from the exposure has been reduced one. The difference between ticks average number at 30 and 60 minutes was only 0.6 ($p<0.1$). The difference between averages at 30 and 90 minutes was significantly higher 3.28 ($p<0.001$), and between 30 and 120 minutes was 5.65 ($p<0.001$).

Between 60 and 90 minutes from the exposure the averages difference was also significant 2.68 ($p<0.001$), as between 60 and 120 minutes 5.05 ($p<0.001$), the surviving rate being smaller after 90 and 120 minutes from exposures. There have been determined significant differences between averages 2.36 ($p<0.001$) in the case of readings at 90 and 120 minutes. All the ticks died after approximately three hours from the exposure, no matter the used dose. It was observed that the feed females were more resistant than the hungry ones or very few feed.

In the case of in vivo testing, mortality percentage at 24 hours varied between 9 and 13 for D. marginatus species and between 0 and 23 for H. punctata species, concluding that: there are no differences of acaricide effect of Euphorbia cyparissias tincture depending on tick's species, the tincture could be used only as an alternative method in controlling the ixodide ticks.

The tincture efficacy is significant different depending on exposure time ($p<0.001$) and dose ($p<0.001$).

Key words: E. cyparissias, efficacy, in vitro, in vivo, H. punctata, D. marginatus

The acaricide substance using in fighting against ixodides remain the main way to follow for most practicing vets, but the chemical methods have two big faults: they are expensive and they action indiscriminate. The risk of appearing resistant populations, which reduce efficacy, on time passing and appearing the medicament residue adds at these.

The researchers reasoned by these considerations endeavor to find new alternatives, more ecologic, cheaper, even if the moment those efficacy is still far to be sufficient. The studies in the sense need a enormous work volume and material meanings that not always are available.

Objective

The study proposed the comparative observation of acaricid effect of *Euphorbia cyparissias* L. tincture, in different concentrations, on ixodide ticks at sheep, *in vitro*, in laboratory and *in vivo*, by sprinkling, in field conditions.

MATERIALS AND METHODS

Tincture obtaining

The tincture was obtained following the indications of Romanian Pharmacopoeia Ed. X (8) 10 grams from dried plant, spreader have been put to maceration extract in ratio 1:10 (m/m), for 10 days, stirring three times per day, in 100 ml alcohol 70 (v/v) in brown glass. After bringing out and pressing the residue each of the extractive liquids rejoined and homogenized have been let to settle down at 5-10ºC temperature, and then have been filtered, underlining the evaporation losses. The obtained tincture has a clear aspect, yellow color and specific aromatized smell, corresponding to Romanian Pharmacopoeia indications referring to tincture. By dilution, the tincture perturbs, it changes its color in milky white opalescent and forms sediment.

Animals

The experiment has been made in period march - april 2007, on 32 sheep from the USAMVB farm, which have been divided in eight lots of four sheep/lot. These formed the testing base for decreasing concentrations of *Euphorbia* tincture, one lot being allotted for each tincture dilution.

The used concentrations have been: 10% (the mother solution), 5%, 2%, 1%, 0,5%, respectively, 0,25% and a witness lot *placebo* treated only with the solvent (alcohol 70°). The treated animals and those from the witness lot grazed together.

Different concentrations of tincture have been applied in sternal region, a region which is easy to examine and where the biggest number of ticks was observed, by aspersing so that the entire region to be covered.

Bio-analysis

For *in vitro* testing Petri plate in which has been put filter paper. On this have been put 1 ml dilution, so that to be uniformly wet on the entire surface. On this were put the ticks, reporting 100 (50/plate), for each concentration, forming a witness lot in the same conditions, on filter paper wet only with diluted alcohol.

Readings

The identification of species has made after the morphological characters and after the Feider (5), Babos (1) and Estrada-Peña and al. (4), determination keys, known in parasitology. The monitoring has made at stereo-microscopic magnifier (resolution 20 x 2,5) at the interval: 30, 60, 90 and 120 minutes, following the ixodides viability, shown by the movement capacity, the amplitude and frequency of appendix movements, *orto*- and *verso*-stasis and exitus installation.

The statistical evaluation moment have been considered the one before exitus (meaning: *versostasis* **+**) after the up mentioned model.

For *in vivo* study, at 24 hours from applying the extract all the ticks have been gathered from the sternal region (that on which have been applied the tinctures) to follow in the laboratory when emerge the exitus.

For *in vitro* testing, the ticks have been put on Petri plates, separately for each case, monitoring the cases evolution.

Statistical analysis

The mortality percentage has effectuated statistically after the *Anova* test with the aid of SPSS-7.5 program, determining: the arithmetic average, standard deviation, variation and middle error of average (standard error).

In Vitro Testing

The tincture acaricide activity on *D. marginatus* and *H. punctata* (photo) females has been tested by direct contact. It considered sufficient and more important *in vitro* testing in different concentrations only on females, they being principaly the respondent for direct and indirect pathogenetic activity, because the males eat enly few, or even not at all and, so, their pathogenetic action is insignificant.

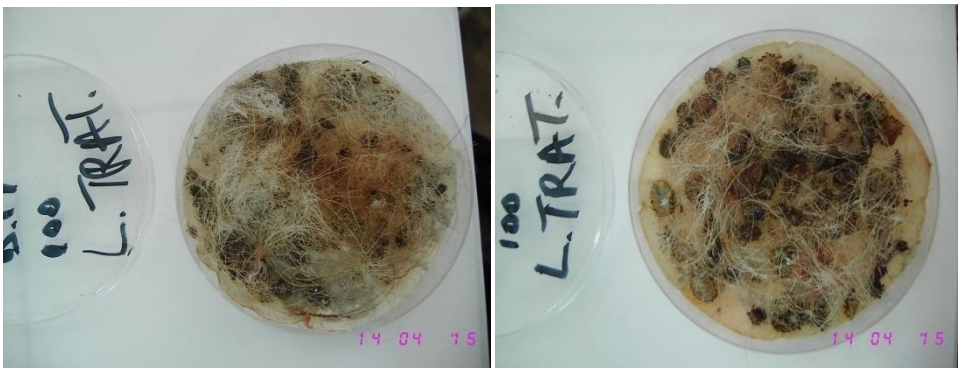

Photo. Dermacentor marginatus and Haemaphysalis punctata females (original)

The tincture efficacy in different concentrations in relation with exposure time is presented in tables 1 and 2, which show, in fact, the average number of *D. marginatus* and *H. punctata* females that survived.

Table 1

Dermacentor marginatus females that survived after the contact (average ± D.S. at 5 testings/dose)

Concentration	**Exposure time (minutes)**			
%	**30**	**60**	**90**	**120**
0 (witness)	50	50	50	50
10	49(9.8±0,4)	47(9.4±0.8)	31(6.2±1.3)	6(1.2±0.8)
5	49(9.8±0,4)	46(9.2±0.4)	37(7.4±1.1)	14(2.8±0.8)
2	50(10±0)	49(9.8±0.4)	36(7.2±0.8)	24(4.8±1.6)
1	50(10±0)	49(9.8±0.4)	32(6.4±1.5)	24(4.8±1.7)
0.50	50(10±0)	50(10±0)	39(7.8±1.7)	26(5.2±1.9)
0.25	50(10±0)	50(10±0)	32(6.4±1.1)	30(6.0±0.7)

Table 2

Haemaphysalis punctata females that survived after the contact (average ± D.S. at 5 testings/dose)

Concentration	Exposure time (minutes)			
%	**30**	**60**	**90**	**120**
0 (witness)	50	50	50	50
10	50(10±0)	44(8.8±0.4)	27(5.4±1.1)	17(3.4±1.1)
5	50(10±0)	44(8.8±0.4)	31(6.2±1)	21(4.2±1.3)
2	50(10±0)	45(9.0±1)	32(6.4±2.1)	23(4.6±0.8)
1	50(10±0)	45(9.0±0.7)	33(6.6±1.1)	23(4.6±1.3)
0.50	50(10±0)	46(9.2±0.8)	35(7.0±1.2)	26(5.2±0.8)
0.25	50(10±0)	47(9.4±0.5)	36(7.2±0.8)	25(5.2±1.2)

At 30 minutes after the contact with the extract, no matter the used concentration, the majority of ticks loss their vivacity and the movement capacity and they are not capable to return to normal position if they fall in dorsal position.

After 60 minutes 1-2 ticks only move at concentration lower than 10%, but most of them only move the feet. The treatment effect after 30 and 60 minutes from the exposure has a low one. The difference between ticks number average at 30 and 60 minutes has only 0.6 ($p<0.1$). After 90 minutes feet movements are slower and the number of ticks that show them is lower.

The difference between averages at 30 and 90 minutes was significant higher 3.28 ($p<0.001$), and between 30 and 120 minutes 5.65 ($p<0.001$). Between 60 and 90 minutes from the exposure the averages difference was also significant 2.68 ($p<0.001$), as also between 60 and 120 minutes 5.05 ($p<0.001$).

Surviving rate was lower after 90 and 120 minutes from exposure. And in the case of readings at 90 and 120 minutes significant differences between averages 2.36 ($p<0.001$) were determined. Because of that, we considered satisfactory the examination at 120 minutes. All the ticks died after appreciatively three hours from the exposure, no matter the used dose. It was observed that fed females are more resistant than the hungry ones or the few fed ones.

Analysing the tables data, and also the variability test results, we can observe that there are no significant differences regarding the efficacy depending on the tick species. The acaricide effect appears after 30 minutes from the exposure by lossing the movement capacity to most ticks, after that the effect become more evident and, so, after 120 minutes from exposure very few ticks move their legs

There have been observed that significant differences regarding ticks number average exist **statistical** that have survived depending the time exposure F = 376.71 df = 3, $p<0.001$, and R^2 = 88.6%. Also, there are significant differences and depending on used concentration F = 6.51, df = 5, $p<0.001$.

Iori and *al.* (6) tested the acaricide effect of oil-like extract of *Melaleuca alternifolia* (tea tree oil, TTO) at different doses (4, 6, 8 şi 10 µl) and exposure periods (30, 60, 90 and 120 minutes) on *Ixodes ricinus* nymphes. There was determined that 8 µl dose was letal for more than 70% ticks and the mortality increased over 80% when the 10 µl dose was used. The effect was correlated with exposure time, being significant after exposure 90 minutes.

In Vivo Testing

At 24 hours from extract application all the ticks were gathered from sternal region, they were numbered and their comportment was monitorized in laboratory. There was observed the viability modification, movement capacity decreasing, vivacity, the incapacity to return if they fall on dorsal face and the cuticle aspect modification (colour and consistence), in comparison with those gathered from the witness or those attached after the application.

After the observation of comportment changes, the ticks gathered from each treated sheep were indentified and numbered. The identified species were *Dermacentor marginatus* and *Haemaphysalis punctata* with the first species predominance. From the incipient studies made in 2005/2006 in area we knoew that the dominant species is *D. marginatus.* Mortality percentage at 24 hours varied between 9 and 13 for *D. marginatus* species and for *H. punctata* species between 0 and 23. From the data in table 3 we can observe that there are no differences regarding the mortality depending on extract concentration, the difference being offered by the number of ticks fixed in sternal region.

Table 3

The ticks gathered from sheeps treated with Euphorbia cyparissias tincture at 24 hours from the first application

Concentration	Species	Ticks number	Alive	Dead	Efficacy %
10	*D. marginatus*	304	293	38	**11**
	H. punctata	11	11	0	**0**
5	*D. marginatus*	332	297	35	**11**
	H. punctata	31	24	7	**23**
2	*D. marginatus*	448	376	72	**16**
	H. punctata	10	7	3	**9**
1	*D. marginatus*	389	339	50	**13**
	H. punctata	2	0	0	**0**
0,5	*D. marginatus*	381	340	41	**11**
	H. punctata	1	0	0	**0**
0,25	*D. marginatus*	282	258	24	**9**
	H. punctata	10	8	2	**20**
Witness	***D. marginatus***	**478**	**478**	**0**	**-**
	H. punctata	**213**	**213**	**0**	**-**

The effect of tincture acaricide is not a spectacular one, type *„knock-down",* the action is slow, the death occurs later, at most of them, after 48-72 hours from the application. The hungry or partial fed ticks proved to be more sensitive than the satiated ones. This observation has been made also *in vitro* testings.The slow action manner, bereft of momentousness, of plant extract could be considered, at first view, as a disadvantage. At second application, seven days period, there were not identified fed satiated ticks on sheeps treated with tincture at concentrations 10%, 5%, 2% and 1%. The number of fixed ticks was lower (2-3 to 5 ticks), all hungry, what means that they did not fixed long time ago or they did not fed properly, it also could suggest an eventual repellent effect. At the concentrations 0,5 and 0,25% it was observed almost satiated females presence.

After second aspersion the ticks were gathered at three days from the application. The number of gathered ticks is presented in table 4, depending on used concentration. A numerical decreasing of ticks was observed, but it was not proportionately with the used concentration.

Table 4

Ticks gathered from sheeps treated with Euphorbia cyparissias tincture at 72 hours from second application

Concentration	Species	Ticks number
10	*D. marginatus*	**33**
	H. punctata	**0**
5	*D. marginatus*	**36**
	H. punctata	**1**
2	*D. marginatus*	**157**
	H. punctata	**15**
1	*D. marginatus*	**51**
	H. punctata	**1**
0,5	*D. marginatus*	**42**
	H. punctata	**2**
0,25	*D. marginatus*	**66**
	H. punctata	**1**
Witness	***D. marginatus***	**176**
	H. punctata	**67**

The studies show that the importance of *in vitro* and *in vivo* results comparison, that often, can be significantly different. If in the case of in vitro testings, the ticks die after one - two hours of contact, no matter the used concentration, in the case of animals applications the situation is different.

In vivo the contact time between ticks and tincture is shorter (being an alcoholic solution it evaporates rapidly and the contact is a short time one, ticks have bigger movement autonomy etc.). This fact must be analyzed closer from the point of view of choosing an excipient base more suitable as remaining on parasitical cuticle.

Because of that, in results interpretation must take in count the manner in which the contact ixodide - acaricide was made. We consider that the studies must be enlarged to affirm that *E. cyparissias* tincture can be used currently in ticks populations control. For the moment it is sure that the tincture can be used only as an alternative method in fighting aginst ticks, avoiding or delaying the installation of different acaricide resistance.

Maybe, by using the oil-like or glicerine-like extracts it may realise a better efficacy. The further studies object must follow and the extract effect on food conversion capacity, reproductive index and larva seclusion.

As an exemple *Borges* and a*l.* (2) found out that *Melia azedarach* alcoholic extract in 0,25% concentration produced 100% mortality for *Boophilus microplus* larvae *in vitro* conditions*,* in the infected cattle the results were more modest (althoogh there was proved the significat decreasing of satiated females at 21 from the treatment).

Webb and *David* (7) in treatments made on Tswana, Brahman and Siemmental bovines infected naturally with ticks, treated with *Azadirachta indica* 5% water like extract from seeds to apply by sprinkling in 5g/kg doses (a very big quantity), lead to numerical decreasing of ectoparasitical, in comprarison with the witness lot, but not to their total elimination.

Cristina and *al.* (3) experiemnted *E. cyparissias* extract *Argas persicus,* proving its efficacy depending on concentration, time contact and dose. Raporting to biology of *Argas* gender species, studied, these results could be more important than the results obtained in the ixodide case.

The results obtained from the experiment, but also the results presented by other authors, suggest that often the plant extracts efficacy is different depending on chosen plant, extract type, concentration, exposure type and time and on evolutive parasitical stage.

Conclusions

- There are no significant differences of *Euphorbia cyparissias* tincture acaricide effect depending on ticks species.
- The tincture efficacy is significantly different depending on exposure time (*p<0,001*) and dose (*p<0,001*).
- The tincture obtained from *E. cyparissias* can be used only as ana alternative method in controlling the ixodide ticks, because it is an natural acaricide, cheap and it play an important role in reducing the use of sintetic acaricide that are harmful for people and ecosystem.

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Efficacy of an antiseptic ear cleanser in dog's erythemato-ceruminous otitis

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Erythemato-ceruminous otitis externa (OE) is frequently reported in dogs.

Although a large number of cases are associated with hypersensitivity, secondary to the microbial proliferations which are essential aggravating factors that should always be controlled.

The study about clinic and antimicrobial activity of an otic solution in dogs with OE was done in October 2006 - March 2007 in 50 from 120 clinically examined dogs. The clinical survey revealed that almost all OE cases were associated with Malassezia, cocci, and/or rods populations.

Dogs with parasitic otitis externa and/or treated with systemic or topical antifungal, antibacterial and anti-inflammatory products within the last week before study were not included in the study.

The clinical status of the ears was evaluated initially at day 0 and after 7 and respectively after 14 days, clinical assessment being based on six clinical parameters: exudates' amount, erythema, stenosis, excoriation on the ear pinnae, pain and discomfort. All six clinical parameters were graded on a scale of 0 to 4 (no signs to severe), ear swabs and otic exudates being investigated for the presence of yeasts and bacteria (cocci and rods).

The reduction of the microbial population (yeast and/or bacteria) in both ear canals, after 1 and 2 weeks of treatment was the main assessment criterion of efficacy; after 1 week of treatment, the clinical discomfort decrease in 65.7% of cases, while the microorganism population decreased in 63.5% of cases. In 34% of cases the treatment was inefficient.

After 2 week of treatment in 94.5% of cases the clinical signs were disappear and the population of microorganism decreased at the limit of normal flora in horizontal ear canals of healthy dogs (1, 2).

The clinical signs of otitis externa were maintained in 5.5% of cases after this period. This study also shows the very good tolerance of the product.

Key words: otitis externa, dog, ear cleanser, efficacy

Objectives

It was already been demonstrated that the ear cleanser tested in this study is *in vivo* effective against bacteria and the yeast *Malassezia spp.*, but the aim of our *in vivo*, study was to evaluate the clinical tolerance and the antimicrobial and clinical activities of an commercial ear cleanser, when is used in erythemato-ceruminous otitis externa.

Materials and methods

Animals

The study about clinic and antimicrobial activity of the otic solution in dogs with OE was done between October. 2006 - March. 2007.

In this period, in 50 from 120 clinically examined dogs of any breed or sex the erythemato-ceruminous otitis externa was clinically established after known cytological and stain methodology.

The almost all OE cases were associated with *Malassezia spp.*, cocci, and/or rods populations. Dogs with parasitic otitis externa and/or treated with systemic or topical antifungal, antibacterial and anti-inflammatory products within the last week before study were not included in the object of the study.

Treatment

Ears were filled with ear cleanser twice a day for 2 week period, and the base of the ear was rubbed after applications, dogs being not allowed to shake their heads for one or two minutes. No additional treatment was allowed.

Studied ear cleanser, **Otisept**® (*Biovet Impex*), has in its composition:

Salicylic acid 1.5 g
Benzoic acid 0.5 g
Lactic acid 1.0 g

Protocol

The clinical status of the ears and healing process was evaluated initially at: day 0, day 7 and respectively after 14 days from treatments.

Clinical assessment was based on six registered clinical parameters:

- exudates' amount,
- erythema
- stenosis,
- excoriation on the ear pinnae and
- pain,
- dog's discomfort, due to the otitis, established by the owners (after registered ear scratchiness, head shaking, pain, and amount of exudate).

All six clinical parameters were graded on a scale of 0 to 4 (from no signs to severe ones).

Examinations

Ear swabs and cytological examinations of the otic exudates obtained from the horizontal ear canal, were investigated for the identification of yeasts and bacteria (cocci and rods).

The specimens were rolled onto a glass microscope slide and Gram stained, after heat fastening. Mean number of each type of micro-organisms was scored for each ear, after the up mentioned scale of 0-4, after cytological examination of 20 and 40 high power fields (studied to x 100 objective).

Cytological specimens were evaluated for the presence, number, and characteristics of three key features: yeast, bacteria, and leukocytes. The presence of more than five yeast elements organisms and 25 bacteria in the microscopic field suggest a significant microbial activity justifying the therapeutic act (1, 2, 3).

Efficacy assessment

The reduction of the microbial population (yeast and/or bacteria) in both ear canals, after 1 and 2 weeks of treatment was the main assessment criterion of efficacy.

Secondary efficacy assessment criterion was the improvement in the clinical condition of the dogs. The evolution of means, score of clinical and microbial parameters are reproduced in table 1, and figures 1 and 2.

Table 1

Evolution of means scores of clinical and microbial parameters on the study

Clinical parameter	Day 0	Day 7	Day 14
1. Exudates	2.45	1.32	0.25
2. Erythema	2.13	1.18	0.31
3. Stenosis	1.22	0.55	1.23
4. Excoriations	1.05	0.49	0.17
5. Pain	2.09	1.17	0.35
6. Discomfort (owner's assessment)	2.27	1.12	0.25
Total clinical score	**11.21**	**5.83**	**2.56**
Microbial parameters			
1. Malassezia spp.	2.35	1.35	0.48
2. Cocci	1.12	0.65	0.18
3. Rods	0.36	0.23	0.17
Total microbial score	**3.83**	**2.23**	**0.83**

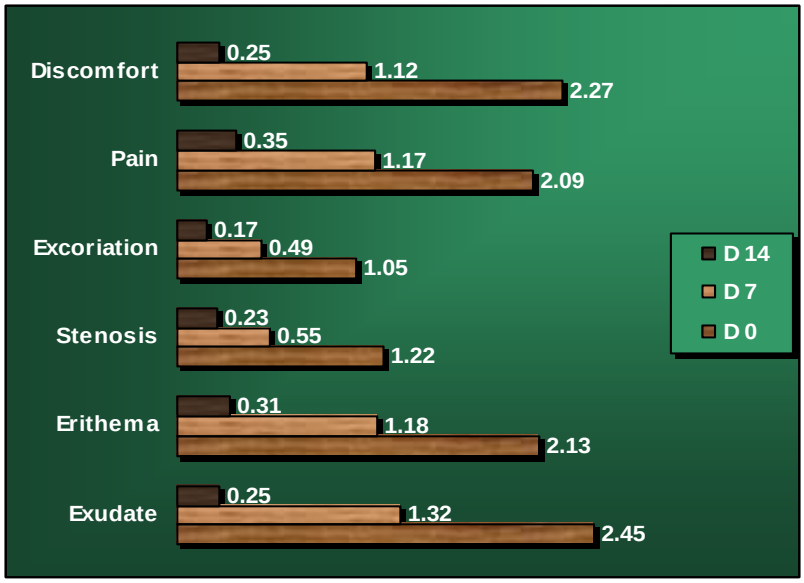

Fig. 1. Evolution of mean scores of the clinical parameters

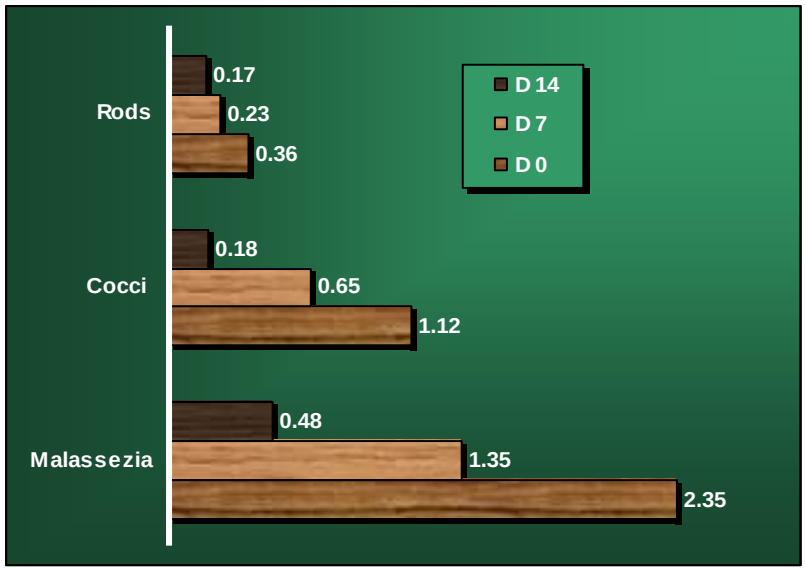

Fig. 2. Evolution of mean scores of microbial parameters

After 1 week of treatment, the clinical discomfort decrease in 65.7% of cases, while the microorganism population decreased in 63.5% of cases. In 34% of cases the treatment was inefficient.

After 2 week of treatment in 94.5% of cases the clinical signs were disappear and the population of microorganism decreased at the limit of normal flora in horizontal ear canals of healthy dogs (1, 2).

The clinical signs of otitis externa were maintained in 5.5% of cases after this period. This study also shows the very good tolerance of the product.

Conclusions

- **Clinical and microbial healing, in two weeks, proven the otic solution’s good activity in the management of dog’s erythemato-ceruminous otitis externa.**
- **Cytology examination is a simple, quick, and helpful diagnostic test, that should be performed as routine test on any patients surveyed for clinical OE.**
- **In combination with clinical signs, the otoscopic evaluation, diagnostic testing of primary disease and serial cytology enhances the ability of veterinarian to diagnose a secondary infection, to predict disease progression, to evaluate the response to therapy, and to make appropriate management decisions.**
- **This study also shows the good tolerance of the product.**

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The detection of enrofloxacin residues in pork meat using HPLC analysis

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The consequences of drug residues presence in animal organism and animal origin alimentary products is an actual subject due to the higher diversity of drugs used in veterinary therapeutics.

In this study we determined enrofloxacin and ciprofloxacin residues from pork meat after 5 days of oral treatments with doses specified by the producer.

The aim of this research was the comparative study of the enrofloxacin and ciprofloxacin residues' decrease in pork meat.

In this study it was used the commercial product Enroflox 10% drinkable solution administered in dose of 5 mg/kg/day to 3 month-aged pigs for 5 days.

The pigs were slaughtered at different time intervals after treatment's interruption. Muscle samples were sampled from different corporal regions; they were minced and then extracted with methanol. Centrifugation was made 10 minutes at 4000 rpm, followed by rotary evaporator concentration. For the chromatographic dissociation of the constituents it was used High Performance Liquid Chromatography method (HPLC).

During this study, the rate of enrofloxacin and ciprofloxacin's concentration decrease was mainly balanced; these substances eliminate from the body preponderant in the first days after treatment's interruption.

Between the 5^{th} and the 6^{th} day after treatment's interruption enrofloxacin and ciprofloxacin residues were not theoretically present any more in pork meat, suggesting that enrofloxacin's waiting period for pork meat is short (5 days).

Key Words: pork meat, enrofloxacin, ciprofloxacin residues

An actual problem with high importance for alimentary hygiene is represented by the existence of toxic substances residues which, in some cases, can affect metabolic and clinic health of the consumers. [6]

The consequences of drug residues presence in animal organism and animal origin alimentary products is an actual subject due to the higher diversity of drugs used in veterinary therapeutics.

Nowadays, the presence of residues is accepted below certain values (Maximum Residue Limit = MRL), which are strictly settled for every type of molecule. [6]

In this study we determined enrofloxacin and ciprofloxacin residues from pork meat after 5 days of oral treatments with doses specified by the producer. [5]

The aim of this research was the comparative study of the enrofloxacin and ciprofloxacin residues' decrease in pork meat.

Enrofloxacin is a chemotherapic substance from the fluoroquinolones group; it has a wide range of action, including both Gram positive and negative bacteria, excluding the anaerobs. Enrofloxacin has a high intestinal absorption rate, maximum blood level attains rapidly and tissue levels are higher than blood levels. [2, 4]

The biotransformation takes place in the liver, resulting both active and inactive metabolites; the inactivated fraction eliminates by urinary route. One of the active metabolites is ciprofloxacin.

Antibacterial action mechanism of quinolones consists in blocking DNA-girase enzyme, disturbing in this way the integrity of bacterial DNA. [1, 3]

MATERIALS AND METHODS

In this study it was used the commercial product Enroflox 10% drinkable solution, administered in dose of 5 mg/kg/day in 3 month-aged pigs for 5 days.

The pigs were slaughtered at different time intervals after treatment's interruption. Muscle samples were sampled from different corporal regions; they were minced and then extracted with methanol. Centrifugation was made 10 minutes at 4000 rpm, followed by rotary evaporator concentration.

For the chromatographic dissociation of the constituents it was used High Performance Liquid Chromatography method (HPLC), with the following characteristics: stationary phase: SS EXSIL ODS 5 µm column (150x4.6 mm); mobile phase A: 4.5 ml trimethilamine in 860 ml distillated water, pH 2.3; mobile phase B: acetonitril Chromasolv HPLC gradient degrees.

Chromatographic conditions were: analysis time: 20 minutes; debit: 1 ml/min; mobile phase A: mobile phase B = 86.3:13.7; wave length: 280 nm; working domain: 5-0.1 mg/kg tissue (ppm).

RESULTS AND DISCUSSIONS

Enrofloxacin is well absorbed absorption is good after oral administration and it determines high tissue concentrations. It is metabolized in the liver and it is eliminated mainly by renal route.

Considering the pharmacokinetics of enrofloxacin, the level of residues in pork meat was presented as the sum of enrofloxacin and ciprofloxacin in mg/kg (Tables no. 1, 2, 3).

Table no. 1

The levels of enrofloxacin and ciprofloxacin residues in pork meat

Day of slaughtering after treatment interruption	Sum (Σ) of enrofloxacin and ciprofloxacin (mg/kg)
1	**0.386**
3	**0.176**
4	**0.028**
6	**< LOD**

Taking into account the equation: Y= -0.2256 Ln(X) + 0.3887:

- ***For day 1: Y= -0.2256 Ln(1) + 0.3887 = 0.3887;***
- ***For day 2: Y= -0.2256 Ln(2) + 0.3887 = 0.2323;***
- ***For day 3: Y= -0.2256 Ln(3) + 0.3887 = 0.1408;***
- ***For day 4: Y= -0.2256 Ln(4) + 0.3887 = 0.0759;***
- ***For day 5: Y= -0.2256 Ln(5) + 0.3887 = 0.0256;***
- ***For day 6: Y= -0.2256 Ln(6) + 0.3887 = -0.0155.***

Table no. 2

The levels of enrofloxacin and ciprofloxacin residues in pork meat calculated through logarithmic equation

Day of slaughtering after treatment's interruption	Sum (Σ) of enrofloxacin and ciprofloxacin (mg/kg) - real values -	Equation value
1	0.386	**0.3887**
2		**0.2323**
3	0.176	**0.1408**
4	0.028	**0.0759**
5		**0.0256**
6	>LOQ	**-0.0155**

**** Only the bold values will be took into account***

Table no. 3

Pharmacokinetic parameters of enrofloxacin

Day of slaughtering after treatment's interruption	Hours after treatment's interruption	Sum (Σ) of enrofloxacin and ciprofloxacin (mg/kg)	Eliminated quantity	Eliminated fraction
1	24	0.3887	0	
2	48	0.2323	0.1564	0.40
3	72	0.1408	0.0915	0.40
4	120	0.0759	0.0649	0.46
5	144	0.0256	0.0503	0.66
6	168	-0.0155		

****Average = 0,48***

The level of enrofloxacin residues decreases gradually after treatment's interruption, so that in the 6th day the level of residues is already below the detectable quantity. During this time interval, the concentration's decrease rate is balanced. (Table no. 1)

A more complete presentation of enrofloxacin's biological circuit is showed in Table no. 3. It can be observed that the highest quantity of eliminated enrofloxacin is registered 48 hours after treatment's interruption and gradually decreases to the 5th day after treatment's interruption.

The depletion of enrofloxacin and ciprofloxacin's residues in pork meat is presented in Figures no. 1 and 2.

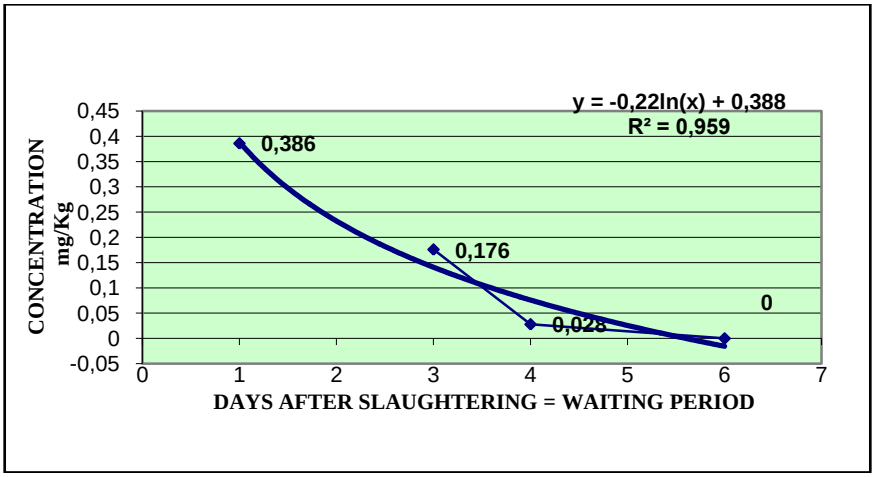

Figure no. 1
The depletion of enrofloxacin and ciprofloxacin residues in pork meat

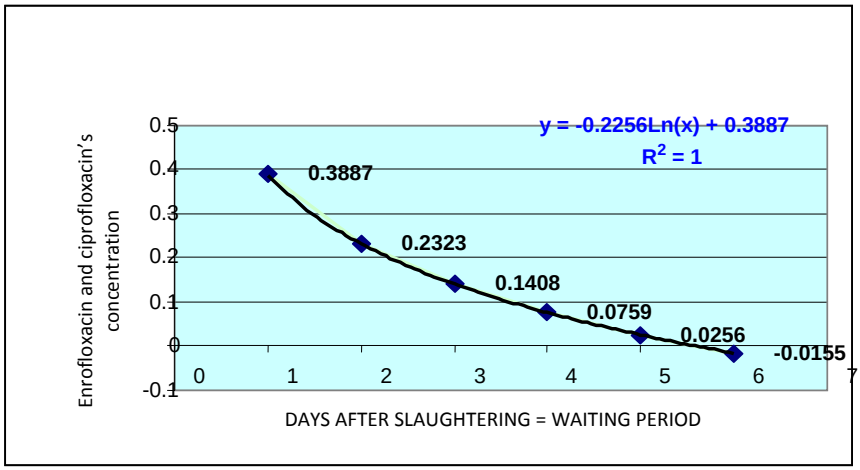

Figure no. 2
The depletion of enrofloxacin and ciprofloxacin residues in pork meat according to the logarithmic equation

It was observed that enrofloxacin and ciprofloxacin residues levels in pork meat constantly reduced after the treatment's interruption. In the 6th day after the treatment's interruption, the curve tends to become tangent to zero; between the 5th and the 6th day, the curve intersects X axis, which suggests that theoretically enrofloxacin and ciprofloxacin residues are not present any more in pork meat.

CONCLUSIONS

1. During this study, the rate of enrofloxacin and ciprofloxacin's concentration decrease is mainly balanced; these substances eliminate from the body preponderant in the first days after treatment's interruption.
2. The levels of enrofloxacin and ciprofloxacin in pork meat tend to become zero in the 6^{th} day after treatment's interruption.
3. Between the 5^{th} and the 6^{th} day after treatment's interruption theoretically enrofloxacin and ciprofloxacin residues are not present any more in pork meat, whereas the curve intersects X axis.
4. Enrofloxacin's pharmacokinetics shows that waiting period for pork meat is short (5 days).

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Studies on pharmacokinetics regarding the toxicity of non-steroidal anti-inflammatory substances

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Non-steroidal anti-inflammatories are analgesic substances used as main medication for therapy in different inflammatory disorders. Their analgesic action is due to the inhibition of prostaglandins biosynthesis- inhibition of the COX 1,COX 2, and COX 3 enzymes at the level of peripheral localization of the inflammatory process.

They are being recommended for a variety of aches of inflammatory origin from low to medium intensity (rheumatic diseases), but also for severe aches such as renal or biliary colic or severe dysmenorhea (intravenously).

Their use in veterinary practice is less due to the secondary effects, especially gastrointestinal and renal ones, sometimes these secondary effects being pretty severe. Rarely are being registered cases of hepatic toxicity and toxicity at the blood level. This relative limitation of their use in veterinary medicine in comparison with their use in human medicine has the explanation that there are different pharmacokinetic parameters on different animal species, being created in this way the possibility to limit the therapeutic effect or an excessive toxicity.

Key words: non-steroidal anti-inflammatories, toxicity digestive, toxicity renal

Non-steroidal anti-inflammatory agents belong to a very active chemical class of drugs, each pharmacodynamic action being demonstrated in "in vivo" and "in vitro" systems.

Some NSAID, due to prolonged treatment or to overdosing, are being accumulated mainly in some organs, for example phenylbutazone is accumulating at the level of the kidneys, muscles, spleen, fact that explains the intoxication with these substances (1, 2).

DISCUSSIONS

Toxicity at the digestive level

Gastric hemorrhage and ulceration are the most severe secondary effects of the NSAID.

In conformity with the theory of Schoen and Vender, non-steroidal anti-inflammatories have direct effect on gastroduodenal mucosa and indirect effect by active hepatic metabolites and by the decrease of the mucosal prostaglandins. The hepatic metabolites are being excreted through bile into duodenum, where they produce lesions of the gastric mucosa by duodenalgastric reflux and lesions of the enteric mucosa by anterograde passage through gastrointestinal tract.

The appearance of the digestive iatrogenic disorder, consecutive to the consumption of the non-steroidal anti-inflammatories is determined by more clinical factors, validated in multiple studies: advanced age (the risk increases at the same time with age: the prostaglandin synthesis at gastric level decreases, the vascular integrity is diminishing, the fragility of the mucosa is increasing); the presence in gastric/duodenal ulceration history or digestive hemorrhage; the use

of a simultaneous corticosteroid or anticoagulant medication; time, period and the dose of the utilized NSAID.

With the time there were propositions related to the hypothesis on the action mode of the NSAID on digestive mucosa. These can produce erosions by the appearance of microhemorrhages (takes place the destruction of the small category blood vessel). The erosion develops at its turn the ischemic infarct. Some lesions are related to the deficiency of glycoproteins from the gastric mucosa. Other observations indicated the destruction of the gastric mucosa barrier towards the redistribution of the hydrogen ions, which can be now considered more as a consequence than a cause of the lesions (4).

The local action (vasoactive), by direct contact with gastric mucosa is not an important factor which induces the ulcerations. Phenylbutazone is equally toxic taken orally or intravenously, and indometacin is more harmful for intestine after being administrated intravenously than after gavage. Salicylates can produce hearing deficiency, vertigo, and in therapeutic dose can produce allergy. Toxic dose of salicylates can produce alkalosis, tachypnea, followed by respiratory depression, acidosis, circulatory collapse, convulsion, coma and death. Aniline derivates are more toxic than the salicylates, especially on cats (3, 7).

Due to the fact that the NSAID have frequently effects at the level of the gastric or intestinal (duodenum and colon) mucosa, index like DU_{50} (ulceration dose 50%), DP_{50} (the dose that produces the perforation of the intestinal mucosa 50%), DE_{50} (pharmacologic efficient dose 50%) are utilized as therapeutic indicators and as assurance limit, when are compared with DL_{50} (8).

Table 1.

The classification of NSAI regarding their ulceration activity on laboratory rats

	The intensity of the ulceration activity	
Low	**Medium**	**Higher**
Azopropazone	Niflumic acid	Aspirin
Sulinda	Ibuprofen	Diclofenac
Fenclofenac	Salicylic acid	Indometacin
	Naproxe	Ketoprofen
	Phenylbutazone	
	Flurbiprofen	

The ulcerogenic potential of NSAID is increased by: concurrent corticosteroids, dehydration, hypovolaemic shock, disruption to normal gut blood flow (8).

Toxicity at the renal level

The chronic nephrotoxic effect of the NSAID is the inhibition result of the renal production of prostaglandin determined by the specific inhibitors of the COX enzymes. There is a new class of drugs, which inhibits selective the COX 2 enzymes (celecoxib, rofecoxib) leaving unmodified the action of the COX 1 enzyme. This feature is essential as the COX 1 enzymes have a cyto-protector effect on the functionality of gastrointestinal mucosa, platelets and renal cells.

Studies made on adult animals (5) showed that, on those that received a long term treatment with NSAID the risk to develop chronic renal insufficiency (CRI) is 2.5 times higher than on those animals on which were not utilized these drugs. Tubular nephritis can be noticed especially in the case of NSAID over dose (paraaminophen derivates: phenacetina, diclofenac) (6).

Some authors presented five examination modalities applied on laboratory animals with the purpose to observe the nephrotoxic potential of these substances:

1. Biochemical evaluation of the blood and urine (9):
   -urea and creatinine – in the case of tubular nephritis there are variations; sanguine lactate dehydrogenase and serumal pyruvic transpeptidase – high values in the first days of administration and slightly decreased or under normal values after the second week of treatment; urine test- on dogs, the NSAID (aspirin), in therapeutic dose induce the decrease of clearance of aminohippuric acid and a slight increase of sodium elimination; more noteworthy signs than gingival ulceration, coma, are registered only in the case of uraemic syndrome (6).
2. Renal biopsy- method utilized when it was administered a high dose of anti-inflammatory to the animal. Tubular nephritis can be associated to cylinder tubular formation, the necrosis of the tubular cells and interstitial inflammation. The regeneration of the tubular epithelium can take place in few days. The fibrosis which appears after necrosis can be seen in extreme cases such as glomerular anoxia and degeneration.
3. The weight of the kidney, taken at the necropsy.
4. Electronic macroscopic and microscopic examination of the renal tissue- the main signs noticed are tubular degeneration associated with tissual degeneration. In the case of chronic treatment interstitial fibrosis is noticed, and sometimes even irreversible glomerular degeneration.

CONCLUSIONS

1. Taking into consideration the latest information, each animal that needs long term treatment with NSAID has to be individually evaluated to prevent the risks that this treatment can have on their health. The choice of the drug is taking into account its efficiency, individual tolerance, the drugs from this group not having the effect of modifying the evolution of the disease but the suffering of the patient.
2. Gastrointestinal and renal toxicity induced by NSAID, that is variable in intensity from dyspepsia to bleedings and nephrotoxicity, are the most common adverse reaction of this drug group and that is why the introduction of new compounds or the introduction of NSAID into carrier type molecules- cyclodextrins, is needed, that have superior profile of gastrointestinal and renal safety, and this fact concerned the scientists and clinicians.
3. The treatment in NSAID intoxication has to avoid problems like: acido-basic equilibrium, dehydration, hypoglycemia, hypertermia, respiratory depression; has to reduce gastrointestinal absorption and to hurry the elimination of the implicated substance by forced diuresis.

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Anesthesia with Xylazine, Ketamine and Midazolam to geriatric dogs

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Xylazine is a very active agonist, efficient and selective at the level of the α_2-adrenergic receptors (from the central nervous system and the peripherical nervous system); it determines some undesirable effects such as: cardiovascular effects (bradycardia, arterial hypertension follow by hypotension and the decrease of the cardiac blood stream), vomiting, muscular, trembling, hypothermia, diminishing of the intestinal peristalsis.

To make possible the prevention of the effects Xylazine was associated with Ketamine and mostly with Midazolam - an imidazolic benzodiazepine -, that induces a proper cardiovascular stability, a rapid anaesthetic induction and an intense ataralgezic effect.

Key words: anesthesia, α_2-adrenergic receptors, geriatric dogs

Alfa-2 agonists stop the release of noradrenaline by linking to the sympathetic adrenoreceptors and block the nervous impulse (1).

MATERIAL AND METHOD

The searching was made on 17 common race geriatric dogs, of weight 10 to 20 kg. The administration was made intramuscular with: Xylazine, 5 mg/kg, Ketamine 10 mg/kg and Midazolam 0,6 mg/kg, as mixture in the same syringe.

It was noticed the time of installation of the anaesthetic effect, the proper time to do the operator act, the anaesthesis duration.

RESULTATS AND DISCUSSIONS

The dogs under anaesthesis were previously examined physical and physiologically. Then they were administrated intramuscular a mixture in the same syringe containing: Xylazine, solution 2% - 0,02 ml/kc: Ketamine solution 10% - 0,05 ml/kc and Midazolam, solution 0,5% - 0,125 ml/kc.

At 5 minutes from administration there were noticed the installation of a deep nervous depression, the animals quickly adopting lateral decubitus and presenting a total indifference to the surrounding noisy stimuli.

Monitorizing the parameters of the great function is displayed in the, table 1.

Breathing is rhythmic, with great amplitude and a slight tendency of reducing the frequency (12 breathings/minute) according as the profoundness of anaesthesis is settled.

Table 1

The monitorisation of the vital parameters in anesthesia with Xylazine, Ketamine and Midazolam to geriatric dogs

The anaesthesia phases	Time	Rectal t^0 (^{0}C)	Heart rate (beats/min)	Respiratory frequency (breaths/min)	Eyeball position	The pupil	Reflexes	Skeletal muscle tone	T.R.C.
Moment 00	0	38,8	88	22	variable	normal	normal	normal	normal
Xylazine Ketamine Midazolam	1	38,8	108	24	variable	normal	normal	normal	normal
Sedation	5	38,8	98	22	central	normal	diminished	diminished	normal
Analgesia and hypnotic sleep	10	38,8	80	20	medial rotation third eyeling evident	myosis	absent, excepting the patellar and palpebral strongly diminished	absent	normal
	15	38,7	80	20					normal
	30	38,4	76	18					normal
	45	38,1	74	16					normal
Awakening	60	38,3	118	18	variable	normal	present slightly diminished	diminished	normal
Recovery	100	38,7	148	20	variable	normal	exaggerated	normal	normal

It is registered a slight diminishing of the heart frequency maintaining in normal limits – 80 – 90 contractions/minute – and the increase of the arterial tension to 186/76 mm Hg during the incipient phase. After 7 minutes from administration in can be noticed a slight progressive diminution of the tension to the limit of 168/74 mm Hg. To be noticed this parameter maintaining in absolutely normal limits, with no rapid, abrupt variations putting in danger the animal's life. This slight variation of the arterial tension can be attributed to the antagonistic action of Xylazine over the α_2-adrenergic receptors, this action is diminished in intensity by Midazolam (a benzodiazepine acting as a strong cardiovascular stabilizer).

The eyeballs present a reduced medial rotation making evident a little the nictitante membrane. The pupil's diameter is little (strong – marked myosis); the pupilar reflex (response to light) is present all through the anaesthesis. It comes and the patellar ones, which are very diminished.

The superficial sensibility is absent, the spare muscles is relaxed (the muscular tone is very diminished) allowing the operator act to be done.

The limits of time for the capillary vases to be refilled (T.R.C.) are normally between 1 and 2 seconds. The oral and conjunctive mucous membranes are hyperemiated at the beginning, and then, after approximatively 40 minutes it can be registered a vague paleness.

The body's temperature varies on an average from 38,8^{0}C to 38,1^{0}C (the most minimal registered) and consequently, the dogs awakening is not associated with muscular trembling or even shiver frequently, in the simple anaesthesis with Ketamine.

The animals awakening was easy and fast, with no incidents. The complete recovering from anaesthesis follows after a medium while of 80 minutes according as the anesthesic agents are metabolized and eliminated by the organism.

CONCLUSIONS

1. This anaesthesic method is recommended in gender dogs anaesthesis for major surgical intervention lasting over 40 minutes.
2. The proper anaesthesic time is of 60 minutes.
3. Xylazine, Ketamine and Midazolam association provides for a good neurovegetative and neuropsychical protection; it does not need a previous premedication with Atropine.
4. Both the induction period and the awakening from anaesthesis are not associated with the apparent excitation phase or with other undesirable vagual phenomena.
5. It is provided for a good cardiovascular stability and also for the other vital parameters (breathing, temperature).
6. This anaesthetic combination offers the great advantage of being very cheap.

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Canine pregnancy diagnosis by fibrinogen and relaxin assay

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Unlike humans and horses that produce a pregnancy-specific gonadotropic hormone that can be quantitated by assay and thus utilized as an indicator of pregnancy, no similar such hormone has yet been found in the dog. Recently, however, a hormone, known as relaxin, which is secreted by the placenta of the dog and functions to relax the pelvis before whelping, has been evaluated as specific marker for canine pregnancy. The aim of this study was to evaluate two recent methods for pregnancy diagnosis in the bitch: measuring fibrinogen level and relaxin assay.

Key Words: dog, pregnancy diagnosis, fibrinogen and relaxin assay

The most methods, except ultrasound, used for pregnancy diagnosis in the bitch, don't offer enough information for a precise establishment of this diagnosis. An early, quick and accurate method is the request of the veterinarians and of the dog breeders as well.

Relaxin hormone is produced by the ovary and placenta of pregnant bitches. The placenta is known to be the primary site of secretion of relaxin in dog. A significant relaxin increase was found in pregnancy at day 24 after ovulation (3). In contrast, relaxin concentration are reported to be undetectable in nonpregnant and pseudopregnant dogs (2). Fibrinogen and serum acute phase proteins, i.e. C-reactive proteins are commonly elevated in pregnancy (1). Serum fibrinogen concentration rise >250mg/dl by day 21-30 of gestation (5).

Material and Methods

Eighteen bitches of several breeds were monitored during oestrus period by observation of sexual behaviour and genitalia, by cytovaginal examination (vaginal smears stained by Harris-Shorr and May Grunwald-Giemsa) to predicte the sexual stage, and by serum progesterone assay (DRG Progesterone ELISA) for detecting ovulation.The bitches were mated or artificial inseminated. Pregnancy diagnosis was performed on day 23-24 of gestation using three methods: measuring fibrinogen level, relaxin assay and ultrasonography.

Measuring fibrinogen level. Blood samples were collected in test tubes with sodium citrate (1 part sodium citrate solution 0.11 mol/L with 9 parts venous blood). Samples were centrifuged immediately at approximately 1500x g for 10 minutes. After supernatant plasma removal, the specimen may be used for the quantitative determination of fibrinogen in plasma. The fibrinogen measurements were made in the Bioclinica Laboratories from Timisoara using MultifibrenU technique (Dade Behring Inc. U.S.A.).

Relaxin assay. For relaxin detection it was used a commercial test kit "FASTest RELAXIN" (Mega Cor Dianostik, Austria). This is a rapid test based on rapid immunochromatography technique using two unique monoclonal antibodies. Blood samples were collected in test tubes with activator gel. After 15 minutes it was centrifuged and serum was used to detect the relaxin according to the instructions provided by the test-kit producer .

Ultrasonography. The bitches were imaged during pregnancy for an accurate identification of pregnancy status. Imaging was performed from the ventral midline using a 4.5MHz or a 7.5MHz sector transducer.

The results of the three methods of pregnancy diagnosis were correlated to the information provided by the breeders referring to parturition and prolificity.

Results

Twelve from the eighteen bitches monitorized were pregnant and gave birt. The results of the study for pregnancy diagnosis in the bitch are presented in table 1.

Table 1.

The results of oestral period monitorisation and pregnancy diagnosis tests (fibrinogen, relaxin and ultrasound)

No crt	Breed	Day of heat	Cytologic stage of sexual cycle	P4 ng/ml	M / AI (day)	Fibrinogen (g/l)	Re-laxin	Ultra-sound	Nr. of puppies
1	Husky	9 12 14 15	Pe Pe E E	- - - -	- - M M	2,52	+	+	9
2	Basset	3 4 8 9	Pe Pe E E	0,24 1,62 9,37 -	- - AI AI	2,43	+	+	8
3	Husky	5 7 11 12	Pe Pe E E	0,31 0,91 13,2 -	- - M M	4,66	+	+	9
4	Labrador Retriever	9 11 12 13	Pe E E E	- 25,4 - -	- M M M	1,55	-	+	-
5	Husky	7 13 15 17	Pe Pe E E	- - 14,3 -	- - AI AI	1,89	-	-	-
6	Schnautzer	7 10 12 14	Pe Pe E E	- 0,85 10,7 -	- - M M	2,35	+	+	7
7	Rottweiler	6 11 13 15	Pe Pe E E	1,72 2,13 5,85 91.7	- - M M	1,50	?	-	-
8	Cane Corso	8 10 13 14	Pe Pe E E	- - - -	- - AI AI	1,36	-	-	-
9	Dog Bordeaux	12 13	E E	- -	AI AI	2,85	+	+	13
10	Husky	8 11 12	Pe E E	1,56 - -	- M M	2,18	+	+	7
11	Belgian Sheepdog	8 10 12 13	Pe Pe E E	4,98 14,6 - -	- - M M	2,15 3,2	+	+	12

Table 1(continued)

No crt	Name/ breed	Day of heat	Cytologic stage of sexual cycle	P4 ng/ml	M / AI (day)	Fibrino-gen (g/l)	Re-laxin	Ultra-sound	Nr. of puppies
12	Mops	9	Pe	14,9	-				
		10	E	-	M				
		11	E	-	M	1,23	-	-	-
		12	E	-	M				
13	Labrador	10	Pe	10,7	-				
		14	E	-	AI	1,23			
		16	E	-	AI		-	-	-
		17	E	-	AI	1,22			
14	Doberman	9	E	13,5	M				
		10	E	-	M	2,43	+	+	11
		11	E	-	M				
15	Husky	10	Pe	3,3	-				
		11	Pe	-	-				
		13	E	19,2	M	2,97	+	+	6
		14	E	-	M				
16	German Sheepdog	8	Pe	1,7	-				
		11	Pe	-	-				
		12	E	11,2	M	2,13	+	+	7
		13	E	-	M				
17	Rottweiler	9	Pe	1,7	-				
		11	E	11,2	M				
		12	E	-	M	2,17	+	+	6
		13	E	-	M				
18	Rotweiler	10	E	-	M				
		11	E	-	M	1,60	+	+	5
		12	E	-	M				

Legend:

Pe-proestrus; E-oestrus; M-mount; AI-artificial insemination; + positive results relaxin ; - negative results relaxin; + positive ultrasonography results; - negative ultrasonography results; - no analysis; ? - uncertain result.

Fibrinogen level measurement in the eighteen bitches showed the following results:

- to 11 bitches fibrinogen concentrations ranged from 2.13 to 4.66 g/l, these females being proved pregnant by the other two methods too (relaxin and ultrasound). The results have also been confirmed by parturition;

- to 7 bitches fibrinogen concentration value ranged from 1.22 to 1.89 g/l, these females being proved nonpregant by the other two methods.

Unconncordance among results was noticed in case number 18 from table 1 which registered a fibrinogen value of 1.60 g/l –value under positive level of pregnancy but ultrasonography and relaxin test indicated the presence of pregnancy. This result may show the absence of acute phase reaction of female organism correlated to pregnancy, phenomenon presented by Gentry and Liptrap (4), or a technical error of fibrinogen assay. Taking into account that the female is at the fourth pregnancy (with no problems at anterior gestations), perhaps the female's organism wouldn't respond by inflammatory reaction post implantation. This situation remains an unanswered question that must be solved in further studies.

Interpretation of test- kit "FASTest RELAXIN" showed twelve positive results (two pink-purple coloured line), five negative results (only one pink-purple line, in the control window) and one invalid result because there are no coloured line neither in the test window nor in the control

window (only an irregular pink band appeared on the entire surface). There were not registered any false positive or false negative results.

Conclusions

The results of the study reported here proved that fibrinogen and relaxin assay may be a reliable marker for pregnancy. For veterinary practice, we recommend both tests, either alone or together, especially when ultrasonography is not available.

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Studies about the synergic effects of enrofloxacin-gentamicin, enrofloxacin-amoxicillin, amoxicillin-gentamicin and sulphamethoxidiazine-tylosin combinations on *Listeria strains*

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Combinations of two antibiotics were accomplished in order to observe the synergic or additive effect on Listeria strains. A comparative study of the activity of some antibiotics used alone and in combination was achieved through dilution method and minimum inhibitory concentrations was determined for each antibiotic and for the combinations. The results were interpreted according to the National Committee for Clinical Laboratory Standards.

Amoxicillin, gentamicin, enrofloxacin, suphamethoxidiazine and tylosin and associations: enrofloxacin-gentamicin, enrofloxacin-amoxicillin, amoxicillin-gentamicin and sulphamethoxidiazine-tylosin were tested on 28 bacterial strains.

The fractional inhibitory concentration index was calculated and showed that the phenomenon of synergism appeared in a high percentage – 71,43 % - when enrofloxacin-amoxicillin and amoxicillin-gentamicin combinations were used. The enrofloxacin-gentamicin combination showed synergism only in 57,14 % of cases, in 28,57 % of strains being observed the additive phenomenon and in 14,29 % the indifference between the two antibiotics. There was no synergism in the case of sulphamethoxidiazine-tylosin combination, only addition in 28,57 % strains and for 71,43 % of the strains was noted indifference between the associated antibiotics.

Key Words: Listeria strains, synergic effects of enrofloxacin-gentamicin, enrofloxacin, amoxicillin, gentamicin, sulphamethoxidiazine-tylosin combinations

Prudent use of antibiotics is an integral part of good veterinary practices. It is very important to maximize therapeutic efficacy and minimize selection of resistant micro-organisms, but it is difficult to have guidelines that could be applied universally. These general principles should not be interpreted so restrictively as to replace professional judgement of practitioners or to compromise animal health or welfare. In all cases, animals should receive a prompt and effective treatment as it is necessary according to every particular situation (FVE, 1999).

Whenever an animal or human is exposed to antibiotics, there will be some degree of selection for resistant bacterial population (SAMMARCO M.L., 2005). Therefore, it is vital to limit therapeutic antibiotic use to those situations where they are needed. The developing of resistance can be minimalized by some measures that are meant to extend the use of antibiotics, both in human or veterinary medicine. One of these measures is the association of antibiotics in therapy (RUBINSTEIN E., 1999).

In this study, the combinations of two antibiotics was accomplished in order to observe the synergic or additive effect on *Listeria* strains and afterwards to recommend these associations, for a better therapeutical efficiency.

Materials and methods

The *Listeria* strains used in this study were isolated from bovine, ovine and swine, the samples being represented by nervous system, abortions, corpses, also conjunctivae and mastitic secretions.

Bacterioscopic and bacteriologic exam were carried out and isolation and identification methods were applied; usual medium, sometimes enriched with serum or blood, were used (RĂPUNTEAN GH. and BOLDIZSAR E., 2001).

Antimicrobial susceptibility testing was carried out by broth dilution method (BOLDIZSAR E. and all, 2002).

Antimicrobial agents from de groups of aminopenicillins (amoxicillin), aminoglycosides (gentamicin), fluoroquinolones (enrofloxacin), macrolides (tylosin) and sulfonamides (sulphamethoxidiazine) were tested, alone and in combination of two antibiotics.

The stock solutions were prepared in sterile distilled water. Serial dilution, begining with 5 µg in enrofloxacin (ENR), 20 µg in amoxicillin (AMX), 10 µg in gentamicin (G), 240 µg in sulphamethoxidiazine (S) and 150 µg in tylosin (T) were performed in 15 tubes for each antibiotic. Every tube contained 1 ml broth in a twofold dilution, comparing with the proximate tubes. One more tube contained no antibiotic and served as a control.

In order to determine the synergism between antibiotics, for each combination of two antibiotics were prepared 15 more tubes of dilutions, by using ½ of the amount of antibiotics, as following: enrofloxacin-gentamicin (the first tube = 2,5 µg ENR + 5 µg G), enrofloxacin-amoxicillin (the first tube = 2,5 µg ENR + 10 µg AMX), amoxicillin-gentamicin (the first tube = 10 µg AMX + 5 µg G) and sulphamethxidiazine-tylosin (the first tube = 120 µg S + 75 µg T).

The bacterial strain grew on broth at 37°C for 18-24 hours and the inoculum was placed in each of the tubes. The minimal inhibitory concentration (MIC) was determined after aerobic incubation at 37°C for 18-24 hours and was considered the lowest antimicrobial concentration that produced no visible bacterial growth (NCCLS, M31-A, 1999).

Fractional inhibitory concentration indices (FIC) were calculated for all isolates with all combinations (BOUANCHAUD D.H., 1992).

Results and discussions

This paper tested the efficacy of amoxicillin, gentamicin, enrofloxacin, suphamethoxidiazine and tylosin, and associations of enrofloxacin-gentamicin, enrofloxacin-amoxicillin, amoxicillin-gentamicin and sulphamethoxidiazine-tylosin on 28 bacterial strains from *Listeria* genus.

The MIC's of the tested antibiotics were determined and interpreted according to The National Committee for Clinical Laboratory Standards (NCCLS, M31-T, 1999) (table 1):

Table 1

MIC values according to international standards

Antibiotic	MIC values (µg/ml)			Standards for dilution susceptibility tests
	S	I	R	
Amoxicillin	≤ 2		>16	Neo-Sensitab Dutch CRG
	≤ 4		>16	Neo-Sensitab „Comité de l'Antibiogramme de la Societé Francaise de Microbiologie"
Gentamicin	≤ 4		≥ 8	Neo-Sensitab Swedish Reference Group of Antibiotics
	≤ 1		> 4	Neo-Sensitab Dutch CRG
	≤ 2		≥ 8	Neo-Sensitab Norwegian AFA Group
	≤ 4	8	≥16	Oxoid Manual Veterinary Pathogens – NCCLS
Enrofloxacin				Neo-Sensitab – NCCLS
	≤ 1		≥4	Neo-Sensitab Veterinary Pathogens – NCCLS
	≤ 0,5/0,25		4/2	Oxoid Manual Veterinary Pathogens – Canine and feline (gram-negative pathogens, *Staphylococcus* spp and other susceptible micro-organisms)
	≤ 0,5	1-2	≥ 4	
Sulfonamide	≤ 32		> 64	Neo-Sensitab Dutch CRG
	≤ 256		≥ 512	Oxoid Manual Veterinary Pathogens – NCCLS
	≤ 16		≥ 256	Neo-Sensitab Norwegian AFA Group
Tylosin	≤ 1		> 2	Neo-Sensitab Dutch CRG
	≤ 0,5		≥ 8	Neo-Sensitab Veterinary Pathogens – NCCLS
	≤ 1		≥ 4	Neo-Sensitab Norwegian AFA Group
	≤ 0,5	1-4	≥ 8	Oxoid Manual Veterinary Pathogens – NCCLS

Synergy studies are interpreted by calculating the fractional inhibitory concentration (FIC) which compares the activity of an agent in combination with the activity of the antibiotic alone according to CF Referral Center for Susceptibility & Synergy Studies (SAIMAN LISA and all, 2003).

The interpretations of the FIC's values are presented in the table 2:

Table 2

Interpretation of the FIC's value

FIC value	Interpretation
≤ 0,5	**Synergistic**
> 0,5 – 1,0	**Additive**
> 1,0 - ≤ 4	**Indifferent**
> 4,0	**Antagonistic or not clinically achievable**

Enrofloxacin-gentamicin combination was synergic in 4 strains from the totality of 7 tested with this combination, meaning 57,14 % of the *Listeria* isolates, 2 strains had values that were interpreted as additive – 28,57 % and only one strain showed indifference between these two antibiotics – 14,29 % (table 3).

FIC values for enrofloxacin-gentamicin combination – Listeria strains

Table 3

Strain no.	**FIC**	**Interpretation**
1	1,5	Indifference
2	0,4999993	Synergism
3	0,3749993	Synergism
4	1	Addition
5	0,2499992	Synergism
6	0,7499987	Addition
7	0,1874994	Synergism

Enrofloxacin-amoxicillin combination presented synergism in a higher percentage – 71,43 % (5 strains), the other two strains being additive – one having FIC at the borderline (1), phenomenon representing a percentage of 28,57 % (table 4).

Table 4

FIC values for enrofloxacin-amoxicillin combination – Listeria strains

Strain no.	**FIC**	**Interpretation**
1	0,3749993	Synergism
2	0,2499996	Synergism
3	1	Addition
4	0,7499987	Addition
5	0,3749993	Synergism
6	0,4999993	Synergism
7	0,3749996	Synergism

Amoxicillin-gentamicin combination had synergic effect also for 71,43 % of the *Listeria* strains, notifying the fact that we calculated low values for three of the strains – 0,1874998, 0,2499996 and 0,25 respectively. FIC in one strain was at the borderline (0,5), this value also being interpreted as synergism and in two strains was noted the addition, a 28,57 % percentage (table 5).

Table 5

FIC values for amoxicillin-gentamicin combination – Listeria strains

Strain no.	FIC	Interpretation
1	0,5	Synergism
2	0,75	Addition
3	0,3749996	Synergism
4	1	Addition
5	0,2499996	Synergism
6	0,25	Synergism
7	0,1874998	Synergism

The phenomenon of synergism was not evident in sulphamethoxidiazine-tylosin combination. These two antibiotic were indifferent in 5 strains and additive in 2 strains, meaning a 71,43 % percentage and 28,57 %, respectively (table 6).

Table 6

FIC values for sulphamethoxidiazine-tylosin combination – Listeria strains

Strain no.	FIC	Interpretation
1	1,25	Indifference
2	0,6249998	Addition
3	1,0625	Indifference
4	1,0625	Indifference
5	0,5624998	Addition
6	1,0625	Indifference
7	1,03125	Indifference

Combinations of penicillins and aminoglycosides were tested by FANTIN B. and CARBON C., in 1992, on some *Listeria monocytogenes* strains, and the results obtained were better than when antibiotics were used alone; the ampicillin-gentamicin combination was more efficient in comparison with penicillin-gentamicin, in their studies.

ORSINI J.A. and all (2004) recommend, also, the association between penicillin-aminoglycosides and the association ampicillin-aminoglycosides, in infections induced by gram-positive pathogens.

On the basis of *in vitro* studies, HERBERT HOF (2003) asserted that a combination between amoxicillin and gentamicin is the best option for treating listeriosis, and POROS G.J. and MARKIEWICZ Z. (2003) recommend the association of amoxicillin with aminoglycosides, especially gentamicin, for a higher efficiency on *Listeria monocytogenes* strains.

Therefore, the recommendation that come off from this research is to use combination of antibiotics when necessary, because of the higher efficacy together, much more than alone, in the therapy.

Conclusions

- Combinations of two antibiotics were accomplished in order to observe the synergic or additive effect on *Listeria* strains
- A comparative study of the activity of some antibiotics used alone and in combination was achieved through dilution method and minimum inhibitory concentrations was determined for each antibiotic and for the combinations, the results being interpreted according to the National Committee for Clinical Laboratory Standards
- Amoxicillin, gentamicin, enrofloxacin, suphamethoxidiazine and tylosin and associations: enrofloxacin-gentamicin, enrofloxacin-amoxicillin, amoxicillin-gentamicin and sulphamethoxidiazine-tylosin were tested on 28 bacterial strains
- The fractional inhibitory concentration index was calculated and showed that the phenomenon of synergism appeared in a high percentage – 71,43 % – when enrofloxacin-amoxicillin and amoxicillin-gentamicin combinations were used
- The enrofloxacin-gentamicin combination showed synergism only in 57,14 % of cases, in 28,57 % of strains being observed the additive phenomenon and in 14,29 % the indifference between the two antibiotics
- There was no synergism in the case of sulphamethoxidiazine-tylosin combination, only addition in 28,57 % strains and for 71,43 % of the strains was noted indifference between the associated antibiotics
- Therefore, the recommendation that come off from this research is to use combination of antibiotics when necessary, because of the higher efficacy together, much more than alone, in the therapy

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The antibiotic resistance phenomenon in some *Morganella* strains, isolated from animals

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Antibiotics from the groups of aminopenicillins, cephalosporins, aminoglycosides, polymyxins, fluoroquinolones, tetracyclines, phenicols and potentiated sufonamides were tested on 19 bacterial strains from the genus Morganella.

The antibiotic resistance phenomenon was noticed for 8 of the 14 tested antibiotics: ampicillin, amoxicillin, gentamicin, trimethoprim/sulphamethoxazole, colistin, tetracycline – 100 %, cephalosporins, neomycin – 50 %.

The most efficient antibiotics were flumequine and cloramphenicol – 100 %, followed by cephalosporins and neomycin – 50 % and in a lower percentage kanamycin – 33,33 %.

There were registered intermediary strains for spectinomycin and enrofloxacin – 100 %, but also for kanamycin – 66,66 %.

Therefore, this study present the susceptibility to antibiotics of the strains belonging to the genus Morganella, in order to help veterinarians to make a better prescription of the drugs.

Key Words: Morganella, antibiotic resistance

The genus *Morganella* was detached from the genus *Proteus* and was made up of one single species (CHOI J.H. and all, 2002). It is a Gram-negative bacillus that has two subspecies: *Morganella morganii morganii* and *Morganella morganii sibonii* (JENSEN K.T. and all, 1992).

MOHR O'HARA CAROLINE and all (2000) assert that, even if all of these microorganisms are ubiquitous in the environment, individual case report indicate that they are capable of causing major infectious disease problems, as digestive disorders, extraintestinale and urinary infections, even osteomyelitis (SCOTT J. STAHL, 2006). So, the pathogenity of these bacteria must be reconsidered (RĂPUNTEAN GH, RĂPUNTEAN S., 2005).

PIGANTO S. and all (1999) classifies *Morganella morganii* as an opportunistic pathogen known to cause infection in humans and animals. Lastly, anticipated antimicrobial susceptibility must be taken into account.

Many of these organisms are easily controlled, but some problems have arisen because of the selection of resistant forms of the bacteria. Therefore, the aim of this study was to present the susceptibility to antibiotics of the strains belonging to the genus *Morganella*, in order to help veterinarians to make a better prescription of the drugs.

Materials and methods

Culture and sensitivity can be utilized to identify the bacterial organisms involved in infections and to determine the most appropriate antimicrobial for treatment. The cultivation was effected on usual and selective media – an example is enteric-agar CMV, obtained by Marica and Răpuntean (RĂPUNTEAN GH., RĂPUNTEAN S., 1999); the biochemical methods were made in order to characterize the strains (MOHR O'HARA CAROLINE and all, 2000).

Most clinical strains were from cats – tears secretion and pus from claws, also from young poultry and snakes – diarrheal faeces and corpses.

The susceptibility of bacteria to antimicrobial agents was effected through agar disk diffusion method that used agar medium and antibiotic discs. The method allowed free diffusion of antimicrobial substances onto inculated agar and the zone of bacterial inhibition around the disk was measured, after 24 hours of incubation at 37 °C (BOLDIZSAR and all, 2002).

Fourteen antibiotics from the groups of aminopenicillins, cephalosporins, aminoglycosides, polymyxins, tetracyclines, phenicols, fluoroquinolones and potentiated sufonamides were tested on 19 strains from the genus *Morganella.*

Results and discussions

The microorganisms were registered as susceptible, intermediate or resistant to a specific antibiotic, based on the size of inhibition area around the disc, in the antimicrobial sensitivity tests. The results were compared to the data offered by the National Committee for Clinical Laboratory Standards (NCCLS, 1999) presented below (table 1):

Table 1

Zone diameter interpretive standards for veterinary pathogens

No.	Antibiotic	Antib. conc./ disc	Diameter of the zone (mm)		
			Resistant	*Intermediate*	*Susceptible*
1	Ampicillin	10 µg	≤ 13	14 – 16	≥ 23
2	Amoxicillin	20 µg	≤ 16	17 – 22	≥ 23
3	Ceftazidime	30 µg	≤ 16	17 – 19	≥ 20
4	Ceftiofur	30 µg	≤ 19	20 – 22	≥ 23
5	Kanamicin	100 µg	≤ 19	20 – 22	≥ 23
6	Neomycin	30 µg	≤ 12	13 – 16	≥ 17
7	Gentamicin	40 µg	≤ 19	20 – 22	≥ 23
8	Spectinomycin	200 µg	≤ 16	17 – 19	≥ 20
9	Colistin	150 µg	≤ 16	17 – 19	≥ 20
10	Tetracycline	30 µg	≤ 14	15 – 18	≥ 19
11	Chloramphenicol	60 µg	< 22	22 – 25	≥ 26
12	Flumequine	30 µg	≤ 16	17 – 19	≥ 20
13	Enrofloxacin	10 µg	≤ 16	17 – 22	≥ 23
14	Trimethoprim/ Sulfamethoxazol	1,25/ 23,75µg	≤ 10	11 – 15	≥ 16

Aminopenicillins – ampicillin and amoxicillin – were tested on the genus *Morganella* and it was noticed that all the strains were resistant.

From the cephalosporins group we tested ceftazidime and ceftiofur, cephalosporins from the third generation, which showed efficiency for 50 % of the bacterial strains, 50 % being resistant to these antibiotics.

The phenomenon of antibiotic resistance appeared in 100 % percentage in the case of gentamicin; other antibiotics in the same group (aminoglycosides), determined a different behaviour of the *Morganella* strains in the presence of antimicrobials: neomycin – 50 % resistant

strains and 50 % susceptible strains, kanamycin – 66,66 % intermediary strains and 33,33 % susceptible strains, spectinomycin – 100 % intermediary strains and 0 % susceptible strains.

The tetracycline, colistin and the combination between trimethoprim and sulfamethoxazole were not efficient at all, determining 100 % resistance in the tested strains; also enrofloxacin determined 100 % intermediary value and no susceptibility in *Morganella* strains.

Although in the same group with enrofloxacin – fluoroquinolones – flumequine was 100 % efficient, as was also the antibiotic from the group of phenicols – cloramphenicol (table 2).

Table 2

Antimicrobial susceptibility of Morganella spp., isolated from animals

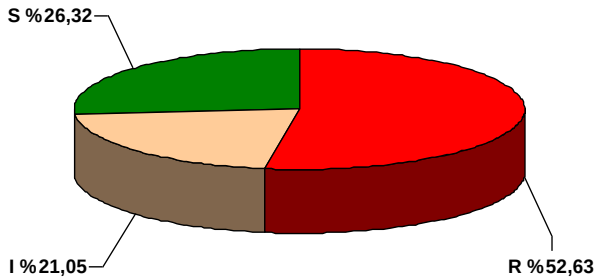
Crt. No.	Antibiotic	No. strains	Susceptibility/resistance					
			R	%	I	%	S	%
1	Ampicillin	2	2	100	-	-	-	-
2	Amoxicillin	1	1	100	-	-	-	-
3	Cephalosporins	2	1	50	-	-	1	50
4	Kanamicin	3	-	-	2	66,66	1	33,33
5	Neomycin	2	1	50	-	-	1	50
6	Gentamicin	1	1	100	-	-	-	-
7	Spectinomycin	1	-	-	1	100	-	-
8	Colistin	1	1	100	-	-	-	-
9	Tetracycline	1	1	100	-	-	-	-
10	Chloramphenicol	1	-	-	-	-	1	100
11	Flumequine	1	-	-	-	-	1	100
12	Enrofloxacin	1	-	-	1	100	-	-
13	Trimethoprim/sulfamethoxazole	2	2	100	-	-	-	-
Total No.		**19**	**10**	**52,63**	**4**	**21,05**	**5**	**26,32**

Like the results in this study, BARROSO H. and all (1999) noticed resistance to ampicilin, amoxicillin, ceftazidime and gentamicin in strains that came from a hospital unit in Lisbon, from children with urinary infections.

Resistance of 100 % to ampicillin and cefuroxim found also TIMKO and KMET, in 2003, when testing the *Morganella morganii* strains isolated from alpine accentor *Prunella collaris*.

In comparison with the present research, GONI-URRIZA MARISOL and all (2000), observed resistance only of 24,3 % to tetracycline and 20,5 % to betalactam antibiotics, lower values than ours, in *Enterobactaeriaceae* strains isolated from Arga river, in Spain, bacteria that came from humans and animals.

From a general point of view, 10 of the 19 *Morganella* strains were resistant – 52,63 %, 4 were with intermediary values – 21,05 %, and 5 were susceptible for the totality of the tested antimicrobials – 26, 32 % (graphic 1).



Graphic 1. Antibiotic resistance in Morganella genus strains

Therefore, the results from the antibiograms give the possibility of prescribing an appropriate antibiotic for the isolated strains, also in the genus *Morganella*, so that the therapy can be succesfull, being of a real help to the veterinarians.

Conclusions

- The testing of 19 bacterial pathogens from the genus *Morganella* used agar disc diffusion method with discs of antibiotics from the groups of aminopenicillins, cephalosporins, aminoglycosides, fluoroquinolones, polymyxins, tetracyclines, phenicols and potentiated sufonamides
- The phenomenon of antibiotic resistance was registered in a 100 % percentage for trimethoprim/sulphamethoxazole, tetracycline, colistin, ampicillin, amoxicillin and gentamicin
- Cephalosporins and neomycin determined 50 % of resistance in the tested strains
- The most efficient antibiotics were flumequine and cloramphenicol – 100 %, followed by cephalosporins and neomycin – 50 %, and in a lower percentage kanamycin – 33,33 %
- There were registered intermediary strains for spectinomycin and enrofloxacin – 100 %, but also for kanamycin – 66,66 %
- The general recommendation is to prescribe an antimicrobial only after the testing of the susceptibility of the identified bacteria, for a better efficiency and to avoid the phenomenon of resistance to antibiotics

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The evolution of antibiotic resistance, in a five-year period, in *Clostridium* strains isolated from dogs

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Antibiotics from the groups of penicillins, cephalosporins, macrolides, tetracyclines, phenicols, fluoroquinolones and potentiated sufonamides were tested on 34 bacterial strains from the genus Clostridium, isolated from dogs diagnosed with enteritis.

The phenomenon of antibiotic resistance had been observed for all antibiotics that were used in antibiograms. The highest percentage of resistance was registered for ampicillin and trimethoprim/sulphamethoxazole – 100 %, followed by penicillin, tetracycline, cloramphenicol – 66,66 %. Although amoxicillin and cephalosporins had had a low percentage of resistance in bacterial strains (33,33 %), these antibiotics presented also intermediary values of the inhibition areas – 33,33 %. The strains showed no sensibility to penicillin and erythromycin, noticing in addition to the resistant strains, also a pretty high number of intermediary strains (erythromycin - 50 %, penicillin - 33,33 %).

During 2001-1005 period, it can be remarked the increasing of antibiotic resistance in Clostridium genus strains: 40 % - 2001, 37,5 % - 2002, 71,42 % - 2003, 63,63 % - 2004, reaching to the value of 100 % in 2005.

Key Words: Clostridium, antibiotic resistance, dog

Antibiotics represent the most important therapeutic arsenal in the fight against pathogen microorganisms. Even in the beggining of their use, there was registered bacterial resistance, phenomenon that became an alarming subject in the last decades (POTTER R. and all, 2002).

The extensive use of antibiotics both in medicine, human or veterinary, and agriculture, bad prescriptions of antimicrobials, non-observance of elementary rules of precaution and hygiene, led and are leading to the selection of resistant forms of bacteria (FVE, 2005).

One of the most common infections seen in many veterinary hospitals is diarrhea from bacteria of *Clostridium* genus (RILEY T.V., J.E. and all, 1991; STRUBLE A.L. and all, 1994). The disease can be acute or it can also be chronic (long term) or recurrent, depending on the particular strain of bacteria the pet has (CAVE N.J. and all, 2002). Opportunistic enteropathogens from *Clostridium* genus may cause diarrhea as a consequence of enterotoxin production under certain environmental conditions, or because of the imbalances that can occur within the normal flora (HALL J. EDWARD, 2004).

The infection caused by *Clostridium* is easily treated with a course of antibiotics and several medications are effective (STARR JOHN, 2005). Nevertheless, it is a necessity the determination of antibiotic resistance for this pathogen, mainly because of the fact that enteric bacteria are those who select more frequently, achieve and spread resistance in bacterial populations (BARBUT F. & J.C. PETIT, 2001). Moreover, there is the concern of transmitting the resistance to humans (BORRIELLO S. P., 1983; O'NEILL G. and all, 1993).

Therefore, the aim of this study was to present the evolution of antibiotic resistance, over the time, in *Clostridium* genus strains obtained from dogs with diarrhea, for a permanent monitoring of this worrying phenomenon.

Materials and methods

The samples examined in this study came from faeces of dogs having diarrhea. The identification of the bacterial genus was made using usual culture medium for anaerobes or sometimes special media, through bacterioscopic and bacteriologic exam, including the testing of biochemical properties.

The *in vitro* susceptibility of bacteria to antimicrobial agents was checked up through agar disc diffusion method. Agar medium and antibiotic discs were used as materials for this method (RĂPUNTEAN GH., E. BOLDIZSAR, 2001).

The susceptibility of the 34 *Clostridium* strains was investigated with antibiotics from the groups of penicillins, cephalosporins, fluoroquinolones, macrolides, tetracyclines, phenicols and also potentiated sulfonamides.

The bacterial inhibition area around the standard antibiotic disc was measured and interpreted after 24 hours of incubation at 37 °C, after the pathogen was disposed on agar plates (BOLDIZSAR E. and all, 2002).

Results and discussions

The bacteria identified as pathogens from the genus *Clostridium* were interpreted as susceptible, intermediate or resistant to a specific antibiotic after the measuring of the inhibition area around the standardized discs, taking into account the National Committee for Clinical Laboratory Standards (NCCLS, 1999) that are presented below (table 1):

Table 1

Zone diameter interpretive standards for veterinary pathogens

Crt. No.	Antibiotic	Antib. conc./ disc	Diameter of the zone (mm)		
			Resistant	*Intermediate*	*Susceptible*
1	Penicillin	5 µg	< 10	10 – 27	≥ 28
2	Ampicillin	10 µg	≤ 13	14 – 16	≥ 23
3	Amoxicillin	20 µg	≤ 16	17 – 22	≥ 23
4	Cephalotin	30 µg	≤ 14	15 – 17	≥ 18
5	Cefuroxime	60 µg	≤ 19	20 – 22	≥ 23
6	Erythromycin	15µg	≤ 13	14 – 22	≥ 23
7	Tetracycline	30 µg	≤ 14	15 – 18	≥ 19
8	Chloramphenicol	60 µg	< 22	22 – 25	≥ 26
9	Flumequine	30 µg	≤ 16	17 – 19	≥ 20
10	Trimethoprim/ Sulfamethoxazole	1,25/ 23,75µg	≤ 10	11 – 15	≥ 16

The results showed that, from the group of penicillins, ampicillin induced resistance in all tested strains, but in the case of penicillin was noticed the fact that there were no susceptible strains to this antibiotic, 66,66 % being resistant and 33,33 % with intermediary values. Even in the same group of antibiotics, there were observed susceptible strains to amoxicillin – 33,33 %, the same percentage being registered as intermediate and resistant strains (table 2).

Cephalosporins, from the group of betalactamins, presented the same percentage – 33,33 %, of resistant, intermediate or sensitive strains as amoxicillin.

PAPICH MARK (2001) revealed good therapeutic results after the administration of penicillin, cloramphenicol, 2nd generation of cephalosporins and other antibiotics in *Clostridium* genus

pathogens, while the activity of the 1st generation of cephalosporins, potentiated sulfonamides and fluoroquinolones could not be predicted in an anaerobic infection.

However, in this study, penicillin, like erythromycin, were not efficient (resistance – 66,66 % and 50 % respectively; intermediary values – 33,33 % and 50 % respectively); cloramphenicol, tetracycline, amoxicillin and cephalosporins determined a low percentage of susceptible strains (33,33 %); potentiated sulfonamide was not efficient at all and for the antibiotic flumequine 50 % of the strains were sensible, 25 % intermediate and 25 % resistant.

The susceptibility of the tested strains was relatively low, observed only in five groups of antibiotics: flumequine – 50 %, amoxicillin, cephalosporins, tetracycline, cloramphenicol – 33,33 % (table 2).

The bacteria in the *Clostridium* genus had a lower percentage of sensibility to antibiotics – 20,59 %, being registered a higher number of resistant and intermediate strains – 79,41 % (58,82 % resistant and 20,59 % intermediate).

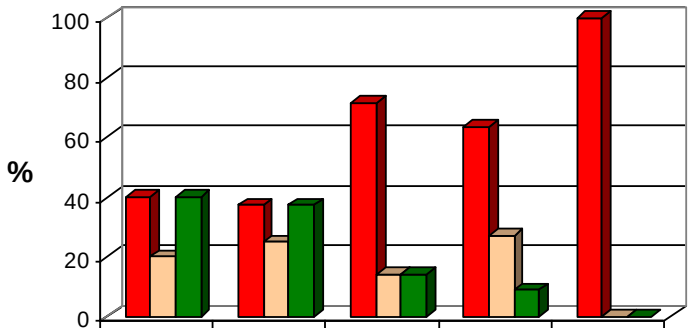
Analyzing the data concerning the totality of used antibiotics, 20 strains of 34 tested were resistant (58,82 %), 7 (20,59 %) were intermediary strains and 7 (20,59 %) were susceptible strains to antimicrobials, in this study.

Table 2

Antimicrobial susceptibility of Clostridium strains

Crt. No.	Antibiotic	No. strains	Susceptibility/Resistance					
			R	%	I	%	S	%
1	Penicillin	3	2	66,66	1	33,33	-	-
2	Ampicillin	4	4	100	-	-	-	-
3	Amoxicillin	6	2	33,33	2	33,33	2	33,33
4	Cephalosporins	3	1	33,33	1	33,33	1	33,33
5	Erythromycin	4	2	50	2	50	-	-
6	Tetracycline	3	2	66,66	-	-	1	33,33
7	Chloramphenicol	3	2	66,66	-	-	1	33,33
8	Flumequine	4	1	25	1	25	2	50
9	Trimethoprim/Sulfamethoxazole	4	4	100	-	-	-	-
Total No.		**34**	**20**	**58,82**	**7**	**20,59**	**7**	**20,59**

Between 2001-2005 were investigated a number of 34 bacterial strains – 11 strains in 2001, 8 strains in 2002, 3 strains in 2003, 5 strains in 2004 and 7 strains in 2005. Because of the intermediary strains noted in 4 of the 5 years taking into account, the susceptibility to antibiotics was as follows: 40 % – 2001 (intermediary strains - 20 %), 37,5 % – 2002 (intermediary strains - 25 %), 14,29 % – 2003 (intermediary strains – 14,29 %), 9,1 % – 2004 (intermediary strains - 20 %), 0 % – 2005 (intermediary strains - 0 %) (graphic 1).



	2001	2002	2003	2004	2005
R %	40	37,5	71,42	63,63	100
I %	20	25	14,29	27,27	0
S %	40	37,5	14,29	9,1	0

Graphic 1. The evolution of antibiotic resistance in Clostridium genus, in 2001-2005 period

During 2001-1005 period, it can be remarked that, even in the first and second year of the study the resistance to antibiotics had close values (40 % - 2001, 37,5 % - 2002), beginning with 2003, the resistance rose pretty much (71,42 % - 2003, 63,63 % - 2004), reaching the value of 100 % in 2005.

Conclusions

- Antibiotics from the groups of penicillins, cephalosporins, macrolides, tetracyclines, phenicols, fluoroquinolones and potentiated sufonamides were tested on 34 bacterial strains from *Clostridium* genus, isolated from dogs diagnosed with enteritis.
- Antimicrobial agar disc diffusion method was used for the testing of susceptibility of bacterial pathogens to antibiotics
- The phenomenon of antibiotic resistance was registered for all antibiotics that were used in antibiograms
- The highest percentage of resistance was registered to ampicillin and trimethoprim/sulphamethoxazole – 100 %, followed by penicillin, tetracycline, cloramphenicol – 66,66 %
- Amoxicillin and cephalosporins had a low percentage of resistance in bacterial strains - 33,33 %, these antibiotics presenting also intermediary values of the inhibition areas – 33,33 %
- The strains showed no sensibility to penicillin and erythromycin, noticing in addition to the resistant strains, also a pretty high number of intermediary strains: erythromycin - 50 %, penicillin - 33,33 %
- In 2001-1005 period, it can be remarked the increasing of antibiotic resistance in *Clostridium* genus strains: 40 % - 2001, 37,5 % - 2002, 71,42 % - 2003, 63,63 % - 2004, reaching to the value of 100 % in 2005

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The dynamics of antibiotic resistance in *Streptococcus* genus, in 2001-2005 period

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In 2001-2005 period there were investigated about antimicrobial susceptibility a number of 172 strains from Streptococcus genus with 14 antibiotics from different groups. The data obtained were analyzed for each antibiotic and also in a global manner, observing the evolution of resistance over time.

The antibiotic resistance phenomenon was noticed for all strains of streptococci. The highest percentage of resistance – 92,31 % – appeared for cephalosporins . Penicillin was not efficient at all, because 81,25 % of the strains were resistant, 18,75 % had intermediary values of the inhibition zones and none of the strains presented sensibility to this antibiotic. A pretty high percentage of resistance was observed also for trimethoprim/sulphamethoxazole (71,42 %), ampicillin (66,66 %), spectinomycin (66,66 %), erythromycin (65,22 %), tetracycline (64,29 %) and amoxicillin (60 %).

Analyzing the data in a global manner it was remarked a 62,79 % percentage of resistance to antibiotics, a 12,21 % percentage of intermediary values of the inhibition areas and only 25 % percentage of sensitive strains to antibiotics.

The dynamics of antibiotic resistance for this 5 year period presented in Streptococcus genus strains an increasing from the percentage of 28,57 % in 2001, to 48,27 % in 2002, to 64,86 % in 2003, to 79,41 % in 2004 and 79, 54 % in 2005.

Key Words: Streptococcus spp., antibiotic resistance

Bacterial resistance can emerge as a consequence of antibiotic use in either humans or animals. Unfortunately, the use of antimicrobials tends to cause bacterial resistance and thus, there is a balance between the need to maintain an animal's health, welfare and productivity with the consumer's requirements for uncontaminated products (BURCH D.G.S., 2005).

Some bacteria are naturally resistant to certain antibiotics, others develop resistance over time (FEDESA, 2000). The most important strategies for controlling antimicrobial resistance among animals include surveillance of antimicrobial use and resistance, effective regulation and the prudent use of antimicrobials in animals (McEWEN S.A., FEDORKA-CRAY P., 2002).

Data about antibiotic resistance evolution is a permanent necessity in any part of the world. Therefore, this study over a five year period joins other researches and data from diagnosis laboratories in Cluj County, in order to supervise the antibiotic resistance in the region.

Materials and methods

The bacterial strains were isolated from samples that came from cattle, swine, horse, poultry, fishes, dogs and cats and were represented by internal organs, brain, abortions, corpses, but also nasal, pharyngeal, conjunctivae, uterine, genital, vaginal, mastitis and skin secretions.

Bacterioscopic and bacteriologic exam were achieved with usual culture medium (broth and agar) that were enriched with serum or blood for the majority of isolated strains.

The laboratory technique for testing *in vitro* susceptibility of bacterial pathogens to antimicrobials agents was agar disk diffusion method.

Agar medium and antibiotic discs were used as materials for this method and also broth for the 24 hours bacterial culture (37 °C) (RĂPUNTEAN GH. and all, 2001).

There were tested 14 antibiotics from the groups of penicillins, cephalosporins, aminoglycosides, fluoroquinolones, macrolides, tetracyclines, phenicols and also potentiated sulfonamides on 172 *Streptococcus* strains.

The diffusion method allow free diffusion of antimicrobial substances onto inoculated agar and after 24 hours of incubation at 37 °C the bacterial inhibition area around the disc was measured and interpreted (BOLDIZSAR E. and all, 2002).

Results and discussions

In 2001-2005 period there were investigated about antimicrobial susceptibility a number of 172 strains from *Streptococcus* genus and the data obtained were analyzed for each antibiotic and also in general, for the totality of antibiotics, observing the evolution of resistance over time.

Susceptible, intermediate or resistant to antibiotics was the interpretation, through diffusion susceptibility test, of the antimicrobial susceptibility to antibiotics of the pathogens, after the inhibition areas around the discs were measured and compared to The National Committee for Clinical Laboratory Standards (NCCLS M31-A, M31-T, 1999).

The antibiotic resistance phenomenon was noticed for all strains of streptococci. The highest percentage of resistance – 92,31 % – appeared for the totality of cephalosporins: from 13 tested strains, 12 strains were resistant and one sensitive. In this study there were used cephalosporins from generation II (cefuroxime), III (cephtriaxone, ceftiofur, cefquinome, cephtazidime) and IV (cephixime); only ceftiofur was efficient, in a 25 % percentage.

It is evident that penicillin was not efficient, because 81,25 % of the strains were resistant, 18,75 % had intermediary values of the inhibition zones and none of the strains presented sensibility to this antibiotic (table 1).

A pretty high percentage of resistance was observed, also, to trimethoprim/sulphamethoxazol (71,42 %), ampicillin (66,66 %), spectinomycin (66,66 %), erythromycin (65,22 %), tetracycline (64,29 %), amoxicillin (60 %).

KOLLIAS-BAKER CYNTHIA ŞI JOHNSON B. (1999) tested, also, the susceptibility to antibiotics of 15 strains of *Streptococcus spp*., and revealed 100 % resistance to tetracycline, 33,33 % - sulfonamide, 26,66 % - enrofloxacin, 13,33 % - potentiated sulfonamides, meanwhile penicillin, ceftiofur and erythromycin were efficient for all investigated strains.

BURCH D.G.S., obtained in 2003, sensibility of 100 % for *Streptococcus suis* tested strains when penicillin, ampicillin and ceftiofur was used in antibiogramms, only 4 % resistance for potentiated sulfonamides and 65 % resistance to tetracyclines.

Different from the results of this study, KASBOHRER A. and all (2006) remarked a low percentage of resistance in *Streptococus pneumoniae* strains for erythromycin – 34 %, cefuroxime axetil – 21 %, penicillin – 15 %, the most efficient antibiotics being fluoroquinolones.

Table 1

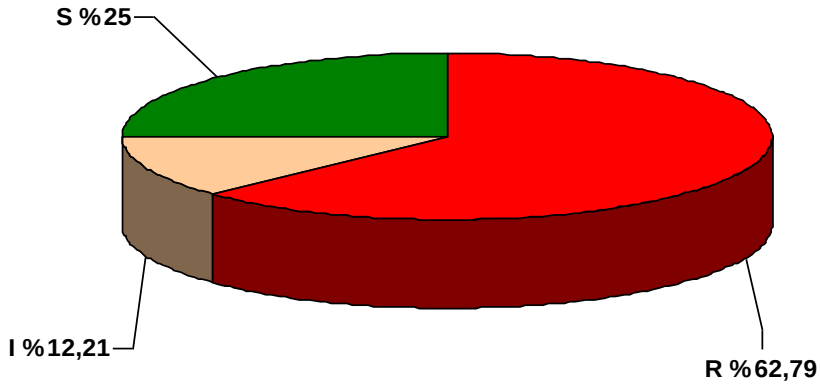
Antimicrobial susceptibility of Streptococcus spp., between 2001-2005

Crt. No.	Antibiotic	No. strains	Sensibility/resistance					
			R	%	I	%	S	%
1	Penicillin	16	13	81,25	3	18,75	-	-
2	Ampicillin	12	8	66,66	1	8,33	3	25
3	Amoxicillin	20	12	60	3	15	5	25
4	Cephalosporins	13	12	92,31	-	-	1	7,69
5	Kanamycin	8	4	50	-	-	4	50
6	Neomycin	5	1	20	1	20	3	60
7	Gentamicin	7	3	42,86	-	-	4	57,14
8	Spectinomycin	9	6	66,66	-	-	3	33,33
9	Erythromycin	23	15	65,22	4	17,39	4	17,39
10	Tetracycline	14	9	64,29	1	7,14	4	28,57
11	Cloramphenicol	4	2	50	1	25	1	25
12	Flumequine	9	5	55,55	-	-	4	44,44
13	Enrofloxacin	11	3	27,27	4	36,36	4	36,36
14	Trimethoprim/Sulphamethoxazole	21	15	71,42	3	14,29	3	14,29
Total number		**172**	**108**	**62,79**	**21**	**12,21**	**43**	**25**

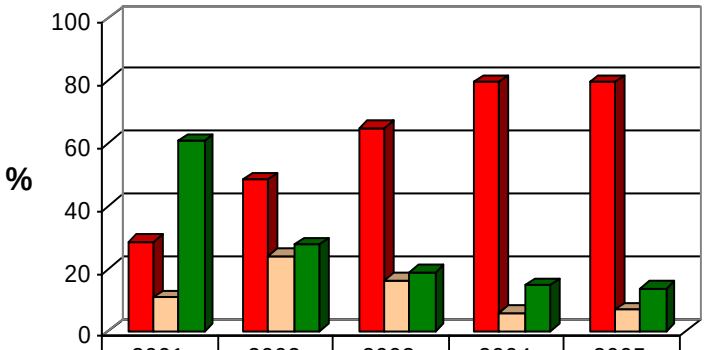
Analyzing the data in a global manner, from the totality of 172 strains, 108 showed resistance to the tested antibiotics, meaning a 62,79 % percentage, 21 of the strains presented intermediary values of the inhibition areas, meaning 12,21 % percentage and only 43 were sensitive to antibiotics, meaning 25 % percentage (graphic 1).

In the 5 year monitorized period, the dynamics of antibiotic resistance presented an increasing from the percentage of 28,57 % in 2001, to 48,27 % in 2002, to 64,86 % in 2003, to 79,41 % in 2004 and 79, 54 % in 2005 (graphic 2).

FENOLL A. and all (1991) also observed the evolution of resistance over a 11 years period, between 1979-1989, notifying an increase of resistance in *Streptococcus pneumoniae* strains to penicillin, from the percentage of 6 % in 1979, to 44 % in 1989.



Graphic 1. Antibiotic resistance in Streptococcus genus strains, between 2001-2005



	2001	2002	2003	2004	2005
R %	28,57	48,27	64,86	79,41	79,54
I %	10,71	24,14	16,22	5,88	6,82
S %	60,72	27,59	18,92	14,71	13,64

Graphic 2. The dynamic of resistance in Streptococcus genus, between 2001-2005

Therefore, the antibiotic resistance is an increasing phenomenon, being imperative to supervise it and the recommmendation is to accomplish the disc diffusion test for every pathogen agent that is isolated from animals.

Conclusions

- The antibiotic resistance phenomenon was noticed for all strains of streptococci tested with antibiotics from different groups
- The highest percentage of resistance was for cephalosporins – 92,31%
- Penicillin was not efficient at all, because 81,25 % of the strains were resistant, 18,75 % had intermediary values of the inhibition zones and none of the strains presented sensibility to this antibiotic
- A pretty high percentage of resistance was observed also for trimethoprim /sulphamethoxazol (71,42 %), ampicillin (66,66 %), spectinomycin (66,66 %), erythromycin (65,22 %), tetracycline (64,29 %) and amoxicillin (60 %)
- Analyzing the data in a global manner it was remarked a 62,79 % percentage of resistance to antibiotics, a 12,21 % percentage of intermediary values of the inhibition areas and only 25 % percentage of sensitive strains to antibiotics
- The dynamics of antibiotic resistance for this 5 year period presented an increasing from the percentage of 28,57 % in 2001, to 48,27 % in 2002, to 64,86 % in 2003, to 79,41 % in 2004 and 79, 54 % in 2005
- The recommmendation is to accomplish the disc diffusion test for every pathogen agent that is isolated from animals having clincal signs of disease, because of the increasing of antibiotic resistance phenomenon

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Entomological expertise in a manslaughter case

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In December 2005 the corpse of a minor of 8 years old was discovered in a cardboard box introduced in an garbage container. The investigations have betray that some groups of insects were found on the corpse.The request of justice was to determine by a entomological expertise details regarding the insect group evolution in this specific environment, to establish some correlations between life-cycle of insects and the rest of data managed, for a good judgement of the case. Our entomological expertise in this manslaughter case has proved that there was involved insects from Calliphoridae familly (Diptera:Oestroidea), regarding posterior stigmae to L-III and and papillae position.

Key words: forensic entomology, blow-flies (*Calliphoridae, Diptera, Oestroidea*)

Insects, are the major group of arthropodes and the most diverse group of animals on the Earth, with over a million described species, more than all other animal groups combined.

There are many types of insects that can be involved in forensic entomology, but the ones listed here are mostly necrophagous and related to medicolegal entomology (directly related to the crime and found on the corpse). This is not a full list; there are many variations due to climate, and many other insects that are necrophagous; this is outlined by different authors; the order in which the insects feed on the corpse is called the faunal succession.

Medicolegal forensic entomology includes arthropod involvement in events such as murder, suicide and rape, but also includes physical abuse and contraband trafficking. In murder investigations it deals with what insects lay eggs when and where, and in what order they appear in dead bodies. This can be helpful in determining the time or post mortem interval (PMI) and location of the death in question.

Forensic entomology deals with the examination of insects in, on, and around human remains to assist in determination of time or location of death. It is also possible to determine if the body was moved after death.

Bow-flies, involved in this study, are member of the family *Calliphoridae* of flies [Gr. *kallos* beauty + *phoros* bearing]. *Calliphoridae* **is** a family of medium-sized to large flies of the order *Diptera*, including the genera *Auchmeromyia*, *Booponus*, *Calliphora* (type genus), *Cordylobia, Cochliomyi, Chrysomia, Lucilia*, Phaenicia and *Phormia*; all species may serve as vectors of pathogens and may also produce myiasis in humans; several are causes of cutaneous myiasis in domestic animals. Bow-flies of this family are often metallic in appearence.

Material and methods

The investigators of this case have expediate for analysis, a sample with fragments of insects, to compare the anatomy of different fragments of the insect body and to establish which family of insects were found on the corpse. The environmental conditions at the time of prelevations was: between 18.10-19.11.05 = 6.2 ^{0}Celsius in air, and the relative humidity in air + 77; between 20.11.05-29.12.05 = 3,4 ^{0}Celsius and the relative humidity +79.

Results an discussions

After the analysis of the entire sample, the preliminary results concluded that :

- in the sample wasn't worms to analyse, but only small parts of insects, with a modest entomological value due of very fragmented pieces and no identifying visible elements (Fig.1);
- considering the morphological aspect of puparium (Fig.2), we apreciate that it belongs to cyclorrapha diptera insects, from the O. *Brachicera*;
- considering the posterior stigma pozition of L III larvae, (Fig.3), we are establishing that is characteristic for *Calliphoridae* Family.

Discussions

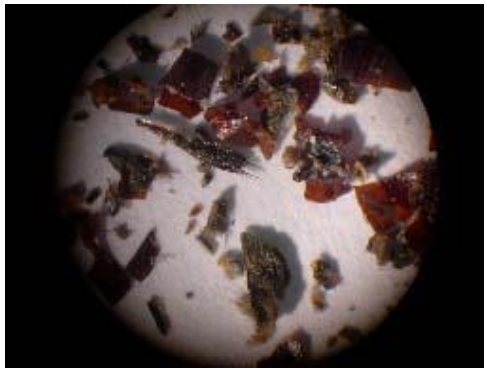

Fig.1.
Sample with parts of insects

The fly life cycle passes through four life stages: egg, larva, pupa, adult. The eggs are approximately 1 mm long and are laid in a loose mass consisting of 50 to 2000 eggs. Group oviposition by several females results in large masses of thousands of eggs that may completely cover a decomposing carcass [6]. The eggs hatch in as little as a day (regarding on air temperature value) and the larvae feed on carrion until they reach maturity. They grow very rapidly and will be full size within a about a week, and then they will normally leave the carrion. The creeping larvae may have an unpleasant appearance, but on the other hand they do reduce the smell of a corpse, over a period of a couple of weeks, which is much worse. Upon maturity, they migrate away from the carrion to search for a suitable pupation site. Pupation usually occurs within the first inch of topsoil or under leaf litter, rocks, or fallen limbs. During this time, the larval skin shrinks and hardens to form the puparium which is dark brown in color (Fig.2).

Fig. 2.
Puparium to Cyclorrapha

This stage may last as long as 12 days; however, the adults can emerge in only seven to eight days depending on temperature. They are immediately able to fly off, mate and start laying eggs.

The larvae is one of the "hairy maggots". They received this name because each body segment possesses a median row of fleshy tubercles which gives it a slightly hairy appearance although it does not possess any true hairs. The puparium is the hardened and shrunken outer skin of the mature maggot. The pupa develops entirely within this hardened shell [10](Fig. 2).

The adults can live up to six weeks. Adults are robust flies metallic green in color with a distinct blue hue when viewed under bright sunlight conditions. The posterior margin of the abdominal tergites are a brilliant blue.There are the first insects to arrive at a fresh carcass in the area. The adults usually arrive within the first 10 minutes after death as long as atmospheric conditions are favorable for activity [12]. The larvae have a shorter development time than other species and their predaceous nature can alter entomological-based postmortem interval estimations, which are founded on the prey species.

Identifying characteristics for the family *Calliphoridae* include: Suborder *Cyclorrapha*: antennae 3-segmented, *aristate;* vein Rs 2-branched, arista plumose for *entire* length. Division *Schizophora*: frontal suture *present.* Section *Calyptratae*: calypters well developed. Two notopleural bristles. Hindmost posthumeral bristle located *lateral to* presutural bristle.

To differentiate the genus and species, must to know: genital apparatus, plume of members, veins of wings.

Distinguishing characteristics: Antennae in *Nematocera* are filiform or feathery; in the *Brachycera*, short and horn-like; in the *Cyclorrhapha*, hairlike.

All larvae are legless. In the *Nematocera*, there is a true head capsule; in the *Brachycera*, a partial head capsule; and in the *Cyclorrapha*, no head capsule. The larvae of the *Cyclorrapha* are the maggots with mouth hooks. They pupate within the last larval "skin" that is hardened to form a puparium [14], Fig. 2.

To differentiate the L III: cephalopharingian skeleton, posterior stigmae (Fig. 3).

The body: *metallic* blue, green, or black.

Figure 3. Posterior stigmae to the Calliphoridae

Conclusions

According the puparium, it belongs to *Cyclorrapha* diptera insects, from the Ord. *Brachicera* [2].

According the position of stigmae on the L III larvae, the insects was from *Calliphoridae* family [3,6].

Environmental conditions sustain the development of these stages of life cycle to the *bow-flies* on the corpse involved in this study.

In our opinion, bees and wasp (*Hymenoptera*), who are not necessarily necrophagous, some are also predatory and eat the insects feeding on the body during the early stages. [1].This may cause problems for murder cases in which larval flies are used to estimate the post mortem interval since eggs and larvae on the body may have been consumed prior to the arrival on scene of investigators [4,7].

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Crayfish pathology in Romania - maybe a programme for the future?

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The devastating affects of disease on European freshwater crayfish are well known as epizootics in wild populations have occurred throughout much of Europe since the mid XVIII[e]s. The fungus Aphanomyces astaci, the cause of the disease named crayfish plague proved in 1934, has concentrated on fugi, especially improving diagnostic techniques,isolation and/or identification for A. astaci. The diagnostic techniques necessary to detect the full range of potential pathogens of crayfish are rarely utilized in the field of crayfish pathology in Europe. Presently, the field is ill-equipped to determine the cause(s) of these epizootics an in the same measure, histopathological analysis, diagnosis of infections by viruses, Rickettsia-like organisms (RLO). Moreover, crayfish conservation strategies may be undermined and even detrimental to the long-term goals; eg., stocking programs may spread undetected pathogens.Therefore, critical limitantions in the field of crayfish pathology have major repercussions in management of freshwater crayfish. Guiding principles and a concept for a trans-European Community research and education program, we intend to adhere to this concept, making a prposal of program for the future for Romania.

Key-words: European freshwater crayfish, disease, pathology, research programme,education

Romanian stock of native species of crayfish is today unknown, even this species are vitally significance for environmental and ecological reasons. Past and recent events and practices in the field highlight the lack of information on general disease, and underline the major difficulties that this situation poses to the effective management of freshwater crayfish.

In Romania, very few data are presenting diseases of crayfish, even in Europe, In Romania, very few data are presenting diseases of crayfish, even in Europe, the first pathogen reported from European freshwater crayfish was *Psorospermium haeckeli* (*H*AECKEL, 1857) followed by *Thelohania contejeani* (HENNEGUY and THELOHAN, 1892).These pathogens are considered to be of much lower pathogenic significance, but actual virulence data for these species are virtually non-existent due to laboratory transmission difficulties. *Aphanomyces astaci* is considered to be the causative agent for the disease termed crayfish plague (microsporidiosis or porcelain disease) (OIE, 2003a), the cause of the panzootic in European freshwater, reported in 1934. However, OIE (2003a) states that for crayfish plague "100% mortality is the norm" in susceptible species. Moreover, there have been cases where different laboratories within the same country have provided different diagnoses from the same samples. Taken together, these observations have resulted in a reduced level of confidence in diagnostic services. For Romania, very late was reported a first case study of aphanomycosis in lake Scheia (MIRON L. & MIRON M., 2002).

Modern genetic-based diagnostic tests for *A. astaci,* such as those utilizing the polymerase chain reaction, have the potential to significantly improve diagnostic capabilities for crayfish plague (CERENIUS *etal.,* 2002).

Frequently the remaining material from diseased crayfish was discarded or was unsuitable for other types of analyses because the only diagnostic analyses contemplated were for fungi. Moreover, a methodology for a broad and comprehensive diagnostic assessment for European freshwater crayfish has not been developed.

Perhaps most significantly, some critical techniques typically used in diagnostic/pathological studies of other animals, such as histopathology, have been under-developed and under-utilised in the field of crayfish pathology in Romania, even the signal of some ectoparasitic crayfish fauna does exist : *Saprolegnia spp., Fusarium, Cephalosporidium, Oidium, Ramularia, Vorticella*(MIRON M & MIRON L., 2006)

Therefore, the current poor level of knowledge of disease in Romania, like in the whole rest of Europe for crayfish presents major difficulties in developing international biosecurity policies (EDGERTON, 2002b). Of greater concern, current European biosecurity policies may be eminently challengeable on the grounds that freedom from certain pathogens has not been demonstrated. Critically, biosecurity policy development is dependent on high quality data on the oceurrence of pathogens.

In 2001 , a paper regarding *Branchiobdella* infestation on crayfish was made (Miron L., MIRON M, 2001), even it is underline that is not a risk posed by these pathogens to be dangerous like endemic in the country.

Moreover, given the extremely low level utilization of techniques required for diagnosis of many diseases, there can be little confidence in even passive surveillance for the detection of potentially important pathogens like viruses and rickettsia-like organisms.

As well as complicating international biosecurity policy, this lack of information on freshwater crayfish pathogens has major implications for regional biosecurity issues. Significantly, in many European countries there are policies for restocking of native crayfish populations to restore fisheries and to enhance conservation efforts. In only a few countries are there mandatory health checks prior to stocking, and in such cases the diagnostic tests applied are for visible signs of disease and the detection of readily identifiable parasites.

It is entirely possible, even likely, that restocking programs for freshwater crayfish are resulting in the dispersal of pathogens, some of which may be seriously detrimental to freshwater crayfish populations.

DISCUSSIONS

It was recognised that other pathogens must also be considered, such as viruses and rickettsia-like organisms. The role of the environment in the expression of disease was also noted. The need for improved diagnostic techniques and widespread adoption of these techniques was seen as critical. As far as practicable, these diagnostic techniques should be quick, cheap and standardised. These diagnostic techniques should then be applied to diagnostic response to disease outbreaks and in pre-stocking health checks in național and regional laboratories on farmed or wild stocks. Data from disease investigations should be incorporated into an EU disease register for freshwater crayfish to assist in the management of crayfish stocks, to prevent spread of disease. The development of the program should involve a network of laboratories throughout Europe, including research and fish disease diagnostic laboratories, EU and national funding, and contain a significant education component to transfer information to regional laboratories, field officers, farmers and the general public.

Epizootics in European crayfish are very frequent, and contribute to significant pessimism that native European crayfish may become extinct in some countries in the foreseeable future.

Often epizootics in native freshwater crayfish do not result in complete mortality, or the mortality in the lake is patchy, suggesting that multiple factors are involved (eg. genetics of host

or *A. astaci,* water currents, or other pathogens). In some of these cases the diagnosis given by authorities was crayfish plague.

Crayfish plague was considered to be an extremely important disease. It was also noted that there are many instances where *A. astaci* can not be associated with epizootics in native freshwater crayfish as attempts to isolate the fungus were negative when material supplied was of high quality. Moreover, there are concerns that diagnostic processes are prolonged, and managers are unable to make timely decisions.

In Scandinavia there have been a number of lakes in which the native noble crayfish, *A. astacus,* and signal crayfish have co-existed for prolonged periods, several decades in some cases. Generally in such instances the native population declined gradually. These observations question two very widely held views, that: 1) all signal crayfish populations are carriers of A *astaci,* and 2) all noble crayfish are highly susceptible to A *astaci.*

Additional constraints in researching and managing disease in freshwater crayfish were discussed. In some countries fisheries legislation hampers our understanding of disease; eg. the closed season on trapping crayfish in some countries, such as Finland, means that fisherman are unaware of an epizootie until after it has oceurred. There is a general difficulty in resourcing (i.e. funding) research and field studies on disease in European freshwater crayfish. This flows through to an inability to retain young and enthusiastic scientists after they have been trained with valuable and rare skills. Significantly, there is a shortage of trained expertise in overall crayfish pathology in research and diagnostic disciplines in Europe.

It is extremely difficult to develop management strategies for disease in European freshwater crayfish with such a low level of confidence in the current level of knowledge. Therefore, a program to address this major deficiency is necessary.

We must have a conceptual framework and a set of guiding principles well developed, to establish a key centre for research which would be responsible for administering the programme and for the primary research role, as well as a strong education role.

Finally, a robust education effort will be required to ensure that the information gathered from the program is presented to the stakeholders (crayfish managers, farmers and general public) in a useful form. This could be in the form of leaflets to raise their awareness to the threat of disease to crayfish populations, and providing information on signs of disease in freshwater crayfish, what to do when sick or dying crayfish are observed, and how to play a role in reducing the impact of disease in freshwater crayfish. The Internet should also be a major tool in the education effort.

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The analgesia with fentanyl and midazolam to geriatric dogs

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Fentanyl, a syntethic opioid analgesic of brief effect in time, similar to petidine (but much more active), in combination with Midazolam - a benzodiazepinic tranquilizer - strives for the therapeutical target of inducing an intense analgesic effect. The combination also presents the advantages of avoiding some secondary effects (following the simple anaesthesia with Fentanyl) such as: respiratory depression, myosis, salivary hypersecretion and vomiting, bradicardia (midazolam determines a good cardiovascular stability, prevention of the postoperator paintfull stress.)

Key Words: geriatric dogs, analgesia, Fentanyl, Midazolam

The vigil anaesthesia is a particular form of general anaesthesia accompanied by a relative vigilent state and which inhibits the pain efficiently, with little modifications of the vegetative equilibrium and of the body's homeostasis.

Such vigile combinations are used mostly to dogs, and rarely to cats as analgesic, postanaesthesic and preanaesthesic sedatives.

MATERIAL AND METHOD

The searching was made in the Pharmacology Laboratory in the Faculty of Veterinary Medicine, Iaşi, on a batch of 15 common race dogs of approximatively the same weight (12-15 Kg).

The administration of the ,,litic'' mixture was made intravenous with: Fentanyl, solution 0,5 %- 0,042 mg/Kc.

The estimate of the anaesthesic effect was made trough clinical examination, that is monitorizing the following data: the general physical status (T.P.R..), the hearth rate and rhythm, the amplitude and the rhythm of breathing, the accidents and the emergency while the anaesthesia, the response tot the external stimulants (digital pressure, percussion, pricking). The registration of data was made before the anaesthesia (representing a comparison base), during the anaesthesia and at awakening, once at 5 minutes.

RESULTS AND DISCUSSION

There ware made measurements of the normal vital parameters in order to have a comparison base (*table 1*); then it was administrated Atropine, in a dose of 0,045 mg/Kc, to prevent the undesirable vagale effects of Fentanyl. After 7 minutes from administration of Atropine (meanwhile there was recorded a slight decrease of the vital parameters) it was injected intravenous the combination Fentanyl - Midazolam, mixture in the same syringe. As early as the time of administration it can be noticed (to all the dogs) the installation of a nervous depression, accompanied by the lost of the voluntary movements (catatonia) and by the attenuation of tendinous and muscular reflexes.

There are also recorded the gradual progression of the central depression, the reducing of the breathing frequency (24 breathings/minute), of the heath rate (100 contractions/minute), and the

diminishing of the muscular tone and reflexes. The breathing is a little irregular (dyspnoeatic to some elder dogs - 12 - 13 years old), being accompanied by reducing of the amplitude of the breathing movements. Its frequency grows during the manipulations or as a response tot the hearth contractions' frequency, on an average of 80- 100 contraction/minute; the pulse is relatively strong and rhythmic.

The eyeball are found in central position; the pupil is a little dilated, with a tendency of myosis; sometimes it is difficult to make evident the pupilar diameter because of the Atropine administration. The pupilar reflex is present (the pupil responses to light by modifying its diameter). The muscular reflexes and the tendinous reflexes are absent, excepting the patellar and palpebral ones, which are very diminished. The muscular tone is intensively diminished which allows the surgical incision to be done.

The mucous membranes are hyperemiated, dry (because of the Atropine); the time needed for refilling the capillary vases is normal- 2 seconds.

After 40 minutes (from the moment of anesthesia's installation) it can be registered a slight decrease of the vital parameters (P= 120 contractions/minute; R= 34 breathings/minute; T= 37 C). The dogs come to their senses, first on the front part of the body propping up on the breast bone. Some of them have spontaneous movements of their tongues, others present a delirium state accompanied with nervousness and vocal manifestations. Only one dog was registered with behavioural modifications as aggressiveness while awakening from anaesthesia.

The complete recovering from the anaesthesia is made after almost 40 minutes from the first signs of awakening..

Table 1

The anesthesia with Xylazine, Ketamine and Midazolam to geriatric dogs

The anaesthesia phases	**Time (min.)**	**Rectal t^0 (^{0}C)**	**Heart rate (beats/min)**	**Respiratory frequency (breaths/min)**	**Eyeball position**	**The pupil**	**Reflexes**	**Skeletal muscle tone**	**Mucous membrane color**	**T.R.C. (second)**
Moment 00	0	38,8	120	24	variable	normal	normal	normal	normal	normal
Atropine	1	38,8	120	24	variable	normal	normal	normal	normal	normal
Fentanyl + Midazolam, i.v.	6	38,8	132	34	variable	midriasys	exaggerated	exaggerated	slightly hiperemiated	normal
Analgesia (Fentanyl) and hypnotic sleep (Midazolam)	7	38,8	121	28	central	myosis	absent, excepting the patellar and palpebral strongly diminished	absent	hiperemiated	normal
	10	38,7	100	24						normal
	15	38,6	114	28						
	30	38,3	120	34					hiperemiated	normal
	45	37,9	136	134					hiperemiated	normal
Awakening	50	38,00	144	38	variable	normal	slightly diminished	slightly diminished	normal	normal
Recovery	130	38,5	107	23	variable	normal	slightly exaggerated	slightly exaggerated	normal	normal

CONCLUSIONS

1. Anaesthesia with Fentanyl and Midazolam proves to be a good combination for the surgical proceeding of short time (less then 30 minutes), to dog.
2. Its is recommended in case of radiologic diagnosis proceedings or in other eaxaminations (vaginal examination, skin biopsy etc).
3. It can be used to old dogs, where the general anaesthesia is not possible.
4. The modifications of the vital parameters are minimal, with a minor risk for the organism subjected to intervention.
5. It can be recommended as preanaesthetic (in the anaesthesia with general anaesthesic when it is reduced the dose eith 50 - 90%) and postoperator analgesic.
6. It presents the disadvantage of a slow recovering period, accompanied by behavioral disturbances to 20% from dogs.

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Therapeutic possibilities in dog's viral papillomas

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Papillomavirus infections are latent and oncogenic type who affects different species of mammals. Taxonomically these viruses belong to Papilomaviridae family, and the dog is affected by "Canine oral papilomavirus".

The papilomas are frequently found in youth and located on the nose, lip, and cheeks.

The following paper presents a therapeutic way to deal this disease using a self vaccine, to a 2 years old Husky.

Key Words: dog, Papillomavirus infections, therapy

Papilomavirozele sunt boli infecțioase latente sau cronice de tip oncogen, întâlnite la diverse specii de animale, produse de virusuri încadrate în genurile *Papilomavirus, subfamilia Papilomavirinae,* familia *Papilomaviridae.*

La carnasiere, papilomatoza este produsă de "virusul papilomului câinelui"(Canine oral papilomavirus). După localizarea procesului, se deosebeşte o formă orală şi una cutanată. Papilomatoza orală, se întâlneşte mai frecvent la tineret şi se caracterizează prin prezența pe mucoasa cavității.bucale (limbă, bucce, vălul palatin, şi chiar în esofag) a multiple papiloame care pot determina disfuncții în masticație şi deglutiție. Papilomatoza cutanată, apare mai frecvent la animalele adulte şi se caracterizează prin prezența unor noduli mici, albicioşi sau mase cenuşii pediculate, localizate mai frecvent pe pielea feței, gâtului, membrelor şi organelor genitale, fără predilecție de sex. Leziunile regresează spontan după 1,5-3 luni.

Prezenta lucrare ilustrează o modalitate terapeutică reprezentată de autovaccin la un câine, rasa Hasky de 1,5 ani, la care au fost identificate formațiuni de tip pedicular atât pe bucce, cât şi pe tegumentul peribucal.

MATERIAL ŞI METODĂ

În urma examenului clinic au fost identificate 12 formațiuni de tip conopidiform din care 3 pediculate. Acestea au fost prelevate în vederea examenului histopatologic şi a realizării autovaccinului cu ajutorul termocauterului respectând regulile de asepsie necesare prelucrării ulterioare.

Imunomodulatorul a fost obținut parcurgându-se o serie de etape :

1. Obținerea unei suspensii virale lipsită de resturi celulare. Eliberarea particulelor virale din celule s-a realizat printr-un tratament mecanic(mojarare în condiții sterile) şi termic (prin congelări şi decongelări succesive). Virusurile fiind ultracentrifugabile la finalul acestei etape, s-a făcut o centrifugare la 6000 turații/minut.
2. Aseptizarea materialului obținut cu antibiotice
3. Purificarea materialului virulent prin ultracentrifugare.

Administrarea inoculului rezultat s-a făcut pe cale intradermică utilizând seringi dozatoare de 0,3 ml.

REZULTATE ŞI DISCUŢII

Produsul imunomodulator obținut a fost administrat conform următoarei scheme:

În prima zi s-a inoculat intradermic, 0,01ml. în zona flancului drept, după tundere şi aseptizare .

În zilele ulterioare, până în momentul căderii primelor papiloamelor(ziua a 11-a) doza a fost majorată cu 0,01ml. zilnic.

Din a 12-a zi s-a continuat administrarea cu dozajul în sens descrescător.

Papiloamele de la nivel cutanat (figura nr. 1) au căzut ultimele, ele având o bază de implantare largă, spre deosebire de cele de tip pediculat de la nivelul mucoasei bucale (figura nr. 2), care au dispărut primele.

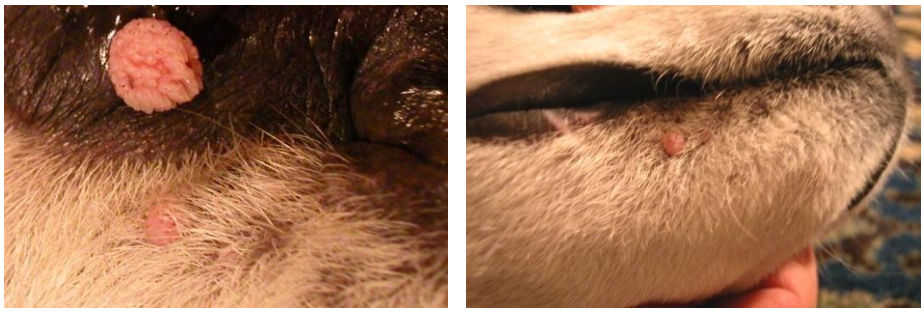

Fig. nr 1 Papiloame la nivel bucal

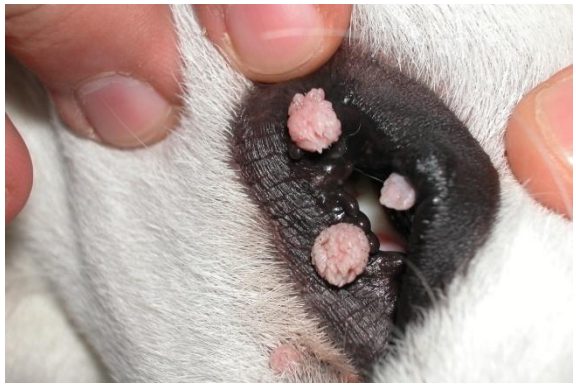

Fig. nr. 2 Papiloame la nivel cutanat

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PRRS virus influence over the reproduction biological markers

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Respiratory and reproduction diseases, revales some of the week points in farms health programs. In spite of modern tehnologies, management techiniques and veterinary prophilactic measures, farmers continue to confrunt with this health problem. The high cost, of these problems, is generated by mortality, weight loss, increased time until delivery, higher costs for veterinary surveillance, and longer time for gaining weight. In the following paper, we present the main losses that occur in a porcine effective after PRRS virus appearance.

Key word: prenatal loss, infectious abortion, PRRS

PRRS syndrome represents a highly contagious disease, causing interstitial pneumonia in all ages (respiratory syndrome) and reproduction disturbances in females (the reproductive syndrome represented by prenatal losses, fetuses' mummification, high abortion rate, piglet's low viability, anestrous and prolonged infertility).

Economical losses are high, mainly because of the reproductive pathology, induced by this disease, and piglet's low viability.

Materials and method

Some of the reproduction marks were analyzed during disease evolution.

In order to appreciate virus influence over reproductive parameters (natality, fertility, prolificacy) and loses thru mortality, data before and after virus outbreak were included too in ours studies.

Table 1

Evolution of natality, returnees to heat and prolificacy

Months	*Natality*	*Returnees to heat*	*Prolificacy*
	%	%	nr.
I 2004	68,3	-	9
II	66,8	-	8,9
III	66,6	-	8,4
IV	66,3	20	9,2
V	66,3	18,5	8,5
VI	68,9	18,7	8,5
VII	69,5	17,8	8,7
VIII	71,0	17,0	8,6
Media	67.9	18,4	8,7
IX	66,2	20,0	6,4
X	61,0	21,3	7,3
XI	55,5	23,0	7,2
XII	55,5	23,0	7,1
I 2005	62,4	19,9	7,9
Media	60,5	21.44	7,2

From data referring to natality we conclude that values previous disease outbreak are higher, about 67.9 % (values between 66.3% and 71%), than values during disease episode, when natality was about 60.5 % (values between 55.5% and 66.2%).

Returnees to heat were more frequent during disease than normal, with values around 21.44% . Before disease occurs, the values were up to 18.4%.

Considering data referring mortality, during virus infection, two distinct periods were determinate.

One period was identified before virus outbreak (when responsible were other pathogens) and another one during disease evolution.

During first period, the mortality values, for all age categories, were low. In piglets the values varied between 7.29% (January 2004) and 18.43% (May 2004), the average value was 11.10%, value recorded before virus outbreak.

In youth mortality varied between 6.5% (may 2004) and 11.9 %(February 2005 – when study ended), the average value was 8.79%.

In adult groups, the average value for mortality was 2.63%.

Virus appearance determinate a new pathological framework, mortality loss rise for all categories, most affected were piglets. In this category mortality rise up to 49.24% for September 2004, value for October was 31.7%. Mortality decreased until January 2005, value recorded was 12.9%. In youth mortality rise starting September 2004 (20.3%), maintained in plateau between October till November (35%-38%) and reaching the maximum value during December (42.6%). To adults mortality rise during September 2004 without significant changes for next months.

Total loss is 3-4 times higher after virus outbreak, which occurred after 8 month of surveillance (January 2004 - August 2004). In the first 8 month, the average mortality value was 22.9%, after virus outbreak rise up to 84.4% during September, 76.7% during October, 64.3% in November and 62.8% in December. The values remained high even after January 2005.

It must be mention that virus appearance and positive serologic exams are closely related. After clinical expression of the disease, mortality and morbidity rise PRRS virus, was serological identified in sectors 1,4,7,8.

After PRRS emerge to all three animal categories body weight gain diminished. Data presented in Table 2, represent average weight gain in period's prior disease and after disease emerge.

Table.2

Body weight gain between: 1 January 2004 – 31 January 2005

Animal category	***Average***	
	01.04-30.08.04	*01.09.04-31.01.05*
Piglets	156 g	151,8g
Youth	355,1 g	339,6g
Fat adults	580,4 g	428,2g

Conclusions

Researches and the findings gathered leaded us to following conclusions:

1. The supplemental losses recorded, during following period: September 2004-january 2005, compared to previous period, are greater mainly because of the PRRS outbreak.
2. Losses caused by mortality are higher in piglets, youth and fat pigs
3. Average weight gain and forage metabolizing decreased. Also encountered was the low efficiency of the antimicrobial drugs.
4. Starting March 2005 the negative effects of the PRRS outbreak diminished, with reproductive and zootechnical indices between normal limits. Because the virus did not disappear from the unit, prophylactic measures must be apply in the following months.

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Treatment with KCND – injectable solution in respiratory afflictions met in community dogs from shelters

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The present paper approaches the problem of sanitary-veterinary assistance assurance at superior parameters in canine respiratory afflictions in a shelter in Bucharest. This unit is sponsored by a foundation and shelters 250 community dogs picked from Bucharest and surroundings, having as purpose to solve the problem of homeless dogs.

For this, the veterinary surgeon had to find those substances in the same time cheap and efficacious. The aim is to decrease the general costs and also the expenses of the therapeutic interventions.

KCND is a complex product with a wide antibacterial range, having as compounds kanamycin, colimycin, neomycin and dexamethasone.

The experiments were made after a preset protocol which had as aim to demonstrate the therapeutic value of the studied medicine comparatively to other substances recommended in the treatment of bacterial respiratory diseases in dogs: Linco-Spectin and Gentamicin 10%.

Key words: KCND, kanamycin, colimycin, neomycin, dexamethasone, lincomycin, spectinomycin, gentamicin

Dog breeding has increased today, but it didn't succeed in finding definitely the dogs' origin, some theories considering that the dog comes from hyena, jackal, fox, wolf or wild dog.

It is considered that the actual dogs evolution would have been placed 20.000 years ago, when they started to join man at hunting, guard the houses and then the herds.

Thus, man has answered the canine devotion by assuring it a shelter, food and carrying. In time, man-dog relation has evolved, not being conditioned by the existence earning any more, but assuring man's company and some specialized services.

MATERIALS AND METHODS

To carry out the different treatments in this unit, students of Faculty of Veterinary Medicine Bucharest take part as volunteers, under the teaching of a veterinary surgeon. Complex medical interventions, special investigations or other manipulations that could not be done in the shelter are made up in some veterinary units in Bucharest.

Each dog's evidence is very strict, every dog having an individual file where there is written the age, sex, approximate date of birth, if the dog is sterilized or not, data about the owner or the person who brought the dog, vaccinations and anti-parasitical treatments and also every dog has got a health notebook which will be given to the future owner.

The dogs used in this experiment were distributed in 3 groups, each one being treated with an antibacterial product: the first group, consisting in 10 dogs, was treated with KCND, the second group, consisting in 4 dogs, was treated with Linco-Spectin, and the third group, consisting in 2

dogs, was treated Gentamicin. Linco-Spectin and Gentamicin 10% injectable solution are basic medicines, initially used in this shelter. That is why for this experiment was used more than one type of injectable antibiotics, in order to compare their efficaciousness.

The dogs in the first group presented cough, dyspnea, fever, congested mucouses, lack of appetite, nasal leak, pain at palpation; some of them presented also noisy breathe, nasal pruritus (due to the continuous mucous-purulent leak), hard vesicular sound. The diagnosis was made upon the clinical signs; there were diagnosed the acute cataral bronchitis and cataral bronchopneumonia.

The treatment was represented by subcutaneous injections of KCND product composed by kanamycin, colimycin, neomycin and dexamethasone in a dose of 2 ml/10 kg, for 3-5 days and even 7 days in severe cases, till the clinical signs disappeared.

The second group (the 4 dogs) which presented the same clinical signs, but more, discreet received Linco-Spectin product, intramuscular, in a dose of 1 ml/5 kg.

The third group, represented by 2 dogs with average clinical signs, was treated with Gentamicin 10% injectable solution, intramuscular, in a dose of 0,4 ml/10 kg.

RESULTS AND DISCUSSIONS

The respiratory afflictions of dogs in a shelter sponsored by a private foundation, diagnosed as acute cataral bronchitis and cataral bronchopneumonia were treated with KCND, Linco-Spectin and Gentamicin 10% products.

In the case of KCND administering, after 2-3 days of treatment there were noticed the following aspects: fever decreased to normal, local pain, pseudo-membranes and sneezing disappeared, after the fifth day of treatment disappeared also the nasal pruritus and the congestion of mucouses; after 4-5 days it was noticed the complete disappearance of the respiratory clinical signs.

In the dogs treated with Linco-Spectin and Gentamicin 10%, in the first 3 days it was recorded an improvement of the general health status; after 5 days disappeared almost all clinical signs, less the cough, which became more moderate and rare than in the third day of treatment. After the sixth and the seventh day of treatment, the animals recovered completely or almost completely.

CONCLUSIONS

1. The treatment with KCND product is a proper one for respiratory afflictions treatment, because it mixes 3 antibiotics (kanamycin, colimycin and neomycin) with an antiphlogistic product, dexamethasone. In addition, dexamethasone presents, according to the studied references, the effect of increasing the antibiotics action.
2. The effectuated treatments and the obtained results give the possibility to consider that the complete cure of the individuals treated with KCND produces after 4-5 days of treatment.
3. The usage of KCND product determined a higher capacity of regeneration and defense of the treated animals' organism.
4. The treatments carried out with Linco-Spectin and Gentamicin 10%, which are basic medicines used initially in the shelter, offered worse results than the ones carried out with KCND.
5. The KCND product could be considered an almost ideal antimicrobial medicine from the price/quality ratio point of view.

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Apiphytotherapy with Propolis and Aloe Vera gel in canine hepatic diseases

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The apiphytotherapy with propolis and Aloe vera barbadensis gel is a guarantee for health maintenance and treatment of some disease, by detoxifing the organism and by stimulating the liver functions. The project was built by tracing and comparising the evolution of the cases that were diagnosed with some type of liver illness (liver failure, cholestasis liver cirrhosis, acute hepatitis), treated both by classical (traditional) and alternative methods of therapy, during 44 days of treatment, in 27 dogs of different ages and breeds, monitoring the efficiency by clinical and paraclinical repeated tests (bilirubin levels, transaminase enzymes activities: ALAT, ASAT, GGT and alkaline phosphatase, total protein, albumin).

The laboratory analyses results proved the apiphytotherapy efficiency in canin liver failure: the bilirubin levels decreased from 0,7 – 1,0 mg/dl to 0,3 – 0,5 mg/dl, ALAT activities decreased from 69 – 85 U/l to 33 – 51,5 U/l, alkaline phosphatase activities decreased from 107 – 115 to 60 – 91 U/l.

The nutritional apiphytotherapy methods were at least equaly efficient as the traditional therapy, which proved the importance of nonagresive support of liver cell functions and regeneration.

In cholestatis, bilirubinemy decrease from 0,8 – 2,5 mg/dl to 0,5 – 0,85 mg/dl, ALAT activity from 102 – 107 U/l to 36 – 79 U/l, GGT activity from 10 – 13,3 U/l to 4,1 – 6,4 U/l show nutritional propolis and Aloe vera gel apiphytotherapy superior efficiency in comparision with classical drug therapy after 14 days of treatment.

In acute hepatitits and liver cirrhosis the results of apiphytotherapy are near of classical (traditional) treatment, but necessitated a much longer period of therapy (months), the principal biochemical ranges analyzed show the curative tendency (decreases of bilirubine levels and ALAT activity, significant increases of proteinemy and albuminemy).

In conclusion, the apiphytotherapy results proved by laboratory exams the therapeutical efficiency in liver failure and cholestatic disorders, but in acute hepatitis and liver cirrhosis are necesare over 2 months of treatment until body clinical and paraclinical normalisation, existing the possibility to utilise the association between apiphytotherapy and drug therapy.

Key words: apiphytotherapy, propolis, Aloe vera barbadensis gel, liver failure, cholestasis liver cirrhosis, acute hepatitis

The development of the interest on the world plane for alternative and complementary medicine, esspecialy for apiphytotherapy , made us to study more the Aloe vera gel therapy efficiency in some type of (diseases) ellness in dogs.

Unlike classical apiphytotherapy with fresh plants who uses fresh gel, obtained from Aloe vera barbadensis plant, the modern apiphytotherapy uses gel, without chemicals as Aloe vera gel obtained and stabilized by Forever living Products.

Favorable results on tissues and organs have been studied after 6 weeks treatment in three daily doses, the daily dose being as 15 – 20 ml/10 kJ/day, propolis in Aloe vera gel..

Researches have been made for the experimental efficiency of apiphytotherapy in animals, so as detoxifing and cure the organism in different types of illness.

Material and methods

Clinical investigations, echograpy exams and paraclinical exams have been made in the Faculty of Veterinary Medicine in Bucharest on 27 experimental cases.

Researches have been made in 27 dogs of different ages and breeds, with some type of hepatobiliary illness, which have been treated with classical methods and alternative with Aloe vera barbadensis gel and propolis.

Tabel 1.

Hepatic illness diagnosed in dogs and treatment

Disease	***Medical Treatment***	***Apiphytotherapy***
Acute hepatitis	*Case 1,18*	*Cases 11,13,20*
Cholestasis	*Case 4,21*	*Cases 8, 10, 12,27*
Liver failure	*Case 3,19*	*Cases 6, 14, 17,22,26*
Liver cirrhosis	*Case 2,24*	*Cases 15, 16,23,25*

Tabel 2.

Apiphytotherapy – daily doses

Details	***Liver failure***	***Cholestasis***	***Liver cirrhosis***	***Acute hepatitis***
Small dogs	*20ml/10kg*	*25ml/10kg*	*25ml/10kg*	*25ml/10kg*
Small dogs	*15ml/10kg*	*15ml/10kg*	*20ml/10kg*	*20ml/10kg*
Small dogs	*5ml/10kg*	*15ml/10kg*	*20ml/10kg*	*20ml/10kg*

The laboratory exams have followed the values of bilirubin, transaminase activity (ALAT, GGT) and alkaline phosphatase from blood and plasma, and the determination of a complete exams to the urine (COMBUR – TEST). The tests had been repeated weekly.

The normal values in dogs were as follow:

- bilirubin: < 0,61 mg/dl;
- ALAT transaminase: < 40 U/l;
- GGT transaminase: < 5U/l;
- alkaline phosphatase (PAL): 20 – 150 U/l;
- total protein: 5,4 – 7,7 g/dl;
- albumin: 45 – 3,7 g.dl;
- bilirubinuria +.

Therapeutic protocol was setted depending on each cases for classical treatment and the apiphytotherapy with Aloe vera gel from Forever Living Products as follow 15 – 20ml/10 kg body weight daily in three “pro dosis” with 30 – 60 minutes before meal , during 44 days.

Results

The evaluation of apiphytotherapy efficiency in dogs with pancreatic diseases, with different degrees of illness was made based on laboratory analyses results, before and after 44 days treatment.

Biochemical investigations were made before classical treatment and apiphytotherapy and after 44 days of classical and apiphytotherapy treatment.

Tabel 3

The results of biochemical investigations before and after 44 days treatment in dogs with liver failure

Parameter	*Initial value*					*Medical treatment*		*Apiphytotherapy*				
	Case 3	*Case 6*	*Case 9*	*Case 14*	*Case 17*	*Case 3*	*Case 9*	*Case 6*	*Case 14*	*Case 17*	*Case 22*	*Case 26*
Bilirubin (mg/dl)	*0.7*	*0.9*	*1*	*1*	*0.8*	*0.6*	*0.5*	*0.3*	*0.3*	*0.5*	*0,4*	*0,5*
ALAT (U/l)	*78*	*80*	*85*	*83*	*69*	*67.8*	*60*	*51.5*	*41.2*	*33*	*36*	*49*
PAL(U/l)	*105*	*108*	*100*	*115*	*107*	*76*	*62*	*60*	*91*	*84*	*86*	*79*

Tabel 4

The results of biochemical investigations before and after 44 days treatmemt in dogs with cholestasia

Parameter	*Initial value*					*Medical Treatment*		*Apiphytotherap*			
	Case 4	*Case 21*	*Case 8*	*Case 10*	*Case 27*	*Case 4*	*Case 21*	*Case 8*	*Case 10*	*Case 12*	*Case 27*
Bilirubin (mg/dl)	*1.6*	*2.1*	*1.1*	*0.8*	*2.5*	*0.8*	*0.85*	*0.6*	*0.5*	*0.85*	*0,75*
ALAT (U/l)	*103.5*	*108*	*102*	*105*	*107*	*86.8*	*68*	*79*	*55*	*36*	*49*
PAL(U/l)	*107*	*112*	*105*	*60*	*115*	*64*	*58*	*41*	*53*	*60*	*51*
GGT (U/l)	*11.24*	*12*	*10*	*10.2*	*13.3*	*9.8*	*7.5*	*4.1*	*6.4*	*4.2*	*5,1*
Bilirubinuria	+++	++	++	+++	+	+	+	+	+	+	+

Interesting results in apiphytotherapy were observed in dogs with liver failure where bilirubin and transaminase values were normal and even superior to classical treatment values.

Tabel 5

The results of biochemical investigations before and after 44 days treatment in dogs with acute hepatitis

Parameter	*Initial value*					*Medical Treatment*		*Apiphytotherapy*		
	Case 1	***Case 18***	***Case 11***	***Case 13***	***Case 20***	***Case 1***	***Case 18***	***Case 11***	***Case 13***	***Case 20***
Bilirubin (mg/dl)	*2,7*	*2,2*	*3*	*2,5*	*2,7*	*2,2*	*1,9*	*0,85*	*1,1*	*0,9*
ALAT (U/l)	*160*	*180*	*165*	*140*	*180*	*88*	*109*	*68*	*36*	*48*
PAL(U/l)	*105*	*130*	*107*	*105*	*128*	*77*	*88*	*69*	*85*	*7,5*
GGT (U/l)	*11*	*12*	*12*	*11,3*	*12*	*9,1*	*8,1*	*9,4*	*8,7*	*7,8*
Albumin (g/dl)	*1,2*	*1,5*	*2,1*	*2,5*	*1,6*	*2,6*	*2,7*	*2,44*	*2,95*	*2,75*
Bilirubinuria	*++*	*++*	*++*	*++*	*++*	*+*	*+*	*+*	*+*	*+*

Tabel 6

The results of biochemical investigations before and after 44 days treatment in dogs with liver cirrhosis

Parameter	*Initial value*				*Medical Treatment*		*Apiphytotherapy*			
	Case 2	***Case 15***	***Case 16***	***Case 24***	***Case 2***	***Case 24***	***Case 15***	***Case 16***	***Case 24***	***Case 25***
Bilirubin (mg/dl)	*2,7*	*2,2*	*3*		*2,2*		*0,8*	*0,9*	*0,7*	*0,8*
ALAT (U/l)	*160*	*180*	*165*		*88*		*59*	*56*	*58*	*59*
PAL(U/l)	*105*	*130*	*107*		*77*		*101*	*94*	*100*	*99*
GGT (U/l)	*11*	*12*	*12*		*9,1*		*2,4*	*2,8*	*2,7*	*2,5*
Albumin (g/dl)	*1,2*	*1,5*	*2,1*		*2,6*		*4,96*	*4,7*	*4,8*	*4,8*
Bilirubinuria	*++*	*++*	*++*		*+*		*+*	*+*	*+*	*+*

In cholestasia apiphytotherapy results were more significant then classical treatment (studied parameters were normal).

In acute hepatitis and cirrhosis after 44 days treatment with *Aloe vera gel,* the laboratory results showed a significant decrease, but the necessity of continuing the apiphytotherapy still exists till the values are normal and the healing is observed.

The laboratory analyses results proved the efficiency of apiphytotherapy in all 4 casses of hepatic diseases.

The analyses results can't be reported to another experiments (studies) in dogs with hepatic diseases by using propolis and *Aloe vera gel*, but some of them can be written only after 14 days of classical treatment.

The biochemical investigations results in dogs (table 3) with liver failure showed after 14 days apiphytotherapy a decreased level of bilirubin (0.3 – 0.5 mg/dl) compare to medical treatment values (0.5 – 0.6 mg/dl) and ALAT transaminase values in apiphytotherapy (33 – 51,5) compare to values from medical treatment (60 – 67,8 U/l).

In dogs with cholestasis the biochemical investigations showed almost the same values both in classical and *Aloe vera gel* apiphytotherapy of bilirubin from 0.5 – 0,85 mg/dl to 0.8 – 0.85 mg/dl.

GGT values were between 4.1 – 6.5 U/l in classical treatment and between 7.5 – 9.8 U/l in fitotherapy. ALAT transaminase showed a decreased value in apiphytotherapy (36 – 79 U/l) compare to values of 68 – 86 U/l in medical treatment. Alkaline phosphatase showed an important decrease through both methods of treatment: classical and propolis *Aloe vera gel* treatment. This showed that the apiphytotherapy is efficient after 6 weeks treatment.

In dogs with acute hepatitis the decreased values of bilirubin were 0.85 – 1.1 mg/dl compare to 1.9 – 2.2 mg/dl and of ALAT transaminase were 36 – 38 U/l compare to 88 – 101 U/l.

Albumin, alkaline phosphatase, GGT and bilirubinuria values are almost the same in both ways of treatment, apiphytotherapy and classic, showed us the necessity in continuing the therapy with *Aloe vera gel* as long as it is necessary for healing the organism.

In dogs with liver cirrhosis apiphytotherapy results showed a decreased of ALAT transaminase value of 56 – 59 U/l (40 U/l in classical treatment) and an increased of protein values and under normal values to albumin and a decreased of bilirubin value of 0.9 mg/dl.

The efficiency of classical treatment and Aloe vera gel therapy can't be proved after 44 days treatment, so that it is necessary for a long period of time of treatment, even months, till a full recovery of the body.

The decreased values are correlated to the clinical state of the animal from the experiments.

The investigations should be made on a larger number of animals and for a longer period of time in animals with hepatic liver cirrhosis, but the biochemical results suggest an associated treatment: apiphytotherapy and classic.

Conclusion

From the blood tests evaluation, the nutritional *Aloe vera gel* therapy results and the classical treatment results in dogs with liver or hepatic diseases, we came to the conclusion that:

1. Te apiphytotherapy methods with *Aloe vera gel* in hepatic diseases showed almost the same efficiency as in classical treatment;
2. I dogs with liver failure, after 14 days treatment, both apiphytotherapy and classic, it's showed a value of bilirubin as under 0.61 mg/dl and a decreased value of ALAT activity (33 – 51,5 U/l) compare to 60 – 67,8 U/l values in medical treatment.
3. In dogs with cholestasis it's showed a decreased value of bilirubin in apiphytotherapy (0.5 – 0.85 mg/dl) compare to values from classical treatment (0.8 – 0.85 mg/ dl), and GGT decreased values in apiphytotherapy (4.1 – 6.4 U/l) compare to medical treatment values (7.5 – 9.8 U/l) and ALAT transaminase values (36 – 79 U/l) compare to 68 – 86 U/l values from medical treatment.
4. In dogs with acute hepatitis, after apiphytotherapy the bilirubin values were 0.85 – 1.1 mg/dl compare to 1.9 – 2.2 mg/dl, and a decreased ALAT value (36 – 68) compare to 88 – 109 U/l in classical treatment.
5. In liver cirrhosis, in dogs apiphytotherapy and classical treatment showed a decreased values of ALAT (56 – 59 U/l) and bilirubin (0.8 – 0.9 mg/l) and an increased values of

albumin (2.4 – 2,8 g/dl) and protein (4.7 – 4.96 g/dl) values. It's showed that the results are almost the same in both ways of treatment, with the necessity of lasting the treatment to months;

6. Fitoterapia used a principle based on detoxifing and regenerating efects of *Aloe vera gel* to the hepatic cell, so as to stimulate the immunity of the organisme;
7. *Propolis and Aloe vera gel* apiphytotherapy (barbadensis) as a nonagresive support of hepatic cells functions and regeneration does not exclude the using of classical therapy, the purpose being the full recovery of the organism.

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Apiphytotherapy with Propolis and Aloe Vera gel in canine pancreatic diseases

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The apiphytotherapy with propolis and Aloe vera barbadensis gel is a guarantee for health maintenance and treatment of some disease, by detoxifing the organism and by stimulating the pancreatic functions. The project was built by tracing and comparising the evolution of the cases that were diagnosed with some type of pancreatic illness (acute pancreatic failure, chronic pancreatic failure, diabetes mellitus), treated both by classical (traditional) and alternative methods of therapy, during 14 days of treatment, in 18 dogs of different ages and breeds, monitorising the efficiency by clinical and paraclinical repeated tests (glucose levels, total amylase levels and pancreatic amylase and ALAT activities creatinine, cholesterol, HDL cholesterol and calcium seric concentrations).

The laboratory analyses results proved the efficiency of propolis and Aloe vera gel therapy in canine acute pancreatits: total amylasaemia decreased from 751 – 1800 to 267 – 615 U/l, pancreatic amylase activity from 251 – 618 U/l to 70 – 213 U/l, the glucose levels decreased from 110.7 – 164.5 mg/dl to 88.6 – 116.8 mg/dl, ALAT activities decreased from 48 – 85.8 U/l to 30 – 42.1 U/l, and cholesterol from 120 – 355 mg/dl to 101 – 217 mg/dl.

The apiphytotherapy methods were at least equaly efficient as the traditional therapy, which proved the importance of nonagresive support of pancreatic cell functions and regeneration.

In chronic pancreatitis, total amylase decreased from 1592 – 2333 U/l to 545 – 683 U/l, pancreatic amylase astivity from 311 – 628 U/l to 170 – 205 U/l, seric glucose decreased from 91 – 144.5 mg/dl to 78 – 96.5 mg/dl, ALAT activity from 38.6 – 109 U/l to 12.1 – 45.1 U/l, and total seric calcium from 12.1 – 14.4 to 9.4 – 11.6 mg/dl show nutritional Aloe vera gel and propolis therapy superior efficiency in comparision with classical drug therapy after 14 days of treatment.

In diabetes mellitus the results of nutritional propolis and Aloe vera gel therapy are near of classical (traditional) treatment, but necessitated a much longer period of therapy (months), the principal biochemical ranges analised show the currative tendency (decreases of total amylase and glucose levels, pancreatic amylase and ALAT activity, signifiant decrease of tryglicerid and total cholesterol).

Key Words: apiphytotherapy, propolis, Aloe vera, pancreatic diseases

The development of the interest on the world plane for alternative and complementary medicine, especially for fitotherapy, made us to study more the Aloe vera gel therapy efficiency in pancreatic disease in dogs.

Unlike clasic fitotherapy with fresh plants who uses fresh gel, obtained from *Aloe vera* plant (barbadensis), modern therapy uses gel, withont chemicals as *Aloe vera gel* obteined and stabilized by Forever living Products, associated with propolis extract.

Favourable results on tissues and organs have been studied after six weeks treatment in three daily doses, the daily dose being as 15 – 30 ml/10 kg/day apiphytoterapeutic product.

Researches have been made for the experimental efficiency of *propolis and Aloe vera gel* in animals, so as classical therapy in detoxifying and cure the organism, and a short time for recovery in different diseases.

Materials and methods

Clinical investigations, echograpy exams and paraclinical exams have been made in the Faculty of Veterinary Medicine in Bucharest on 18 experimental cases.

Researches have been made in 18 dogs of different ages and breeds, with some type of pancreatic illness, which have been treated with classical methods and alternative with Aloe vera gel (barbadensis) and propolis.

Tabel 1.

Pancreatic diseases diagnosed in dogs and the treatment

Disease	***Classical Treatment***	***Propolis and Aloe vera gel apiphyitotherapy***
Acute pancreatitis	*Cases 2,4*	*Cases 8, 10, 12, 16*
Chronic pancreatitis	*Cases 3,5*	*Cases 9, 11, 18, 15*
Diabet mellitus	*Cases 1,6*	*Cases 7, 13, 14, 17*

Tabel 2.

Propolis and Aloe vera gel apiphyitotherapy – daily doses

Details	***Chronic pancreatitis***	***Acute pancreatitis***	***Diabet mellitus***
Big dogs	*40ml/10kg*	*80ml/10kg*	*60ml/10kg*
Middle dogs	*25ml/10kg*	*50ml/10kg*	*40ml/10kg*
Small dogs	*15ml/10kg*	*30ml/10kg*	*20ml/10kg*

The laboratory exams have followed the values results of total amylaseaemia, of pancreatic amylase, glucose levels and ALAT activities from the blood and plasma. The tests had been repeated weekly.

The normal values in dogs were as follow:

- total amylase: 100 – 600 U/l (37^0C)
- pancreatic amylase: 50 – 200 U/l (37^0C)
- glucose: 75 – 126 mg/dl
- ALAT: 5 – 40 U/l (37^0C)
- tryglicerid: 50 – 100 mg/dl
- cholesterol: 115 – 315 mg/dl
- HDL cholesterol: 20 – 40 mg/dl
- creatinine: 0.5 – 1.8 mg/dl

Therapeutic protocol was setted depending on each cases for classical treatment and the apiphytotherapy with *propolis and Aloe vera gel* from Forever Living Products as follow 15 –

30ml/10 kg body weight daily in three “pro dosis” before meal with 30 – 60 minutes , during 14 days.

Results

The evaluation of *propolis and Aloe vera gel* fitotherapy efficiency in dogs with pancreatic diseases, with different degress of illness was made based on laboratory analyses results, before and after 14 days treatment.

Biochemical investigations were made before and after 14 days of classical and apiphytotherapy treatment (table 3 – 5).

Tabelul 3

The results of biochemical investigations before and after 14 days treatment in dogs with acute pancreatitis

Parameter	***Initial Value***						***Classical treatment***		***Propolis and Aloe vera gel apiphytotherapy***			
	Case 2	***Case 4***	***Case 8***	***Case 10***	***Case 12***	***Case 16***	***Case 2***	***Case 4***	***Case 8***	***Case 10***	***Case 12***	***Case 16***
Total amylase U/l (37^0C)	1200	751	1330	1600	991	1800	605	601	101.1	114.2	105.5	88.6
Pancreatic amylase U/l (37^0C)	420	251	505	601	388	618	186	226	198	140	70	213
Glycemia mg/dl	110.7	150.2	132.4	156.2	164.5	141.4	95.1	125.5	101.1	114.2	105.5	88.6
ALAT U/l (37^0C)	56.1	49.4	48.2	76.4	85.8	59.4	44.1	39.5	30.2	38.5	42.1	33.6
Total cholesterol mg/dl	120.5	318.1	298	336	217	355	98.5	156	101	115	156	217
HDL cholesterol mg/dl	20.4	28.6	30.1	25.2	34.6	21.5	29.5	35.5	39	42	44.5	52
Tryglicerids mg/dl	56.1	49.4	48.2	76.4	85.8	59.4	44.1	39.5	30.2	38.5	42.1	33.6

Tabel 4

The results of biochemical investigations before and after 14 days treatmemt in dogs with acute pancreatitis

Parameter	***Initial Value***						***Classical treatment***		***Propolis and Aloe vera gel apiphytotherapy***			
	Case 3	***Cas 5***	***Case 9***	***Case 11***	***Case 18***	***Case 15***	***Case 3***	***Case 5***	***Case 9***	***Case 11***	***Case 18***	***Case 15***
Total amylase U/l (37^0C)	1688	911	1900	1891	1592	2333	712	688	615	606	545	683
Pancreatic amylase U/l (37^0C)	428	311	565	609	388	628	204	233	180	199	170	206
Glycemia mg/dl	144.5	121.6	90.7	130.2	112.4	136.8	95.5	91.1	81.2	96.5	78.2	89.2
ALAT U/l (37^0C)	58.8	88	38.6	79.3	46.2	109	38.8	48.8	12.1	33.4	21.6	45.1
Calcium seric mg/dl	12.1	14.4	12.9	11.6	10.2	13.3	9.4	12.1	11.6	9.4	11.1	11.5

Tabel 5

The results of biochemical investigations before and after 14 days treatment in dogs with diabet mellitus

Parameter	***Initial Value***						***Classical treatment***		***Propolis and Aloe vera gel apiphytotherapy***			
	Case 1	***Case 6***	***Case 7***	***Case 13***	***Case 14***	***Case 17***	***Case 1***	***Case 6***	***Case 7***	***Case 13***	***Case 14***	***Case 17***
Glycemia mg/dl	180	165	191	141	205	185	155	135.8	138.5	126.8	143.5	130.8
Total cholesterolmg/dl	424	328	301	350	497	398	325	270	256	160	288	255
Tryglicerids mg/dl	175	150	148	166	198	181	105	99.1	81	68	98	59
ALAT U/l (37^0C)	68.1	59.5	50.1	45.4	56.1	60.8	52	42.1	36	39	26.6	39.4
Total amylase U/l (37^0C)	1620	998	517	417	1788	1612	665	495	189	177	621	505
Pancreatic amylase U/l (37^0C)	501	400	298	206	594	569	196	120	88	70	204	180
Creatinine mg/dl	1.92	1.88	1.66	2.05	1.76	2.22	1.66	1.23	1.41	1.75	1.1	1.5

Interesting results in *Aloe vera gel and propolis* apiphytotherapy were observed in dogs with acute pancreatitis, where the values of total amylase, pancreatic amylase, glycemia, cholesterol and HDL cholesterol were normal and even superior to classical treatment values.

In chronic pancreatitis apiphytotherapy's results were more significant then classical treatment (studied parameters were normal).

In diabetes melletus after 14 days treatment with *propolis and Aloe vera gel,* the laboratory results showed a significant decrease, but the necessity of continuing the apiphytotherapy still exists till the values became normal and the healing is observed.

The laboratory analyses results proved the efficiency of propolis and Aloe vera gel therapy in all 3 casses of pancreatic diseases.

The analyses results can't be reported to another experiments (studies) in dogs with pancreatic diseases by using *propolis and Aloe vera gel*, but some of then can be written only after 14 days classical treatment.

The biochemical investigations results in dogs (table 3) with acute pancreatitis showed, after 14 days apiphytotherapy, a decreased level of total amylase (267 – 615 U/l)compare to classical treatment (601 – 605 U/l), pancreatic amylase showed a decreased level (70 – 213 U/l) compare to medical treatment (186 – 226 U/l), ALAT transaminase level (30 – 42.1 U/l)showed a decrease compare to classical treatment (39.5 – 44.1 U/l) and a normal level of blood sugar (glucose) in *Aloe vera gel* treatment (88 – 114.2 mg/dl) compare to medical treatment (95.1 – 125.5 mg/dl).

In dogs with chronic pancreatitis the biochemical investigations showed almost the same values both in classical and *propolis and Aloe vera gel* therapy: glycemia (blood sugar) 81.2 – 96.5 mg/dl and 91.1 – 95.5 mg/dl calcemia 9.4 – 11.6 mg/dl and 9.4 – 12.1 mg/dl, pancreatic amylase 170 – 206 U/l and 204 – 233 U/l.

Total amylase activity values were between 545 – 683 U/l in dogs treated with propolis and Aloe vera gel and between 688 – 712 U/l in dogs after a classical treatment. ALAT transaminase showed a decreased from 12.1– 45.1U/l to 38.8 – 48.8 U/l. After six weeks of propolis and Aloe vera gel apiphytotherapy the efficiency is almost the same as in classical treatment.

In dogs with diabetes mellitus the blood sugar values showed a decreased in fitotherapy (127– 143.5 mg/dl) compare to values showed in classical treatment (135.8 – 155 mg/dl) and also a decreased value of the total amylase activity in fitotherapy (177 – 621 U/l) compare to values from classical treatment (495 – 665 U/l).

The values of pancreatic amylase activity, cholesterol and tryglicerid are almost the same in both ways of treatment, apiphytotherapy and classic, showed us the necessity in continuing the *propolis and Aloe vera gel* apiphytotherapy as long as it necessary for healing the organism.

The efficiency of classical treatment and propolis and Aloe vera gel apiphytotherapy can't be proved only after 44 days treatment, so that it is necessary a long period of time of treatment, may be months, till a complete healing.

The decreased values are correlated to the clinical state of the animals from this experiment.

The investigations should be made on a number of animals and for a long period of time in animals with diabetes mellitus, but the biochemical results suggest an associated treatment: apiphytotherapy and classical one.

Conclusions

From the blood tests evaluation, the nutritional *propolis and Aloe vera gel* therapy results and the classical treatment results in dogs with pancreatic diseases, came to the conclusion that:

1. The apiphytotherapy methods with *propolis and Aloe vera gel* in pancreatic diseases showed almost the same efficiency as in classical treatment;
2. In dogs with acute pancreatitis, after 14 days of apiphytotherapy and classic treatment , it's showed a decreased of blood sugar values under 125.5 mg/dl, total cholesterol under 217

mg/dl, total amylase activity under 615 U/l, pancreatic amylase under 226 U/l and ALAT activity under 44.1 U/l.

3. In dogs with chronic pancreatitis are showed normal values of calcemia (under 12.1 mg/dl) and creatinine (under 1.66 mg/dl) and total amylase activity showed a decreased in *propolis and Aloe vera gel* apiphytotherapy (545 – 683 U/l) compare to medical treatment values (688 – 712 U/l) and a decreased value of pancreatic amylase in fitotherapy (170 – 206 U/l) compare to values showed in classical treatment (204 – 233 U/l).
4. In dogs with diabetes mellitus after apiphytotherapy the glycemia values showed a normal tendency of recovery (under 143.5 mg/dl), the cholesterol, tryglicerid and pancreatic amylase activity values become normal after *propolis and Aloe vera gel* apiphytotherapy.
5. Apiphytotherapy used a principle based on detoxifing and regenerating efects of *propolis and Aloe vera gel* to the hepatic and pancreatic cells, so as to stimulate the immunity of the organisme;
6. Apiphytotherapy as a nonagresive support of pancreatic cells functions and regeneration does not exclude the using of classical therapy, the purpose being the full recovery of the organism.

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Multimodal approach of dehorning pain management using metamizol in two month old calves

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There is a lack of information on acute and long term dehorning pain management with pyrazolone drugs and in the same time there is an increased interest in human treatment of animals. Because dehorning is considered today a "high pain" procedure researches into ways for reducing pain and the associated stress improve finally calf welfare.

As part of the multimodal approach to treating pain in the intra- and postoperative period it be used a mild analgesic metamizol. The objectives of study was to evaluate the capacity of metamizol associated with regional block and sedation to reduce the immediate pain associated with dehorning and the pain experience in the hours that follow measuring changes in plasma cortisol concentrations and assessing the behavior changes.

The study was carried out on 16 two month old Friesian cross breed calves, distributed in two groups (control and experimental). Blood samples were collected before and after dehorning and at 2, 4 and 6 hours later. Plasma cortisol was determinated using an enzyme immunoassay kit. All calves were sedated with diazepam. The corneal nerve block was performed with lidocaine. At the same time and at 2 hours after dehorning metamizol was administrated by intravenous route in the "experimental" calves. The combined technique (surgical removal followed by chemical cauterization) it was used for dehorning. Calf behavior was recorded. Feeding or drinking and rumination were recorded as being present or absent. Student's t-tests, and nonparametric chi-square test were used for analysis of collected data. Significance was set at $P < 0,05$.

The increase in cortisol concentration and behavior modification in the control group shown the pain and stress effects of used dehorning procedure. Dual administration of metamizol and local anesthetic represent a useful way for an efficient long-term pain control in dehorning two month old cattle.

Key Words: calves, pain management, metamizol

By one hand there is a lack of information on acute and long term dehorning pain management with pyrazolone drugs and by the other hand, there is an increased interest in human treatment of animals. Because dehorning is considered today a "high pain" procedure (2, 3, 5, 14, 15), researches into ways for reducing pain and the associated stress improve finally calf welfare.

As part of the multimodal approach (1, 12) to treating pain in the perioperative period it be used a mild analgesic metamizol (pyrazolone drug group).

The objectives of the present study was to evaluate the capacity of metamizol associated with regional cornual nerve block and sedation to reduce the immediate pain associated with dehorning and the pain experience in the hours that follow, in young dairy calves, measuring changes in plasma cortisol concentrations and assessing the behavior changes.

Materials and methods

The study was carried out on 16 two moth old Friesian cross breed calves (body weight (bw) = 52±3 kg), distributed in two groups (control and experimental), housed in individual pens having free access to hay and water. A 16 gauge catheter was inserted into the jugular vein of each individual 24 h before the beginning of the experiment. Blood samples were collected before and after dehorning and at 2, 4 and 6 hours later. All calves were sedated with diazepam 0,05%, 1 mg/bw, administrated by intravenously (IV) route for reducing the handling stress. When sedation was obviously and a with 10 minutes prior to start of surgical procedure, the regional corneal nerve block was performed with 3 ml of lidocaine 2%. At the same time and at 2 hours after dehorning metamizol (500 mg/ml) was administrated by IV route at a volume of 5 ml/animal in the "experimental" calves. The combined technique (6) (surgical removal using a scalpel followed by 20 seconds of chemical cauterization of the site) it was used for dehorning.

Blood was collected in heparinized tubes and centrifuged. The tubes containing plasma were stored at –20°C until cortisol was determinated using an enzyme immunoassay kit (Cortisol ADALTIS). All samples were tested in duplicate.

Calf behavior was recorded in individual watching sheet for 6 hours, in each hour 20 minutes of 5-minute period of time. Behavioral responses to pain, such as ear flicking, head shaking, head rubbing and vocalization, were recorded as the number of times each activity occurred during each 5-minute period of observation. Feeding or drinking and rumination were recorded as being present or absent in observation period.

Student's t-tests and nonparametric chi-square test were used for analysis of collected data. Significance was set at $P < 0,05$ and all values are given as the mean ± standard deviation of the mean.

Results

Plasma cortisol levels measured before and after dehorning in the experimental and control group are shown in figure 1. Compared to baseline (before dehorning), cortisol increased rapidly and significantly immediately after dehorning in both groups, and reach their maximal levels after dehorning in experimental group (5,18±1,92 ng/ml) and at 2 hours lather in the control group (19,08±1,37 ng/ml). Afterward, a gradual decrease in cortisol concentration was observed in experimental group, the difference being significant at 2, 4 and 6 hours later (p=0,0008, 0,0001 respectively $4,3x10^{-5}$). In the control group plasma cortisol concentrations were still significantly higher, than basal and the level reached immediately after dehorning, at 4 and 6 hours later (11,7±4,25 respectively 9,18±1,64 ng/ml) having the significance of pain.

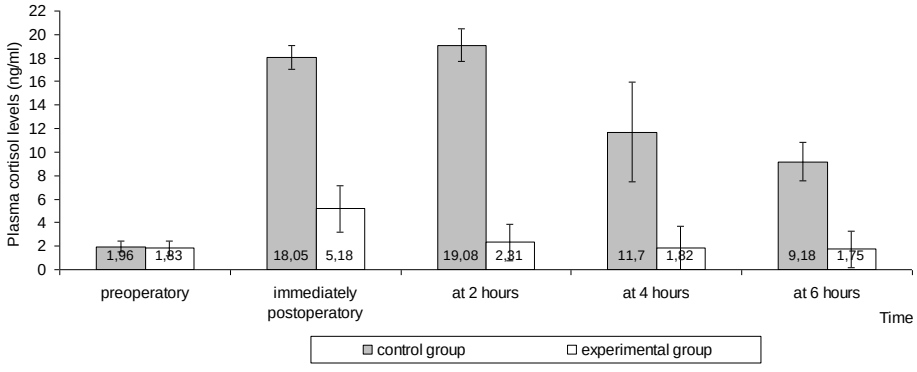

Figure 1. Plasma cortisol levels during six hours observation period (mean ± standard deviation of the mean)

Differences between groups regarding head shaking or ear flicking was no statistically significant, but control animals demonstrated higher frequencies of these behaviors, both responses peaked between 2-4 hours interval of time after dehorning. A low frequency of head rubbing was observed in both treatment and control groups, and only few vocalizations were recorded in the control group, but without statistical significance. There is little response during the first few hours by either group, as all animals in this experiment received both a sedative and a local anesthetic before dehorning. A statistically significant effect of metamizol treatment on the other behavioral measures, feeding and rumination, was recorded. One half of calves belong to this group start rumination and feeding in 2 hours after dehorning, and only one in 6 hours, compared with control group in which nobody were interested in eating and do not ruminate. However as the behaviors of all calves were similar along 6 hours after dehorning, it suggest that these agents reduced the pain experienced, but measured cortisol level suggest that did not eliminate it. In the control group lake of rumination and absence of food intake are considered to characterize an animal in pain, this is supported by the increased levels of cortisol as well too.

Our results has shown that a systemic sedative (diazepam) eliminate calf response to the injection of the local anesthetic and the need for physical restraint during this injection and during dehorning. The sharp peaks in cortisol level assessed immediately after dehorning indicates a physiological response to surgical stressors and these results were similar to those observed by other authors (3, 5, 7). Our results that the cortisol level is still high 6 h after dehorning in control group is alike with that of other studies (7, 8, 10, 14).

Physiological and behavioral changes registered in control group shown that lidocaine, amide local anesthetics, reduce both the physiological and the immediate behavioral responses, but this effect lasts only for 1,5-3 hours (14) and does not provide pain relief in the hours following dehorning. In the experimental group response to combined procedure dehorning performed under treatment with a metamizol, sedative and local anesthetic is different. Behavioural changes corelated with cortisol dynamic indicate the efficacy of used drugs combination, the pain responses are greatly reduced. The cortisol response was significantly lower after metamizol and local anesthetic dual administration and comparable with results obtained by NSAIDS using (9). This confirms the concept of the multimodal approach to preventing and treating pain. The mechanism of action for the mild analgesics is controversial (11), metamizol inhibit the cyclooxygenase (COX) enzyme of arachidonic acid metabolism, COX-3, may be a site of action of these drug, resulting in a number of antiinflammatory, antipyretic, and analgesic effects, and may represent with other analgesics (paracetamol, salicylate and pyrazolone drugs) a distinct class of atypical NSAIDs. (4). In some studies (10, 13, 14) there is the evidence that cauterization may help to decrease postoperative pain, and because the used dehorning method includes chemical cauterization we assume therefore that the beneficial effect of metamizol could be increased.

Conclusions

1. The increase in cortisol concentration and behavior modification in the control group shown the pain and stress effects of used dehorning procedure.
2. Dual administration of metamizol and local anesthetic represent a useful way for an efficient long-term pain control in dehorning two mo-old cattle.

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Clinical signs of otitis externa at carnivores

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Otitis externa reperesents an acute or cronic inflammation of the external auditory meatus, that occur in response to any insult to the ear canal epithelium.

Otitis externa at carnivores is an frequent and frustrating problem that can lead to otitis media if is not adequately treated. For an adequate treatment it is important to establish the causative factors and the evolutive stage. Depending on evolutive stage and clinical signs, otitides are classified in: erytematous otitis, ceruminous otitis, exsudative otitis, suppurative otitis, ulcerative otitis and proloferative otitis.

General clinical signs of otitis externa are auricular and periaural pruritus and pain, head shaking, and progressive inflammatory changes of epithelium.

Key Words: otitis externa, dog, cat, clinic

Otitis externa is the most common disease of the canine and feline ear canal, which involves an acute or cronic inflammation of the epithelium of the external auditory meatus.

GRONO, 1980, classify cases of otitis as reactive or infective. Reactive otitides are characterized by acute erytematous reactions, but also may include proliferative forms. Infective otitides include acute or chronic purulent inflammations, ulcerative form and parasitic or fungal infections.

Although this classification is suitable for describing morphologic changes, it is not suitable for etiopathogenesis of this disease. Therfor, it is preferable to classify cases of otitis externa based on etiopathogenesis, pathologic changes and secondary microbial infections (1,3,5,6).

This paper includes investigations of clinical signs, corelated with etiopathogenesis, to elaborate an classification of otitides, necessary in medical management.

MATERIAL AND METHODS

The researche was perfomed on dogs and cats, attended in Clinics of Veterinary Medicine Faculty, Iasi.

To establish the evolutiv stage of otitis externa at carnivores we used general semiologic methods corelated with animal behaviour. By otoscopic examinations we determinated the presence of foreign bodies, the quantity and the aspect of otic exudate and the presence of ulcerations.

RESULTS

Although the investigations conducted to establish clinical signs of otitis externa at carnivores, reveals a great variety, the earliest clinical signs of otitis are auricular and periauricular pruritus and head shaking (foto 1). The head is tilt on affected ear in cases of unilateral otitis. The epithelium of external auditory meatus has an erytematous aspect (erytematous otitis-foto2). Erytematous otits is frequently the result of an hypersensitivity disease or keratinization disorder, therfor we have to identify the primary factors before treatment as GOTTHELF, 2005 recommends.

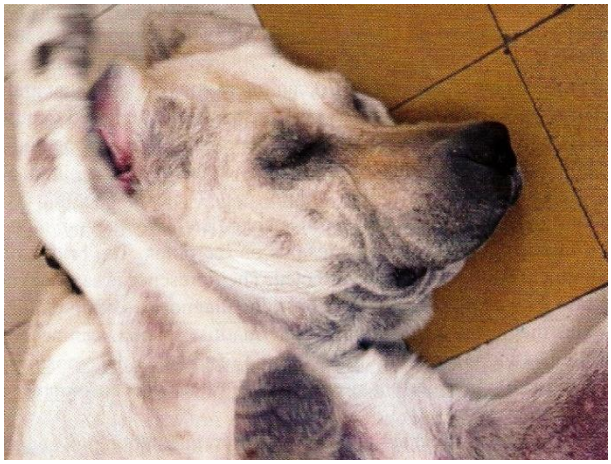

Foto 1. Auricular and periauricular pruritus

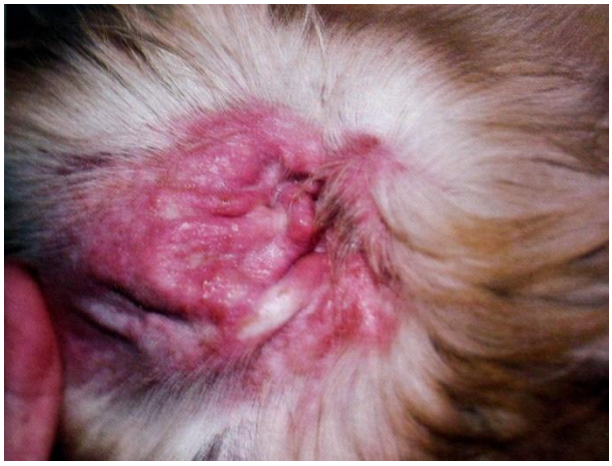

Foto 2. Erytematous otitis

If the inflammatory process evolves, the ear becomes hot and painful, the internal epithelium is congested and covered with an oily and yellow exudate (ceruminous otitis- foto 3).

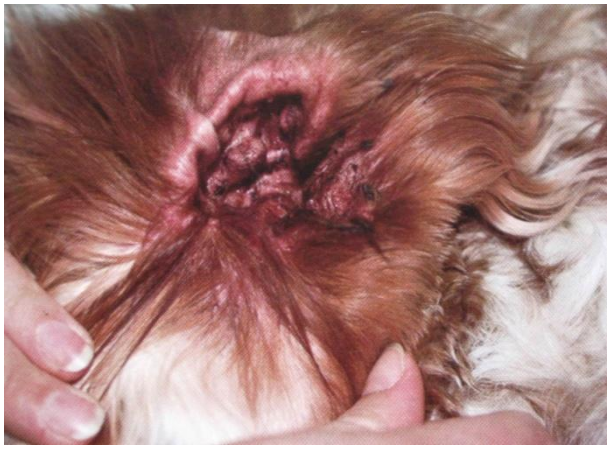

Foto 3. Ceruminous otitis

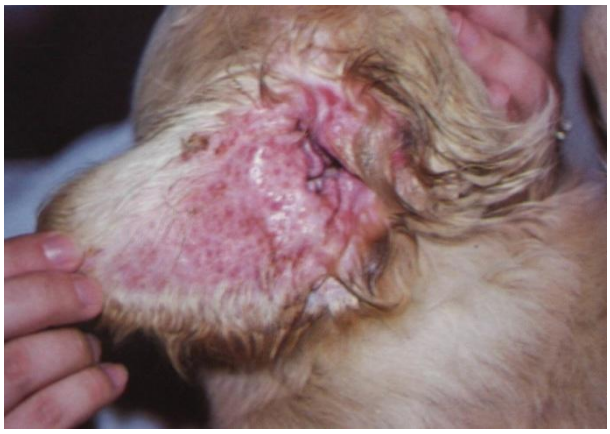

Foto 4. Exudative otitis

Exudative otitis is characterized by increases of fluid exudate discharge (foto 4). Violent head shaking and scratching the periaural region are clinical sings of this stage. Palpation of affected ear is very painful and we distinguish an specific sound produced by the fluid exudate (BURTAN,2000). An otoscopic examination reveals the presence of exudate and the congested aspect of internal epithelium. Depending on aspect of the exudate, we can suspicionate the causative factor (tabel 1).

Table 1

Corelation between aspect of exudat and causative factor

Aspect of exudate	Posibil causative factor
Dry, brown exudate	***Otodectes cynotis***
Oily, adherent, brown	***Malassezia canis***
Creamy, yellow-green	***Pseudomonas aeruginosa***
Purulent, grey, malodorous	***Staphilococcus ssp., Streptococcus ssp.***
Increases of ceruminous secretion	**Hypersensitivity diseases or disorders of keratinization**

If secondary microbial infections occur, otitis externa advance to suppurative stage. In suppurative otitis a malodorous liquid discharge is present. Some patients produce so much purulent exudate, that is overflows onto the periaural region (foto 5). In floppy-ear dogs, there will be dried exudate on the ear flap, adjacent to the external opening of the auditory canal.

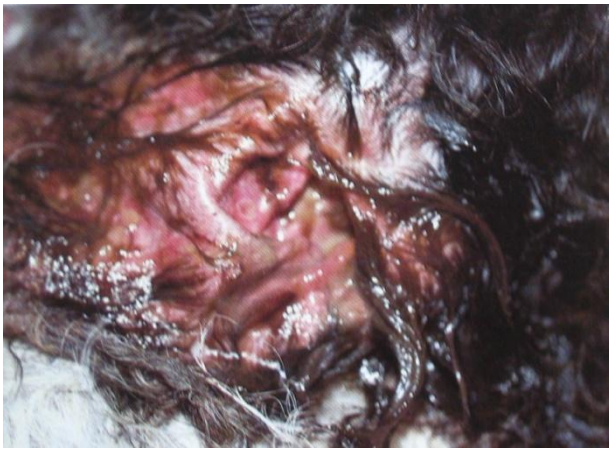

Foto 5. Suppurative otitis

General status of the patient is alterated, manipulation of the pinna or palpation of the base of the ear canal is very painful and the purulent exudate is malodorous and yellow-brown. Internal epithelium of the distal external ear canal becomes ulcerated, and the pain is maximum (ulcerative otitis-foto 6).

Ulcerative stage occurs in some cases of autoimmune diseases (Pemphigus vulgaris) or frequently, consequently the erosive actions of purulent exudate (1,4,7). The ulcerated zones are hiden under the thick pus, that is stored among internal tegument folds. Ear cleaning is very painful and at the cotton-tipped aplicator we can distinguish blood. The exudate is thick and brown to black because of the pus and the blood.

If the inflammatory process persist, obstructive proliferations occur (proliferative otitis-foto 7). Chronic inflammation within the ear canal causes dermal hyperplasia and atrophyof the sebaceous glans. The proliferative tissue changes resulting from persistent inflammatory otitis are a potent factor in the perpetuation of the disease, as AUGUST, 1988, noticed.

The results of this researche allow us to elaborate an classification of otitides, necessary in medical management. Depending on etiology, evolutive stage and clinical sings, otitides are classified in: erytematous otitis, ceruminous otitis, exudative otitis, suppurative otitis, ulcerative otitis and proliferative otitis.

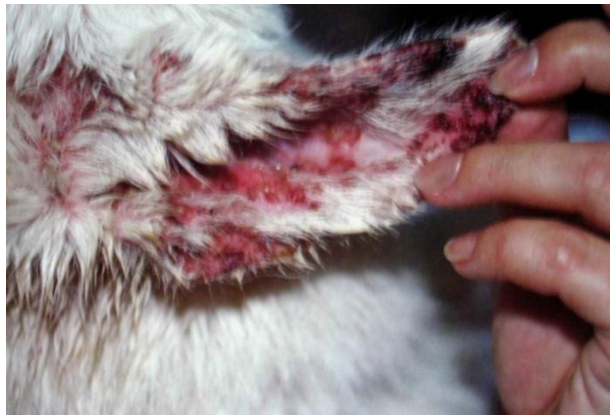

Foto 6. Ulcerative otitis

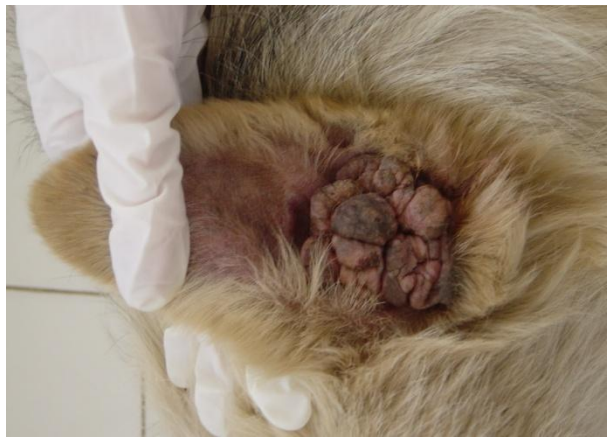

Foto 7. Proliferative otitis

CONCLUSIONS

1. Depending on evolutive stage and clinical signs, otitides are classified in: erytematous otitis, ceruminous otitis, exsudative otitis, suppurative otitis, ulcerative otitis and proloferative otitis.
2. The earliest clinical signs of otitis are auricular and periauricular pruritus and head shaking
3. Erytematous otits is frequently the result of an hypersensitivity disease or keratinization disorder
4. Depending on aspect of the exudate, we can suspicionate the causative factor.
5. If secondary microbial infections occur, otitis externa advance to suppurative stage.
6. Ulcerative stage occurs in some cases of autoimmune diseases (Pemphigus vulgaris) or frequently, consequently the erosive actions of purulent exudate.
7. The proliferative tissue changes result from persistent inflammatory otitis.

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Post-crisis surveillance for avian influenza in Romania

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An epidemiological survey of the influenza virus in wild birds has been conducted in Romania in October 2006, in the framework of the Technical Cooperation Programmes (TCP) of FAO, entitled "Emergency assistance for early detection and prevention of avian influenza in the Eastern Europe and Caucasus regions". Objectives were to evaluate the Avian Influenza prevalence, in particular highly pathogenic strains, among wild bird populations, including both migratory and resident bird species; and to provide technical support to the national surveillance programme through capacity building of national counterparts on sampling techniques.

Key Words: avian influenza, post-crisis surveillance

Incidence and characterization of some strains belonging to the genus *Streptococcus* coming from swine

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The research was carried out on a number of 283 swine, between 0 and 14 months old, originating from the individual households of the inhabitants across the area of Suceava county, over the period 2003 – 2005.

There were identified 3 strains of Streptococcus suis, and 2 strains of Streptococcus porcinus respectively, isolated from 55 samples, coming from 5 swine.

In order to identify the bacterial species, there were used the classical bacteriological methods and the rapid confirmation methods (The rapid ID 32 STREP system and the Lancefield method of rapid typing the A, B, C, D, F and G beta-hemolytic streptococci through agglutination in a special latex: SLIDEX STREPTO-KIT), being established an agreement between the results obtained from the 2 categories of tests.

The study of the spectrum and frequency of the infections caused by the bacteria of the genus Streptococcus at swine is in continuous dynamics and progress; it has both an economic importance, resulting from the fact that in the intensive swine breeding, the streptococcal infections lead to losses due to mortality, defects at animals, expenses with treatments and prevention, and a sanitary importance, mirrored by the worldwide registration of numerous human diseases (meningitis and, more recently, the streptococcal toxic shock syndrome) as effect of the contamination with streptococci from swine.

Some strains from the genus Streptococcus originating from swine were characterized completely and unambiguously by using the classical bacteriological methods and those of rapid confirmation, the diagnosis being thus established rapidly and accurately in view of initiating some treatments targeting the stocks of origin.

Key Words: pork meat, Streptococcus spp

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Some strains from the genus *Streptococcus* originating from swine were characterized completely and unambiguously by using the classical bacteriological methods and those of rapid confirmation, the diagnosis being thus established rapidly and accurately in view of initiating some treatments targeting the stocks of origin.

MATERIAL AND METHOD

The research was carried out on samples coming from a number of 283 swine, mixed breed animals from the breeds: Great White, Landrace, Bazna, Great Black, of which 208 were represented by dead animals.

The examined material was represented by pharyngeal secretions, cervix-vaginal secretions, abortions and placental linings, liquids from puncture in the lymph nodes, exudates from wounds, liquids from cavities, secretion from the conjunctiva and ears, organs (heart, lung, liver and spleen), brain and marrow.

The used work methods have been the following:

1. The authorized method of isolating and identifying the species within the genus *Streptococcus* (code 1.24).
2. The RAPID ID32 STREP System;
3. The Lancefield method of rapid stereotyping of A, B, C, D, F and G beta-hemolytic streptococci through rapid agglutination in special latex (Slidex Strepto-kit).

We proceeded to the isolation of the bacteria in the pathological materials, then to the identification of the species based on the cultural, microscopic, biochemical, serological and pathogenic characteristics for the experiment animals. The identification of the streptococci was done upon 32 enzymatic tests in miniature. Moreover, with the help of some antiserums, it was carried out the enzymatic extraction and the identification of the specific group antigens, the corresponding latex reactive remaining in homogenous suspension, being agglutinated respectively, depending on the absence or presence of the antigen.

RESULTS AND DISCUTIONS

Prevalence

There have been identified 3 strains of *Streptococcus suis*, and 2 strains of *Streptococcus porcinus* respectively, isolated from 55 samples, coming from 5 swine, the number of studied swine being 283, the total of examined samples being 1154. In percentages, those 5 cases of swine streptococci represent 1.77% of the studied cases.

Therefore, the incidence of streptococci at swine with *Streptococcus suis*, and *Streptococcus porcinus* respectively, in the individual households of the inhabitants across the area of Suceava county is of sporadic nature.

Those 5 isolated strains were initially named S1 – S5, being isolated from the dead swine marked with P1 up to P5, with the age of 6 weeks, 5 weeks, 11 weeks, 9 months, and 7 months respectively.

From the point of view of the necropsy, there have been found the following lesions:

Swine P1: fibrinous purulent pericarditis, myocardial degeneration, enlarged liver and spleen, with increased firmness and small necrotic centers in the parenchyma, congestion of the renal medullar area and a catarrhal-hemorrhagic enteritis, the mesenteric lymph nodes being increased in volume and hemorrhagic.

Swine P2: catarrhal-purulent inflammation of the nasal septum, purulent pleuro-broncho-pneumonia and hyperemia of the lymph nodes in the bronchia and mediastinum.

Swine P3: fibrinous purulent meningitis, with accumulation of exudates in the cerebral ventricles and hydrocephalus; the liver had necrotic centers.

Swine P4: in the mass of the lymph nodes under the mandible and at the back of the pharynx there were abscesses, with the diameter of 10 cm, containing creamy, grayish and smelly puss.

Swine P5: purulent inflammation of the lymph nodes under the mandible.

Identification of the isolated strains

Cultural characterization

The cultural features of the isolated strains were observed both in solid and in liquid medium, the incubation being made at 37°C, under conditions of aerobiosis, for 24 hours. The cultures in culture medium exhibited reduced or discrete turbidities, with many flocculent or abundant-flocculent deposits, and in the case of those in culture medium with serum, the medium remained clear with slight deposits; the cultures in jelly have generated small, round, mucous colonies with regulated edges, and small, convex, semitransparent, rare colonies respectively, while the cultures in Columbia agar have produced small, point-like, round colonies with regulated edges.

Morphological-tinctorial characterization

The morphological-tinctorial features of the isolated strains were observed in direct smears (created from pathologic materials) and in smears from cultures stained by means of Gram method. In the direct smears there were observed small, isolated, Gram-positive stained cocci grouped two by two, in short chains or in piles. The seams from the cultures in the culture media with serum exhibited small, Gram-positive cocci, grouped in chains. The seams from the cultures on solid media exhibited Gram-positive cocci, grouped two by two, in piles, in short chains or isolated.

Physiological and biochemical characterization

Table 1: Main identification tests of the species in the genus Streptococcus used in experimental research.

Increase to 10ºC	(-)	(-)	(-)	(-)	(-)
Increase to 45ºC	(-)	(-)	(-)	(-)	(-)
Increase with 6,5%NaCl	(-)	(-)	(-)	(-)	(+)
Increase with 0,25% optochin	(-)	(-)	(-)	(+)	(+)
Increase with 40% gall	(+)	(+)	(+)	(+)	(+)
Beta hemolysis	(-)	(+)	(-)	(+)	(+)
Alpha hemolysis	(+)	(+)	(+)	(-)	(-)
Hypurate hydrolysis	(-)	(-)	(-)	(-)	(-)
Esculin hydrolysis	(+)	(+)	(+)	(+)	(+)
Acid from D-ribose	(-)	(-)	(-)	(+)	(+)
Acid from D-mannitol	(-)	(-)	(-)	(+)	(+)
Acid from D-sorbitol	(-)	(-)	(-)	(+)	(+)
Acid from D-lactose	(+)	(+)	(+)	(-)	(-)
Acid from D-trehalose	(+)	(+)	(+)	(+)	(+)
Acid from D-raffinose	(+)	(+)	(+)	(-)	(-)
Acid from D-saccharose	(+)	(+)	(+)	(+)	(+)
Acid from D-arabitol	(-)	(-)	(-)	(-)	(-)
Acid from D-maltose	(+)	(+)	(+)	(+)	(+)
Acid from D-salicine	(+)	(+)	(+)	(+)	(+)
Acid from D-innuline	(+)	(+)	(+)	(-)	(-)
Acid from L-arabinose	(-)	(-)	(-)	(-)	(-)
Lancefield serogroup	D	D	D	E	E
Species	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5

In the end, all the species were tested with the RAPID ID 32 STREP System, for the confirmation of the identifications obtained.

Table 2: Identification of the studied strains with RAPID ID 32 STREP System.

Active ingredients	RAPID ID 32 STREP				
	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
ADH	(+)	(+)	(+)	(+)	(+)
βGLU	(+)	(+)	(+)	(+)	(+)
βGAR	(-)	(-)	(-)	(-)	(-)
βGUR	(+)	(+)	(+)	(+)	(+)
αGAL	(+)	(+)	(+)	(+)	(+)
PAL	(-)	(-)	(-)	(+)	(+)
RIB	(-)	(-)	(-)	(+)	(+)
MAN	(-)	(-)	(-)	(+)	(+)
SOR	(-)	(-)	(-)	(+)	(+)
LAC	(+)	(+)	(+)	(-)	(-)
TRE	(+)	(+)	(+)	(+)	(+)
RAF	(+)	(+)	(+)	(-)	(-)
VP	(-)	(-)	(-)	(+)	(+)
APPA	(+)	(+)	(+)	(+)	(+)
βGAL	(-)	(-)	(-)	(-)	(-)
PYRA	(-)	(-)	(-)	(-)	(-)
βNAG	(-)	(-)	(-)	(-)	(-)
GTA	(-)	(-)	(+)	(-)	(-)
HIP	(-)	(-)	(-)	(-)	(-)
GLYG	(+)	(+)	(+)	(-)	(-)
PUL	(+)	(+)	(+)	(+)	(+)
MAL	(+)	(+)	(+)	(+)	(+)
MEL	(-)	(-)	(-)	(-)	(-)
MLZ	(-)	(-)	(-)	(-)	(-)
SAC	(+)	(+)	(+)	(+)	(+)
LARA	(-)	(-)	(-)	(-)	(-)
DARL	(-)	(-)	(-)	(-)	(-)
MβDG	(+)	(+)	(+)	(+)	(-)
TAG	(-)	(-)	(-)	(-)	(-)
βMAN	(-)	(-)	(-)	(-)	(-)
CDEX	(-)	(-)	(-)	(-)	(-)
URE	(-)	(-)	(-)	(-)	(-)

Key:
(-) represents negative reaction; (+) represents positive reaction.

Fig. 1: RAPID ID 32 STREP Gallery incubated for 4 hours at 37ºC with Strain 5.

Testing the studied strains with RAPID ID 32 STREP System, we found out, as a result of consulting the identification table attached to the method, that there were confirmed the results obtained from the biochemical tests previously fulfilled.

Antigenic characterization

In order to establish if the studied strains belong to the Lancefield serogroups, it was used the rapid test for typing the A, B, C, D, F and G beta-hemolytic streptococci through rapid agglutination in a special latex.

The strains 1, 2, 3 gave imprecise results, and the strains 4 and 5 gave negative results. In conclusion, the studied strains do not belong to the A, B, C, D, F and G groups of beta-hemolytic streptococci.

The serotyping carried out after that, with group precipitated serums, lead to the following results: strains 1, 2 and 3 belong to D Lancefield serogroup, while the strains 4 and 5 belong to E Lancefield serogroup.

Experimental pathogenicity

In order to test the pathogenicity, 2 mice were inoculated in the peritoneum with cultures in culture media from those 5 strains incubated for 24 hours. In all the cases, the death of the mice occurred in 2-3 days.

At the necropsy, it was established that the mice had septicemia.

Testing the sensitivity to the anti-infectious substances

It was established that the sensitivity of the studied bacterial strains to the anti-infectious substances varied slightly, the strains being sensible to most of the used substances. It was observed the increased sensitivity to amoxicillin with clavulanic acid and to macrolides.

Table 3: Testing the sensitivity of the studied strains

Anti-infectious substance (µg)	Standard diameter of the inhibition area (mm)			Measured diameter of the inhibition area (mm)				
	R	I	S	1	2	3	4	5
Amoxycillin with clavulanic acid	< 14	14 ÷ 18	> 18	20	18	16	20	19
Ampicillin	< 18	19 ÷ 25	> 26	22	20	22	18	20
Azithromycin	< 13	14 ÷ 17	> 18	18	16	18	15	17
Cefotaxime	< 25	26 ÷ 27	> 28	30	28	26	24	28
Ceftiaxone	< 24	25 ÷ 26	> 27	26	25	27	26	24
Clarythromycin	< 16	17 ÷ 20	> 21	21	18	20	18	16
Clyndamicin	< 15	16 ÷ 18	> 18	16	16	18	20	17
Cloramphenicole	< 12	13 ÷ 17	> 18	17	18	14	16	18
Erythromycin	< 15	16 ÷ 20	> 21	18	21	20	18	20
Ofloxacin	< 13	13 ÷ 15	> 16	16	12	14	14	14
Penicillin			> 20	18	20	22	18	20
Rifampicin	< 16	17 ÷ 18	> 19	16	14	17	17	15
Streptomycin	< 11	12 ÷ 14	> 15	10	12	16	11	14
Tetracycline	< 18	19 ÷ 22	> 23	17	19	16	18	16
Trimethoprim	< 15	16 ÷ 18	> 19	17	14	16	14	18
Vancomycin			> 17	16	18	16	18	14

CONCLUSIONS

1. The incidence of the streptococci at swine with *Streptococcus suis* and *Streptococcus porcinus* in the individual households of the inhabitants across the area of Suceava county has a sporadic nature, the ration between the number of sick animals and the number of the studied animals being 5:283 (1,77%). Concretely, the ratio between the number of animals with streptococcal infections caused by *Streptococcus suis*, and *Streptococcus porcinus* respectively and the number of the studied animals is 2:283 (0,71%), and 3:283 (1,06%) respectively.
2. The isolation of the bacterial strains was done easily, these ones growing both in usual media and in media enriched with serum or blood.
3. The criteria used in identifying the species *Streptococcus suis* and *Streptococcus porcinus* have lead to similar results to the data existing in the specialized literature.
4. The isolated and identified strains of *Streptococcus suis* and *Streptococcus porcinus* were characterized by an accentuated virulence, the clinical forms under which the streptococci manifested being sub-acute in the case of infections with *Streptococcus suis*, and chronic in the case of infections with *Streptococcus porcinus.*
5. By testing the sensitivity to the anti-infectious substances it was emphasized that the strains of *Streptococcus suis* and *Streptococcus porcinus* show sensitivity to antibiotics, sulfonamides and substances for chemotherapy, being moderately sensible to classical antibiotics, frequently used in the treatment of streptococcal infectious.
6. By identifying the bacterial species belonging to the genus *Streptococcus* it was confirmed the importance of the bacteriological tests in establishing the diagnosis with certainty.

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Preliminary researches concerning the isolation of APEC strains in broilers

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The researches have been performed on 47 strains of E. coli isolated from broilers with different ages, from two farms, and from the shed air, only from one farm.In order to detect the genes ompA, iss and fimH, we have applied the PCR technique, the multiprimer variant.

This technique, with its multiprimer variant (multiplex), has made evident the three genes which encode the synthesis of some virulence factors, characteristic to the APEC strains, in 29 (61.70%) of the strains tested.

The gene iss which encodes a protein of the external membrane inducing resistance to the complement was present in 27 of the strains tested, the gene ompA which encodes a protein of the external membrane responsible with the bacterial attachment was present in 21 of the strains tested and the gene fimH which encodes the synthesis of the type-1 fimbre was present in 21 of the strains tested.

These 3 genes which encode the main pathogenity factors of the APEC strains were associated to 16 strains; only two or even one gene were associated to the other strains.

Key Words: E. coli, APEC strains, pathogenity factors

Avian colibacillosis is an infectious disease, extra-intestinal, caused by *E.coli* strains belonging to the pathotype APEC (Avian Pathogenic *E. coli*). More frequently, it develops in young poultry with septicemia, post-septicemia sequels and localized infections (1, 4).

The strains belonging to the pathotype APEC have different phenotypical and genotypic features compared to the *E. coli* strains belonging to the pathotypes pathogen for mammalians. So, these strains belong to the serogroups O1, O2 and O78, bind Congo Red, have the capsular antigen K1, produce colicines and use the aerial sacks and the lungs as penetrable situses within the sanguine circulation (2, 4).

The major virulence factors, present in the APEC strains, are: adhesins (the fimbriae F1, P and curl), iron-purchasing system through aerobactin, complement-resistance, thermo-sensible hemagglutinin and endotoxins (2, 4).

The researches carried out have tried to make evident some phenotypical and genotypic features of the isolated APEC strains, in broilers within disease focuses.

Materials and methods

The researches have been performed on 47 strains of *E. coli* isolated from broilers with different ages, from two farms, and from the shed air, only from one farm.

The strains have been identified according to their biochemical characters, and then we have tested their hemolytic activity on agar with 5% sheep defibrined blood, fixed the Congo Red in agar TSA and the profile of resistance to antibiotics (3, 4).

In order to detect the genes *ompA*, *iss* and *fimH*, we have applied the PCR technique (Polymerase Chain Reaction), the multiprimer variant, within the Laboratory for Molecular Biology, S.N. Institute Pasteur S.A. (3).

Results achieved

Successive to the primary inseminations, we have isolated 47 strains (43 from bodies and 4 from air), which, according to their biochemical characters, were determined to be *E. coli* strains. The fixation of Congo Red was present in 29 strains (61.70%), and the hemolysis was present in one single strain.

Most isolated strains were sensible to florfenicol and ciprofloxacin, moderately sensible to colistin sulphate, spectinomycin and enrofloxacin and resistant to neomycin, tetracycline, doxycycline and erythromycin.

The results achieved through the PCR technique are presented in Table 1. This technique, with its multiprimer variant (multiplex), has made evident the three genes which encode the synthesis of some virulence factors, characteristic to the APEC strains, in 29 (61.70%) of the strains tested.

The gene *iss* which encodes a protein of the external membrane inducing resistance to the complement was present in 27 of the strains tested, the gene *ompA* which encodes a protein of the external membrane responsible with the bacterial attachment was present in 21 of the strains tested and the gene *fimH* which encodes the synthesis of the type-1 fimbre was present in 21 of the strains tested.

These 3 genes which encode the main pathogenity factors of the APEC strains were associated to 16 strains; only two or even one gene were associated to the other strains (Table 1).

The results achieved make evident the presence of the APEC strains in broilers; these strains may be transmitted through direct or indirect contacts with different sources within sheds. This fact was proved by the identification of 4 APEC strains within the shed air.

The economic importance of *E.coli* infections in broilers, associated to the more increased frequency of the strains involved in the etiopathogenesis of these infections, requires a control upon these strains and a genotypic characterization allowing, eventually, to the definitive design of the APEC genotype.

Rodriguez-Siek, K.E. et al. (2005) have performed a genotypic study on 451 *E. coli* strains isolated from poultry and have established the prevalence of some genes encoding the virulence factors, which, at the same time, characterize the pathotype APEC (4).

In Romania, Virgilia Popa et al. (2004) have elaborated a variant of PCR multiplex with which we may detect the main genes encoding the virulence factors of the APEC strains (3).

Table 1

The characteristics E. coli strains

Nr. crt	Characterizing		No.	%
1.	Strains tested		47	100
2.	Fermentation of lactose	S-S agar	47	100
		Levine agar	47	100
3.	Hemolytic activity		1	2,13
4.	Congo Red in agar		29	61,70
5.	mPCR	iss	27	57,45
		ompA	21	44,68
		fim H	21	44,68
6.	1 gene (iss)		5	17,24
7.	2 genes	(iss+fim H)	3	10,34
		(iss+ompA)	3	10,34
		(omp A+fim H)	2	6,90
8.	3 genes(iss+fim H+ iss+ompA)		16	55,17
9.	mPCR		29	61,70

Conclusions

The PCR technique with its multiprimer variant allows the establishment of an early pathogenity diagnose of the APEC strains.

The *E. coli* strains isolated from focuses of aviary colibacillosis and from shed air have had within their genotypic structure three genes encoding the main virulence factors of the APEC strains.

The presence of these three genes is correlated with the fixation of the Congo Red, a phenotypical character specific to avian-origin *E.coli* strains

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The electronic documentary products and the actual scientific research

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In an informational society, like the one that we live in, the current scientific documentation is closely and inevitably addicted on IT. Giving the information explosion, the multiplicity of concerns with a direct effect on the diminution of the time given to the information's research, the digitized document is the only one who can answer the demands of the modern documentation. The Iasi USAMV Library is launching the first product for the direct, free and quick long distance access: DirAgroDig – the prototype of an electronic library based on digitized documents, the support for the modern scientific education and research.

Key words: current scientific documentation, long distance access, digitization

Analiza de risc pentru influenţa aviară – identificarea elementelor globale de risc

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Avian influenza is one of the most serious diseases in animals. Formerly known as a poultry and birds disease, it become a cosmopolite disease, affecting in very specific conditions other animals species and humans. Romania was one of the most affected country in the world, fortunately the disease being located mainly only in animals, especially poultry and birds. A risk analysis is going to be performed in the framework of the research project initiated by National Sanitary Veterinary and Food Safety Authority, Institute for Diagnosis and Animal Health and other public and non-governmental organisations – called "Prevention and control of avian influenza dissemination" with the aim to point out risk elements identification as origin of risks, risk itinerary, intermediate risk zones, risk destination, main elements of risk and their description as well as risk emission, risk exposure, and risk dissemination ways and factors.

This paper, as part of main author's theses to obtain the professional degree "doctor in medicine – veterinary speciality" has as main purpose to describe the firs stage of risk analysis for avian influenza – the identification of potential and plausible of risk for the disease concerned.

Key words: risk analysis, risk identification, avian influenza, avian migrations

Introducere

Influenţa aviară este una din cele mai grave boli ale animalelor. Cunoscută iniţial ca fiind o boală transmisibilă a păsărilor domestice şi sălbatice, aceasta şi-a schimbat, în special după anul 2000, caracterul evolutiv şi caracterele de patogenitate, afectând grav păsările domestice şi sălbatice dar şi alte specii de animale, fie prin exprimare clinică, lezională, morbiditate şi mortalitate cât şi prin infecţii acute inaparente la diferite specii de păsări şi/sau conversiuni serologice ori modificări genomice, la alte specii, inclusiv omul.

România a fost cea mai afectată ţară din Europa de evoluţia influenţei aviare şi una dintre cele mai afectate din lume în special prin ce de-al treilea val de boală ce a evoluat în lunile mai-iulie 2006, primul val evoluând în lunile octombrie – decembrie 2005, în judeţele Tulcea şi Constanţa, iar ce de-al doilea val, în perioada ianuarie-aprilie 2006.

În vederea pregătirii serviciilor sanitare din România de a face faţă eventualelor focare de influenţă aviară şi a serviciilor sanitare umane de a preveni şi a limita o potenţială pandemie umană de gripă aviară H_5N_1, conducerea Autorităţii Naţionale Sanitare Veterinare şi pentru Siguranţa Alimentelor împreună cu Institutul de Diagnostic şi Sănătate Animală, Facultatea de Medicină Veterinară din Bucureşti, unele direcţii sanitare veterinare şi pentru siguranţa alimentelor judeţene şi organizaţii non-guvernamentale (Societatea Ornitologică Română, Organizaţia Mondială pentru Protecţia Animalelor) au elaborat un program de cercetare intitulat

„Prevenirea şi controlul apariției şi extinderii gripei aviare". Lucrarea prezentă constituie o parte a unei secțiuni a programului vizând analiza de risc în relație cu această boală.

Material şi metodă

În vederea perfectării acestei lucrări au fost studiate informațiile existente pe site-urile Organizației Mondiale pentru Sănătatea Animalelor, Organizației Mondiale a Sănătății, Organizației Mondiale a Comerțului, Organizației Națiunilor Unite pentru Agricultură şi Alimentație – Oficiul Subregional pentru Europa Centrală şi de Est „Whooper Swan Project" în Mangalia, „Recomandările Conferinței Științifice Internaționale FAO & OIE privind Influența Aviară şi Păsările Sălbatice" ce a avut la Roma, Italia, în perioada 28 – 31 mai 2006, „Cursul de Instruire privind Epidemiologia Influenței Aviare" organizat de FAO la Tulcea, România, „Programul de Monitorizare a Păsărilor Sălbatice în UE", bazat pe „Liniile directoare privind implementarea programelor de supraveghere pentru influența aviară la păsările domestice şi sălbatice ce trebuie să fie implementate în Statele Membre, în 2007" nr. 10268/2006 Rev 5 al Comisiei Europene. Au fost utilizate, de asemenea, informațiile şi materialele primite de la Societatea Ornitologică Română, Societatea Regală pentru Protecția Păsărilor din Marea Britanie. Au fost consultate lucrările „Păsările din România şi Europa" editată de Societatea Ornitologică Română, „Birds of Europe" editată de BirdLIfe International, „Aves Histrial" scrisă de Peter Weber, „Handbook of Risk Analysis" al Organizației Mondiale pentru Sănătatea Animalelor şi manualul „Concepte Moderne în Medicina Veterinară – Analiza de Risc, Cadru Metodologic" a Prof. univ. dr. Gheorghe Onțanu.

Metoda utilizată a constat din estimarea comparativă a informațiilor preluate de la Direcția de Inspecții şi Control din cadrul Autorității Naționale Sanitare Veterinare şi pentru Siguranța Alimentelor privind activitățile de import şi tranzit derulate la nivelul posturilor de inspecții şi control sanitar veterinare de frontieră privind păsări de fermă, păsări şi animale de companie, carne de pasăre, produse şi preparate din carene de pasăre, furaje pentru păsări, precum şi datele preluate de la Societatea Ornitologică Română referitoare la migrațiile de păsări ce implică teritoriul. Au fost luate în considerație şi micul trafic de frontieră.

Rezultate

Evaluarea datelor referitoare la informațiile referitoare la activitățile de import şi tranzit care s-au derulat în anul 2005 prin posturile de inspecție şi controale veterinare la frontieră ne conduce la concluzia că prin aceste activități nu s-au introdus elemente de risc în relație cu influența aviară.

Din analiza acestor date şi a destinației produselor „susceptibile" se poate concluziona că destinația acestora au fost județele neimplicate în cele trei focare de influență aviară sau implicate târziu fără corelare pozitivă între data realizării acestor activități de import cu apariția focarelor de influență aviară.

Au fost eliminate, ca origine a focarelor de influență aviară din România aceste mărfuri. Ținând cont de locația apariției primelor focare de boală malul lacului Razelm – Tulcea în relație cu Delta Dunării şi faptul că virusul influenței aviare înalt patogene fusese izolat inițial de la păsări sălbatice acvatice, investigațiile au fost focalizate asupra acestor aspecte, în ipostaza în care păsări ar fi putut introduce virusul influenței aviare în România. Din datele şi informațiile primite de la Societatea Ornitologică Română au putut fi stabilite sau identificate principalele zone sau căi de migrare pentru păsările migratoare ce realizează micromigrații, adică migrații de peste 1000 km, ca fiind: zona de migrare – America de Nord – Mississippi – America de Sud, localizat de la fluviul Mississipi către zonele centrale ale Americii de Nord, America Latrină, America de Sud, de-a lungul celor două continente americane; zona Pacific - Americi care se întinde pe coastele de vest ale Americii de Nord, Americii Centrale şi ale Americii de Sud; zona Atlantic – Americi – pe coastele de vest ale Americii de Nord, ale Americii Centrale şi ale Americii de Sud; zona Atlanticului de Est –

Nord Europeană – Nord Asiatică care cuprinde zona arhipelagică de la Nord de Goful Hudson, Groenlanda, Islanda, litoralul țărilor maritime vest-europene, țările scandinavice, nordul Siberiei până la fluviul Lena; zona Pericaucaziană – Marea Neagră – nord – Africa; zona Vest-Centrală Asia – Africa de Est; zona Asia pericaucaziană – Asia Centrală şi de Sud-Est, zona Asia de Est – Australia.

Ținând cont că migrația păsărilor este definită ca o mişcare regulată, în mod curent sezonieră a unei părți a unei populații de păsări sau a întregii populații de la şi către zone determinate precis, în mod ciclic şi predictibil şi că această mişcare este corelată cu necesitatea de hrănire şi de reproducție care sunt afectate sau influențate de condițiile climaterice, am putut să alcătuim, pe baza informațiilor primite de la Whether Forecast media globală a temperaturilor vara şi iarna pentru cele două emisfere: în majoritatea uscatului din emisfera sudică temperaturile sunt pozitive atât vara cât şi iarna pe când în majoritatea uscatului din emisfera nordică temperaturile sunt negative iarna şi pozitive vara. Aşa se explică de ce marile zone de migrare au orientare toamna de la nord la sud, iar primăvara de la sud la nord.

Evoluția comparativă a volumului de migrare, ca număr de specii migratoare, între cele două emisfere relevă că 85% din speciile migratoare realizează macromigrații între Asia, Europa şi partea de nord a emisferei sudică (Africa, Asia de Sud Est), 63 % din acestea realizează macromigrații în zona Mării Negre – Marea Caspică, 45% din cele care ajung în aceste zone realizează în continuare macromigrații în nordul şi nord-estul Africii şi numai 6% ajung în partea centrală şi zona de sud a Africii. 31% din speciile de păsări migratoare realizează migrații doar în emisfera nordică. Nu am luat în considerare cele trei zone de migrare ale Americii din lipsa datelor relevante şi oricum acestea nu respectă patern-ul migrațiilor longitudinii estice.

Au fost stabilite apoi principalele rute de migrare, definite ca culoarul pe care majoritatea indivizilor dintr-o specie de păsări sălbatice îl urmează din motive de reproducere a speciei, corelate cu teritoriul Europei şi teritoriul țării noastre. Astfel s-a stabilit că foarte multe păsări sălbatice pleacă din marele bazin de păsări sălbatice care este peninsula Indochina, în lunile de primăvară timpurii, datorită temperaturilor ridicate şi migrează pe trei rute principale, în funcție de specie. Unele zboară pe „culoarul chinez”, din țările Asiei de Sud - Est în zona estică şi est-nordică a Chinei, altele migrează în zona centrală a Asiei – Siberia, iar a treia categorie migrează pe ruta Asia de Sud - Est – partea estică şi nord estică a Munților Ural, în cele trei zone având loc reproducția şi clocirea (temperaturi mai scăzute propice). Toamna timpuriu, păsările ce au migrat pe „culoarul chinez” revin în Asia de Sud - Est şi de aici în zona de migrare Asia de Est – Australia, cele din Siberia migrează în zona între Marea Caspică şi Marea Neagră în jurul Mării Negre în special în Crimeea şi Delta Dunării, iar cele care au migrat în apropierea Uralilor, cam jumătate se reîntorc în Asia de Sud – Est aproximativ 40% ajung în aceeaşi zonă a Mării Caspice şi Mării Negre, iar aproximativ 10% realizează migrații în țările scandinavice şi Marea Britanie, Danemarca, Olanda, Belgia şi nordul Franței. Toamna în aria Caspică – Marea Neagră şi aria Asiei de Sud - Est se înregistrează cele mai mari populații de păsări migratoare.

Toamna târziu de pe teritoriul Europei se formează trei rute de migrare: ruta de vest – cu originea în țările scandinave, Danemarca, Marea Britanie, iar itinerariul de migrare pe teritoriul Marii Britanii, Olandei, Belgiei, Franței, Spaniei, Portugaliei, Maroc, Mauritania, Senegal, Siera Leone, Gabon, Camerun ruta Europei Centrale, cu originea în țările scandinavice, Germania, Austria, Slovenia, Croația, Italia – nordul Africii – Algeria, Libia, Tunisia, iar ruta Est – Europeană cuprinde păsările din bazinele Caspicii şi Mării Negre – Delta Dunării care migrează, în parte (45%) peste Bulgaria, coastele Turciei, Cipru, Siria, Israel, Iordania, Egipt, Sudan, Etiopia, iar unele ajung în Kenya, Tanzania, Mozambic – Africa de Sud. Primăvara păsările migratore care au migrat la Sud de Europa se reîntorc pe cele trei culoare şi refac spre vară rutele „ruseşti”.

Analizând zonele şi rutele de migrare prezentate au fost identificate „zonele de oprire şi şedere” (stopover sites) ale păsărilor sălbatice migratoare. Principala zonă de oprire şi şedere este Asia de

Sud - Est (Indonezia, Malaezia, Vietnam, Laos, Tailanda, Hong - Kong, Myanmar, China de Sud). Al doilea mare situs de oprire şi şedere este cel din jurul Mării Negre, în special Delta Dunării şi Crimeea extinzându-se până la Marea Caspică, urmează apoi situsul fluviului Mississippi şi situsul siberian care cuprinde zone din Siberia rusească centrală, Kazahstan, Kirghizstan, Uzbekistan, Tajikistan.

Evaluând zonele, rutele şi situsurile de oprire ale păsărilor migratoare din zonele de migrare ce acoperă Asia, Europa şi Africa putem explica apariția focarelor de boală în România şi în Europa. Tragedia H_5N_1 a început în 1996 în China cu câteva cazuri cantonate în provincia Guangzhau. Până în primăvara anului 2003 situația a fost oarecum sub control, însă după macromigrațiile din primăvara acelui an (2003) influența aviară s-a diseminat pe rutele de migrare, astfel că în cursul anului 2003 şi 2004 erau contaminate 10 state din Asia de Sud Est – Indonezia, Malaezia, Tailanda, Laos, Vietnam, Cambodgia, Hong – Kong, Myanmar, Pakistanul de Est, precum şi Japonia şi Pakistan.

Influența aviară a migrat apoi spre vest, astfel încât în iunie este prezentă în zona Siberiei Centrale, în iulie în zona munților Ural, iar odată cu migrația de toamnă, boala este depistată în Kazahstan, Turkmenistan, pericaspian şi în Turcia în Septembrie 2005. Odată cu sosirea primelor păsări migratoare din Siberia centrală şi din zona estică şi nord-estică a munților Ural boala apare în România şi Ukraina şi în Bulgaria. Migrarea păsărilor migratoare pe cele trei rute europene explică focarele de influență aviară apărute în Marea Britanie şi în țările din vestul şi centrul Europei. Condițiile de temperatură din iarna 2006/2007 din Europa şi Asia ce au facilitat rămânerea păsărilor migratoare în bazinul siberian a prevenit reluarea lanțului infecțios între Asia şi Europa.

Concluzii

1. Păsările migratoare joacă un rol primordial în transmiterea influenței aviare la mari distanțe.
2. Zona Asiei de Sud – Est este originea endemică a focarelor de Influență Aviară apărute pe rutele de migrare ale păsărilor sălbatice migratoare, constituind principalul element de risc pentru diseminarea bolii la mari distanțe.
3. Situsurile de oprire şi odihnă de pe rutele de migrare ale păsărilor migratoare constituie un alt element major de risc pentru apariția şi diseminarea bolii (vezi Delta Dunării).
4. Condițiile de temperatură şi ecologia speciilor de păsări sălbatice migratoare constituie, de asemenea, elemente majore de risc pentru apariția şi diseminarea influenței aviare.

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Researches concerning water quality in Snagov lake

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There were sampled water tubes from Snagov Lake in four checkpoints: Tancabesti, "Peace" Complex, "Mansion 23" Complex and downstream monastery.

From the samples there were carried out the chemical and physical parameters (temperature, pH, dissolved oxygen, CBO5, CCO-Mn, CCO-Cr, ammonium, nitrite, nitrates, nitrogenous, phosphorus, phosphates, the dry matter in oven at 105 °C, Cl^-, SO_4^{2+}, Ca^{2+}, Mg^{2+}, Na^+).

The establishing methods were the ones stipulated by the actual standards. The results interpretation was made according to 1146/2002 Order, regarding the quality of the surface water.

Following the researches, there were concluded:

- the physical indicators (pH, temperature) are bordered within the limits stipulated by 1146/2002;

- the oxygen regime borders water of Lake Snagov by the whole in the second quality class;

- from nutrients group, ammonium and nitrites border the water of Lake Snagov, in almost all sampling points, in the third quality class;

- nitrates, total nitrogen, orto-phosphates and the total phosphorus recorded values which border the water of Lake Snagov in the first quality class;

- chlorines and sulphates in all sampling points had values which border the water of Lake Snagov in the second quality class;

- general ions border the water in Lake Snagov in the first quality class for calcium and the third quality class for natrium;

- magnesium recorded value for the fifth quality class in the sampling point Downstream Manastirea;

- the critical points of Lake Snagov are: Tancabesti for oxygen and nutrients regime; downstream monastery for general ions (Mg^+ and Na^+).

Key words: quality class, water, limits, nutrients, oxygen regime, general ions

The surface water pollution has serious effects on biosphere, affecting the aquatic life but also the terrestrial animals and plants health.

Depending on the intensity and kind of pollution, water utilization as physiologic, hygienic, industrial way could be diminished or cancelled.

Surface water pollution could be the base of illness by direct contact or by ingestion.

This paper has as aim the assessment of quality of Snagov Lake water from the physical or chemical point of view and of the corrective measures too, in the case of water pollution.

Material and methods

From the water of Snagov Lake there were sampled water amounts having in view its quality establishment. There were carried out physical and chemical indicators (temperature, pH); indicators which reflect the oxygen regime in water (dissolved oxygen, CBO5, CCO-Mn, CCO-Cr); nutrients (ammonium, nitrates, nitrites, total nitrogenous, orthophosphates, total phosphorus) and general ions (filterable dry matter at 105 °C, chlorines, sulphates, calcium, magnesium, natrium).

The methods used for establishing the parameters were the ones stipulated by the standards.

The results interpretation was made according to 1146/2002 Order, regarding the surface water quality.

There were established four checkpoints: Tancabesti, “Peace” Complex, “Mansion 23” Complex and downstream monastery.

Results and discussions

As physical indicators in the table, it is noticed that the water pH is bordered within the limits stipulated by 1146/2002 Order for the fifth quality class, trending to an alkaline one.

Table no. 1

Physical indicators of water in Snagov Lake (average values)

Sampling point		Assessed indicators	
		Temperature (°C)	pH (pH unit)
Tancabesti		19	7.9
“Peace” Complex		19.5	8.1
“Mansion 23” Complex		17	8
Downstream monastery		19	8.1
Admitted limits according to 1146/2002 Order	The quality class		
	I	No provision	6,5 – 8,5
	II		
	III		
	IV		
	V		

In table 2, there are shown the average values of chemical indicators which constitute the oxygen regime of water in Snagov Lake.

Within this group there were established the dissolved oxygen, the oxygen biochemical consumption (CBO5) and the chemical consumption of oxygen (CCO-Mn and CCO-Cr).

Table no. 2

Oxygen regime of water in Snagov Lake (average values)

Sampling point		Assessed indicators			
		Disolved oxygen (mg/l)	CBO5 (mg O_2/l)	CCO-Mn (mg O_2/l)	CCO-Cr (mg O_2/l)
Tancabesti		7.1	8.1	10.8	25.2
"Peace" Complex		10.5	5.5.	8.5	22.6
"Mansion 23" Complex		8.4	4.1	9.7	24.7
Downstream monastery		9.4	4.3	9.9	20.1
Admitted limits according to 1146/2002 Order	The quality class				
	I	9	3	5	10
	II	7	5	10	25
	III	5	7	20	50
	IV	4	20	50	125
	V	<4	<20	>50	>125

Analyzing the obtained results regarding the parameters it is noticed that the dissolved O_2 borders the water in Snagov Lake in the first quality class in three of the checkpoints, respective: "Peace" complex, "Mansion 23" Complex and downstream monastery and in the second quality class the one from Tancabesti.

Regarding the biochemical consumption of oxygen (CBO5) in Tancabesti checkpoint water is framed within the third quality class, and in the other three sampling points in the first quality class.

The chemical consumption of oxygen (CCO-Mn and CCO-Cr) recorded values included in the second quality class for all the samples, no matter the sampling point.

The average values of nutrients in Snagov Lake water are shown in table 3.

Table no. 3

Nutrients of the water in Snagov Lake (average values)

Sampling point		Assessed indicators					
		Ammonium NH_4^+ mg N/l	Nitrite NO_2^- mg N/l	Nitrate NO_3^- mg N/l	Total nitrogen mg N/l	Ortho-phosphates PO_4^{3-} mg P/l	Total phosphorus mg P/l
Tancabesti		1.239	0.062	0.883	1.520	0.240	0.361
"Peace" Complex		0.841	0.061	1.217	1.145	0.033	0.174
"Mansion 23" Complex		1.028	0.038	0.917	1.213	0.039	0.238
Downstream monastery		1.106	0.055	1.125	1.298	0.050	0.107
Admitted limits according to 1146/2002 Order	The quality class						
	I	0.4	0.01	1	1.5	0.1	0.15
	II	0.8	0.03	3	7	0.2	0.4
	III	1.2	0.06	5.6	12	0.4	0.75
	IV	3.2	0.3	11.2	16	0.9	1.2
	V	>3.2	>0.3	>11.2	>16	>0.9	>1.2

Within the nutrients group there were established the following parameters: ammonium, nitrates, nitrites, total nitrogenous, orthophosphates and the total phosphorus.

Regarding the data in the table, it is noticed:

ammonium recorded values which frame water in Snagov Lake in the third quality class in Tancabesti, "Mansion 23" and downstream monastery checkpoints and in the second quality class in "Peace" checkpoint;

nitrites framed the water in the third quality class for Tancabesti, "Peace" complex and downstream monastery checkpoints and in the second quality class in "Mansion 23" checkpoint.

Nitrates had values that correspond to the first quality class in all the checkpoints.

The total nitrogenous framed the water in Snagov Lake in the first quality class.

The soluble orthophosphates recorded values for the second quality class in Tancabesti checkpoint and in the first quality class for the rest of the checkpoints.

The average values of total phosphorous framed water in the first quality class in "Peace" Complex and downstream monastery checkpoints and in the second quality class in Tancabesti and "23 Mansion" checkpoints.

The average values of the parameters which constitute the group of the general ions or salinity (dry residuum at 105 °C, chlorines, sulphates, calcium, magnesium and natrium) are shown in table 4.

Table no. 4

Water salinity in Snagov Lake (average values)

Sampling point		Assessed indicators					
		Filtered dry residuum at 105°C mg/l	Chlorines mg/l	Sulphates mg/l	Calcium mg/l	Magnesium mg/l	Natrium mg/l
Tancabesti		472.417	90.4	114.23	65.45	18.223	121.567
"Peace" Complex		567.033	134.68	97.45	53.4	25.267	124.2
"Mansion 23" Complex		567.083	137.73	99.53	55.55	23.05	108.117
Downstream monastery		580.167	143.6	92.78	54.483	226.217	124.033
Admitted limits according to 1146/2002 Order		The quality class					
	I	500	25	60	50	12	25
	II	750	50	120	100	50	50
	III	1000	250	250	200	100	100
	IV	1300	300	300	300	200	200
	V	>1300	>300	>300	>300	>200	>200

Analyzing the data in the table it is noticed:

- from the dry filterable residuum at 105 °C, the water quality of Snagov Lake is framed within the first quality class in all samples and checkpoints;
- chlorines and sulphates recorded values for the second quality class in all checkpoints;
- calcium had values for the first quality class;
- regarding the concentration of magnesium in the water samples, there were recorded values for the first quality class in Tancabesti, "Mansion 23" and "Peace" and in the fifth quality class in downstream monastery checkpoint.
- natrium had values for the third quality class in all checkpoints.

Conclusions

1. The physical indicators (pH, temperature) are bordered within the limits stipulated by 1146/2002;
2. The oxygen regime borders water of Lake Snagov by the whole in the second quality class;
3. From the nutrients group, ammonium and nitrites border the water of Lake Snagov, in almost all sampling points, in the third quality class;
4. Nitrates, total nitrogen, orto-phosphates and the total phosphorus recorded values which border the water of Lake Snagov in the first quality class;
5. Chlorines and sulphates in all sampling points had values which border the water of Lake Snagov in the second quality class;
6. General ions border the water in Lake Snagov in the first quality class for calcium and the third quality class for natrium;
7. Magnesium recorded value for the fifth quality class in the sampling point Downstream monastery;
8. The critical points of Lake Snagov are: Tancabesti for oxygen and nutrients regime; downstream monastery for general ions (Mg^+ and Na^+).

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Researches concerning mycotoxic and fungic contamination range of forages in the southern area of the country

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Fungi, by their secondary metabolits could gear to different life conditions, some could be repellents and others are classified as mycotoxins leading to plant metabolism changing and phytotoxins.

Forage mycotoxins and their metabolites are transferred to meat, milk and eggs, influencing consumers' health.

In the present paper there were samples 62 forage samples of different categories and sorts: industrial combined fodder, premixed feed, pellets, concentrated forage (maize, oat, and wheat), animal origin proteic flour, industrial residues, green fodders and fibrous forage.

The samples were harvested in a randomized way from farms, individual households, units for forage processing, storehouses and vehicles in Dolj county.

By samples there were carried out: the fungi charge (total number of fungi - TNF) and the level of mycotoxins (total aflatoxins – B1 + B2 + G1 + G2; B1 aflatoxin and ochratoxin A). The establishing methods were made conformingly SR ISO 7954/2001 methodology (technique of colonies counting at 25 °C), the thin layer chromatography for B1 aflatoxin and A ochratoxin, as well as the immune enzyme technique (ELISA) for total aflatoxins and B1 aflatoxin.

The results interpretation was made according to MAAP and MSF Order no. 249/2003.

The tests ran leads to the following conclusions:

- *The total number of fungi in all the samples harvested in the southern area of the country does not record exceeding of the maximum admitted limit;*
- *The average concentration of total aflatoxin was 0,52 ppb, framing within the admitted limits on national level in all categories and forage sorts;*
- *B1 aflatoxin recorded an average value of 0,6556 ppb (value in normal limits), with a concentration of 0,924 ppb in proteic flours and 0,391 ppb in concentrated forages samples;*
- *A ochratoxin determined in the concentrated forage and premixed feed samples had values under the admitted limits;*

- *Due to the fungic and mycotoxic contamination of the forage samples in the south of the country, the forage using in this area does not imply risks for animals.*

Key words: aflatoxin, forage, ochratoxin, admitted limits, fungic, mycotoxic

The inferior mushroom organisms (fungi, mould) are ubiquitar organisms which live a latent live as spores and vegetate very quickly when they found favorable conditions.

Fungi, by their secondary metabolites could gear to different life environments, could be repellents and others are classified as mycotoxins or having a noxious action by changing plant metabolism and phytotoxins appearance.

Their distribution is different depending their tolerance to humidity, temperature, pH and their preferences for a certain substrate.

After mycotoxins contaminated forages consumption, in animals appear different manifestation, from food consumption and bioconversion reducing to different illness and even animal death.

Mycotoxins in forages or their metabolites transfer to meat, milk and eggs, with involvement on human health.

Material and methods

In the present paper there were samples 62 forage samples of different categories and sorts: industrial combined fodder, premixed feed, pellets, concentrated forage (maize, oat, and wheat), animal origin proteic flour, industrial residues, green fodders and fibrous forage.

The samples were harvested in a randomized way from farms, individual households, units for forage processing, storehouses and vehicles in Dolj county.

By samples there were carried out: the fungi charge (total number of fungi - TNF) and the level of mycotoxins (total aflatoxins – B1 + B2 + G1 + G2; B1 aflatoxin and ochratoxin A). The establishing methods were made conformingly SR ISO 7954/2001 methodology (technique of colonies counting at 25 °C), the thin layer chromatography for B1 aflatoxin and A ochratoxin, as well as the immune enzyme technique (ELISA) for total aflatoxins and B1 aflatoxin.

The results interpretation was made according to MAAP and MSF Order no. 249/2003.

Results and discussions

From the total 62 forage samples for mycotic examination, the total number of fungi was determined in 48, the values widely varying from less than 10 to 29.000.

In 13 samples of combined processed fodder the average value of the total number of fungi was 270/gram of forage, this value being below the maximum admitted limit of 50.000/gram.

The 15 samples of premixed feed recorded a TNF average value of 81/gram of forage, framing in the maximum admitted limits.

The TNF average value for the 9 forage samples of concentrated forage was 9633/gram, framing in the maximum admitted of 50.000/gram, according to Order no. 249/2003.

The maximum admitted limit 1.000 fungi/gram of forage for the 5 samples of protein flours was not exceeded by any samples, the average value being 9 fungi/gram.

In the industrial residues samples, the average value for the 5 samples was 466 fungi/gram, framing more below the maximum admitted limit of 50.000/gram.

The obtained results regarding the analyze of fungic contamination range of forages samples in Dolj county are shown in chart 1.

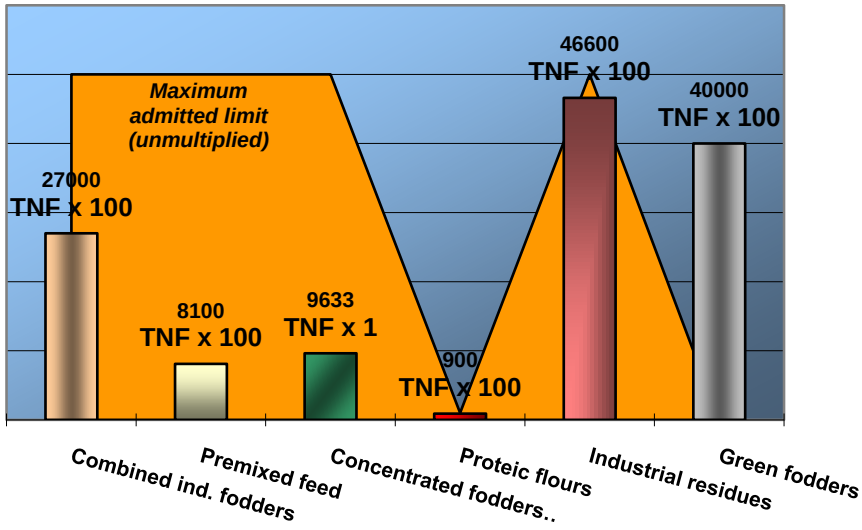

Figure no. 1
Average value of total number of fungi in different forages in Dolj county

Synthesizing the results, in any forage sample was not exceeded the maximum admitted limit for TNF. From all the analyzed samples, the largest charge of fungi (but framing within the maximum admitted limits) was recorded at concentrated forage samples (oat, wheat, maize).

Having in view the mycotoxins screening in forage samples harvested from Dolj county, there were analyzed 30 samples. Among them, there were carried out 27 immuno-enzymatic tests (ELISA) for assessment of total aflatoxins – TAF (B1, B2, G1, G2) and B1 aflatoxin (AFB1) and 5 thin layer chromatography tests for assessing the AFB1 and OTA (A ochratoxin).

The results of the analyzes regarding the total aflatoxins (B1 + B2 + G1 + G2) in forages are shown in chart 2.

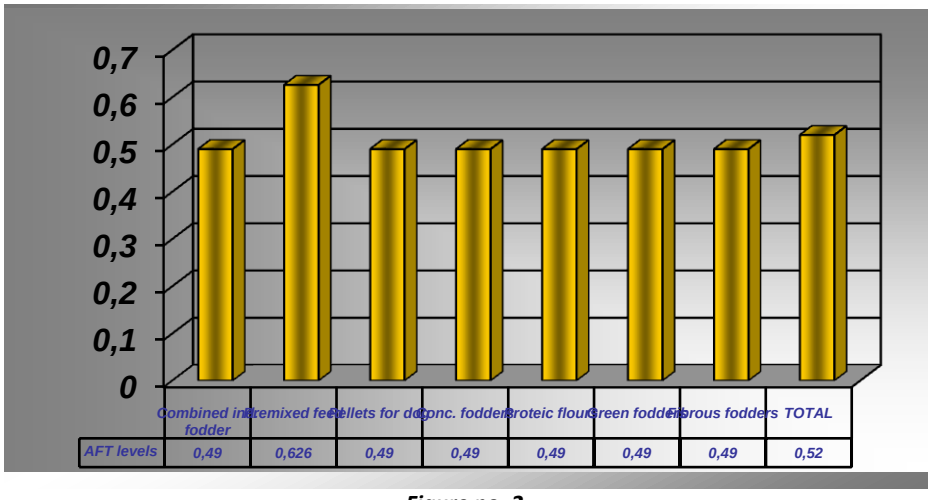

Figure no. 2
Average concentration of total aflatoxins in different forage samples – Dolj county

Analyzing the data it could notice an average concentration of 0,52 ppb (value which is bordered between the maximum admitted limit on national and EU levels).

The highest concentration of total aflatoxins was determined in the case of premixed feed, respective 0,626 ppb – this value framing in the stipulated limits.

The content of B1 aflatoxin was measured on four sorts of forages: premixed feed, concentrated forages, proteic flours and industrial residues (11 samples).

The average concentration of B1 aflatoxin was 0,6556 ppb. Regarding the concentration distribution on sorts, the highest value was recorded in proteic flour samples (0,924 ppb), followed by the one in premixed feed (0,8 ppb) and the industrial residues (0,683 ppb). The lowest concentration of AFB1 was determined in the concentrated forage samples (0,391 ppb). The results regarding AFB1 average concentration are shown in chart 3.

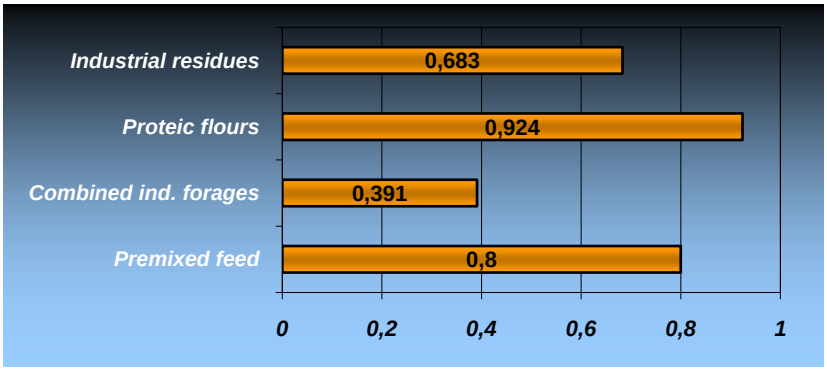

Figure no. 3

Average concentration of B1 aflatoxin in different forages – Dolj county

In two forages samples - one of concentrated forage and one of premixed feed - was determined the ochratoxin by thin layer chromatography. In both samples, the value of OTA was below the maximum admitted limit according the provisions of the orders.

All forages samples in the studied area are framed within the admitted limit by the stipulated standards regarding the fungic and mycotoxic contamination.

Conclusions

1. The total number of fungi in all the samples harvested in the southern area of the country does not record exceeding of the maximum admitted limit;
2. The average concentration of total aflatoxin was 0,52 ppb, framing within the admitted limits on national level in all categories and forage sorts;
3. B1 aflatoxin recorded an average value of 0,6556 ppb (value in normal limits), with a concentration of 0,924 ppb in proteic flours and 0,391 ppb in concentrated forages samples;
4. A ochratoxin determined in the concentrated forage and premixed feed samples had values under the admitted limits;
5. Due to the fungic and mycotoxic contamination of the forage samples in the south of the country, the forage using in this area does not imply risks for animals.

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Definirea conceptului de trasabilitate: elemente de trasabilitate

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Traceability, as relative new concept in animal health, feed and food safety, is defined by Regulation of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (EC) No 178/2002, as 'traceability' means the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution.

Despite the fact that references to the concept of traceability in animal health, feed and food safety are very limited, this paper has as aim to formulate the system of traceability defining the framework of traceability, the elements of traceability, the instruments of traceability and the means of traceability. This paper is particularly designated to propose the identification and definition of traceability elements in animal health, feed and food safety.

At the same time, our proposal also refers to the establishment of criteria used to define the elements of traceability.

Key words: traceability, channel, line, chain and segement of traceability

Introducere

Deşi conceptul de trasabilitate în domeniul sănătăţii animalelor, siguranţa alimentelor şi a hranei pentru animale este definit recent, după perioada marilor crize care au avut loc la sfârşitul mileniului trecut şi primi ani de după 2000, elemente şi instrumente ale trasabilităţii sunt recunoscute încă din antichitate.

Definită de către diferite organizaţii veterinare internaţionale sau cu incidenţă în domeniul veterinar în forme apropiate, trasabilitatea, ca şi concept nou în medicina veterinară, a fost complet şi complex definită prin legislaţia comunitară, iniţial prin Regulamentul Parlamentului şi al Consiliului din 28 ianuarie 2002 ce stabileşte principiile generale şi cerinţele pentru legislaţia privind alimentele, instituind Autoritatea Europeană pentru Siguranţa Alimentelor şi stabilind procedurile în materie de siguranţă a alimentelor (EC) nr. 178/2002.

Definirea conceptului de trasabilitate şi punerea în practică a acesteia este rezultatul efectelor induse de crizele majore care au avut loc la sfârşitul secolului trecut şi în primii ani după 2000. Menţionăm criza encefalopatiilor spongiforme ale animalelor, declanşată după 1986, odată cu prima descriere a encefalopatiei spongiforme bovine, urmată de criza encefalopatiilor spongiforme umane concretizată prin definirea noii variante Creutzfield – Jakobs şi corelarea acestora cu encefalopatia spongiformă bovină, criza utilizării hormonilor în creşterea animalelor între Uniunea Europeană şi Statele Unite, criza dioxinelor din perioada 1999 – 2000, criza febrei

aftoase în perioada 2000 – 2003 sau criza influenței aviare din perioada 2002 – 2006 şi care continuă.

Deşi referirile la conceptul de trasabilitate sunt destul de limitate, se impune o definire mai detaliată a acestui concept pentru a facilita aplicarea concretă a acestuia în domeniul furajelor şi materiilor furajere, a sănătății animalelor, a industriei alimentare şi a comercializării alimentelor şi produselor alimentare. În acest sens, prezenta lucrare are drept scop să propună definirea unor elemente de trasabilitate pentru a facilita aplicarea conceptului.

Material şi Metodă

Pentru elaborarea prezentei lucrări au fost consultate şi preluate majoritatea referirilor la conceptul de trasabilitate existente în actele normative elaborate de organizațiile internaționale de profil veterinar sau de cele cu incidență în domeniul veterinar, precum şi prevederi ale legislației comunitare transpuse. Această abordare a fost necesară pentru stabilirea criteriilor propuse a fi utilizată pentru identificarea elementelor de trasabilitate şi pentru definirea acestora. În vederea soluționării practice a aspectului abordat au fost preluate o serie de documente utilizate în Statele Membre pentru a se urmări producerea, identificarea, transportul şi utilizarea furajelor şi materiilor furajere, identificarea şi înregistrare animalelor pentru gestionarea mişcării acestora, sistemele de identificare şi marcare a alimentelor şi produselor alimentare la nivelul producerii acestora, precum şi sistemele de identificare a acestora în cadrul distribuției şi comercializării en-gros sau en-detail a acestora. Concret, s-au utilizat instrumentele de trasabilitate pentru a se defini elementele de trasabilitate.

Rezultate

Rezultat al prelucrării aspectelor tehnice prevăzute de actele normative ce fac referire la trasabilitate şi al utilizării instrumentelor de trasabilitate pe care le utilizează Statele Membre ale Uniunii Europene, precum şi a unor precizări din literatura de specialitate s-a reuşit definirea criteriilor pentru identificare elementelor de trasabilitate şi definirea acestora.

Criteriile utilizate pentru definirea elementelor de trasabilitate sunt:

- conținutul filierei de trasabilitate
- nivelul de trasabilitate
- sensul de trasabilitate
- structuri de trasabilitate
- direcții de trasabilitate

În funcție de conținutul filierei de trasabilitate se poate defini **trasabilitatea administrativă şi trasabilitatea informațională**. Din punct de vedere al nivelului de trasabilitate se poate defini **trasabilitatea globală şi trasabilitatea individuală, trasabilitatea partajată, trasabilitatea totală şi trasabilitate parțială**. Din punct de vedere al sensului de trasabilitate se poate defini **trasabilitatea ascendentă şi trasabilitatea descendentă**. Ca structuri de trasabilitate se pot defini **etapă de trasabilitate**, **lanțul de trasabilitate, segmentul de trasabilitate, linia de trasabilitate, filiera de trasabilitate, intersecția de trasabilitate şi nod de trasabilitate**. În baza criteriului privind direcțiile de trasabilitate se poate defini **trasabilitatea convergentă şi trasabilitatea divergentă**.

Pentru a exemplifica şi defini elementele de trasabilitate propuse am luat drept exemplu filiera cărnii de bovine, deoarece este cea mai veche şi cea mai bine pusă la punct filieră de trasabilitate. Până la prezența cărnii de bovine sub diferite prezentări în rețeaua de comercializare există mai multe etape, o primă etapă constituind-o furajele şi medicamentele care se administrează animalelor de la care provine acest produs. Furajele se obțin în cadrul unor etape de cultivare, recoltare şi prelucrare a unor materii furajere vegetale şi din prelucrarea unor materii furajere de origine animală. Ansamblul acestor etape constituie **lanțul furajer**, iar pentru produsele

medicamentoase veterinare – **lanţul medicamentos**. Următoarea fază a obţinerii cărnii de bovine este înmulţirea şi creşterea acestora în cadrul unor exploataţii zootehnice comerciale sau necomerciale, această fază constituind **lanţul animalier**. Ulterior, animalele sunt tăiate în abatoare autorizate, iar carnea, produsele şi subprodusele obţinute sunt destinate fie prelucrării, fie distribuţiei şi comercializării sub diferite forme. Această fază constituie **lanţul alimentar**. În continuare carnea, produsele şi subprodusele obţinute, prelucrare sau nu sunt dirijate către reţeaua de distribuţie şi apoi comercializate către unităţi publice sau direct către consumator. Această fază constituie **lanţul de distribuţie şi comercializare**. În cadrul lanţului alimentar, de exemplu, au loc mai multe operaţiuni cum ar fi: tăierea animalelor, eviscerarea, obţinerea carcaselor sau a semicarcaselor, zvântarea, tranşarea, porţionarea, fasonarea, dezosarea, uneori tocarea sau obţinerea mecanică, marcarea, ambalarea, împachetarea, depozitarea. Toate aceste operaţiuni care au loc în cadrul unui lanţ de trasabilitate se definesc ca **etape de trasabilitate**. Succesiunea lanţurilor de trasabilitate de la lanţul furajer la lanţul de distribuţie şi comercializare constituie **filiera de trasabilitate**, în acest caz a produselor de bovine. Această filieră este constituită din lanţul furajer, lanţul medicamentos, lanţul uman situate la acelaşi nivel, apoi lanţul animalier, lanţul alimentar şi lanţul de distribuţie şi comercializare. În afara aspectelor materiale reprezentate de furaje, animale, produse şi subproduse animaliere şi de origine animală, sistemul de distribuţie şi comercializare, toate acestea sunt însoţite de documente oficiale ce constituie instrumentele de trasabilitate specifice fiecărui lanţ de trasabilitate: furajele circulă însoţite de certificate şi etichetate, animalele sunt identificare şi înregistrate, carnea este identificată şi marcată, iar produsele obţinute în lanţul de distribuţie şi comercializare sunt certificate, marcate şi etichetate. Atunci când ne referim la partea materială a filierei de trasabilitate, definim **trasabilitatea administrativă**, iar atunci când ne referim la instrumentele de trasabilitate sau documentele ce însoţesc marfa respectivă, definim **trasabilitatea informaţională**.

Dacă trasabilitatea se realizează pentru toate produsele şi subprodusele care se obţin, de exemplu, de la o bovină, definim **trasabilitatea globală** (trasabilitatea pentru carnea şi toate subprodusele obţinute de la o bovină), dacă trasabilitatea se realizează numai pentru un produs, cum ar fi carnea, definim **trasabilitatea individuală** (de exemplu, trasabilitatea cărnii de bovine, trasabilitatea pieilor de bovine, a grăsimilor, a sângelui, a organelor), iar dacă trasabilitatea se realizează pentru un grup de produse, de exemplu, carne şi oase sau piei sau grăsimi, definim **trasabilitatea partajată** (de exemplu, trasabilitatea cărnii şi a organelor sau a grăsimii de bovine). Atunci când trasabilitatea se realizează de la origine până la nivelul consumatorului, în exemplul prezentat de la furaje până la consumator, definim **trasabilitatea totală** (a se vedea filiera produselor de bovine). Dacă un produs sau mai multe produse sunt supuse trasabilităţii pentru un număr limitat de lanţuri de trasabilitate din cadrul filierei de trasabilitate al acelui produs, de exemplu numai în lanţul animalier şi alimentar, definim **trasabilitatea parţială**, lanţurile de trasabilitate incluse constituie **segmentul de trasabilitate** care este constituit din două sau mai multe lanţuri de trasabilitate din cadrul unei filiere de trasabilitate. Urmărirea unui singur produs, de exemplu a cărnii de bovine, de-a lungul filierei de trasabilitate a produselor de bovine, constituie **linia de trasabilitate** a cărnii de bovine. Între diferite lanţuri de trasabilitate există operaţiuni care nu au o legătură concretă cu lanţurile respective dar fac legătura între acestea. Aceste operaţiuni constituie **noduri de trasabilitate** şi sunt reprezentate prin activităţi de transport. Dacă trasabilitatea unui produs sau a mai multor produse se realizează de la un anumit lanţ de trasabilitate a acestuia către originea acestor produse, definim **trasabilitatea ascendentă, amonte sau calitativă**. Un exemplu concret îl constituie trasabilitatea cărnii de bovine în cazul encefalopatiei spongiforme. Dacă trasabilitatea unui produs sau a mai multor produse se realizează de la un anumit lanţ de trasabilitate către lanţul distribuţie sau comercializare, definim

trasabilitatea descendentă, aval sau cantitativă. Un exemplu concret de trasabilitate descendentă este cea privind criza dioxinelor.

De la un anumit animal, în cadrul lanțului alimentar se pot obține diferite produse sau subproduse precum: carne, piele, oase, grăsime, organe, glande, sânge, ongloane şi fanere, păr. De la acest nivel aceste produse şi subproduse au destinație diferită: unele au destinație pentru consum uman, iar altele au destinație pentru prelucrare industrială. Aceste segmente sunt gestionate de instituții care aparțin unor organe ale administrației centrale diferite. Lanțul de trasabilitate în care se produce o disjuncție a destinației produselor obținute de la acelaşi animal se defineşte ca **intersecție de trasabilitate**.

Pentru creşterea animalelor se utilizează, pe lângă furaje, medicamente, aditivi, hrană furajeră de origine animală şi alte ingrediente. Fiecare din acestea sunt obținute în cadrul unui lanț de trasabilitate (exemplu, lanț furajer, lanțul medicamentos, lanțul aditivilor furajeri, etc.). Lanțurile de trasabilitate care contribuie la realizarea unui produs dintr-un alt lanț de trasabilitate se definesc ca lanțuri de trasabilitate convergente, iar trasabilitatea produselor obținute în aceste lanțuri convergente se defineşte ca **trasabilitate convergentă**. Urmărirea produselor care se obțin de la un animal în cadrul unor filiere ce constituie intersecții de trasabilitate se defineşte ca trasabilitate divergentă (exemplu, trasabilitatea cărnii de bovine destinată consumului uman care urmează filiera alimentară a produselor respective, față de trasabilitatea pieilor de bovine destinate prelucrării industriale şi care urmează filiera industrială a produselor respective).

Concluzii

1. Lucrarea propune criterii pertinente pentru definirea elementelor de trasabilitate pentru domeniul sănătății animalelor, a siguranței furajelor şi a alimentelor.
2. Lucrarea identifică şi defineşte elementele de trasabilitate pentru domeniile menționate la 1.
3. Identificarea şi definirea elementelor de trasabilitate, aşa cum sunt definite de prezenta lucrare constituie elemente benefice şi necesare pentru definirea conceptului de trasabilitate şi în special pentru punerea în aplicare a acestui concept.
4. Identificarea şi definirea elementelor de trasabilitate constituie un suport real pentru clarificarea cu privire la instrumentele de trasabilitate ce trebuie să fie proprii fiecărui lanț de trasabilitate.

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A contribution to information on starvation survival capacity of poultry red mite Dermanyssus gallinae

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The cause and aim of the research was a practical question – what is the length of facility and equipment operation break sufficient to bring about the eradication of present poultry red mite in the conditions of food absence. The research was conducted over a four-year period on 430 samples of poultry red mite, drawn from 268 poultry farms on the territory of Serbia and Montenegro. Placed in test tubes closed with porous egg packaging cardboard, poultry red mites were exposed to various environment conditions. The vitality and viability of poultry red mites was assessed in unopened test tubes, with a magnifying glass. Sources state various data on the starvation capacity of D. gallinae. The longest survival period so far is 55 weeks, i.e. 13 months, and there still are surviving units. The stated D. gallinae starvation results are not deemed as final. In the author's field experience, D. gallinae can be found in farming facilities 1.5 years after flock depopulation. We therefore believe the obtained results to be only a step toward the real answer to the question how long poultry red mites can actually starve.

Based on the obtained research results, we recommend an approach with additional safety period for practical assessment of D. gallinae infestation at poultry farms after facility operation break.

We therefore propose the following estimation: in addition to flock depopulation, the facilities and equipment should be regarded as infested with D. gallinae for subsequent 1.5 years, during which period mite control measures must be applied. After facility operation break between 1.5-2 years, we recommend that the facilities and equipment be regarded as suspected of infestation, and mite control measures should be applied. Only a facility operation break longer than 2 years should be regarded as sufficient for the present D. gallinae population to die out. Only in this case, and when no alternative food sources are present, should mite control measures be regarded as unnecessary.

Key words: Dermanyssus gallinae, starvation survival

Introduction

Among the numerous avian ectoparasites, *Dermanyssus gallinae* takes up the most important place in poultry farming. Parasitism of this haemotophagous mite is demonstrated through disturbance of poultry, irritation, anemia, transfer of diseases, reduced laying ability, and sometimes even death The global medical and economic impact on poultry farming has shown a constant growth. The latest reports quote the infestation of over 80% poultry flocks in northern Europe (Van Emous, 2005), while similar findings were recording in Serbia three years earlier. The high viability of *D. gallinae* species is partly contributed to by its feeding method, i.e. the resulting life cycle pattern. The poultry red mite is an obligate hematophagous ectoparasite.

D. gallinae is a cosmopolitan parasite. It has so far been confirmed on 30 bird species and 20 mammal species (Nordenfors, 2000). Still, *D. gallinae* is primarily an avian parasite (Dobrijević and Petanović 1982; Pavlović 1995; Nordenfors, 2000). In the lack of avian host, *D. gallinae* will infest mammals (Ramsay et al. 1975; el Kady et al. 1995; Bakr et al. 1995; Fitri 2002), including humans (Mullen and Durden 2002; Pavlović, 2004). Mites feeding on mice and rabbits lay eggs, unlike those feeding on humans, which lay very few eggs (Nordenfors, 2000).

In its search for food, *D. gallinae* moves mostly at night, in periodic intervals. Feeding intervals of a single unit range from 1 to 4 days (Emous 2005), usually 3 days (Nordenfors, 2000). The birds' skin and plumage contain a substance attracting *D. gallinae* (Zeman, 1988). To find its host, the mites use body temperature, vibrations caused by the birds' movement and the increased CO_2 concentration (Kilpinen, 1997). They remain on the host from ½ to 3 hours, as long as they need to feed (Šibalić and Cvetković, 1996). At daytime they live hidden, so that their presence is not noticed before their population becomes extensive.

Adult *D. gallinae* have a higher starvation capacity compared to other development stages and this is the key to the population's survival when the poultry cages or facilities are depopulated. There are various data on the starvation capability of poultry red mite. Still, all sources agree that the period is very long and can range from 34 weeks (Wall and Shearer 1977, 2001; Saif 2003) to several months (Babić et al. 1956; Simić and Živković 1958; Bowman 1980; Mullen and Durden 2002). Pavlović states over 5 months; *Merck Veterinary Manual* (1998) states 6 months; there is also data about 8 months (Chauve, 1998), while the longest period of 9 months is stated by Nordenfors (2000).

The reason and aim of the research was to answer the practical question on how long discontinuation of a poultry farming facility can lead to elimination of poultry red mite *Dermanyssus gallinae* due to lack of food.

Materials and methods

Materials. The samples taken from the farms contained all development stages of field populations of *D. gallinae* and a smaller amount of impurity collected. Samples were taken and observed in 18x1.8 cm glass test tubes, pieces of egg packaging cartons, adhesive tape, scalpel blade, markers, paper bags and newspaper.

Time and location. Samples of *D. gallinae* field population were collected over 23 different months from 2002 to 2005, from 268 farms, including those for layer exploitation, meat production and high- and low-intensity breeding lines. The poultry farms had various production capacities, equipment and zoohygienic conditions, and were populated with various poultry hybrid lines. A total of 430 samples were taken.

Sampling procedure. 0.5-1 cm^3 samples were taken from *D. gallinae* accumulations on battery cages with scalpel blades and placed in test tubes. After inserting *D. gallinae*, we first inserted a fragment of egg packaging carton with the tip downward to limit the movement of mites mechanically and prevent them from gathering at the test tube lid. Next we covered the test tube mouth with another fragment of carton, this time with the wider end toward the test tube, and

fixed it to the test tube mouth with adhesive tape. Thus formed sample prevents *D. gallinae* from escaping from the test tube and their dehydration, while providing sufficient breathing oxygen. Each test tube was marked with the essential data: farm name, sampling place and date.

Transport. Test tubes with mite samples were transported in paper bags, wrapped in newspaper. We took special care to keep the samples from direct sunlight. Temperature was not controlled and it depended from daily and seasonal conditions. Transport period varied depending on the distance from the farm, usually ranging from 1 to 3 days.

Sample storage. After transport, samples were stored in unheated and unlit space. Minimum temperature in the sample storage space was 3°C, and maximum was 27°C. Humidity in sample storage space ranged from 62 to 100%.

Sample observation. Samples were observed in unopened test tubes, under magnified glass and artificial lighting. Mite mobility was the decisive factor in assessing their viability. The samples were inspected in random time intervals. At the time of the year when the environment temperature in the sample storage space was below 15°C, the samples were not inspected. In samples with the longest viability we identified mites microscopically as well.

Results and Discussion

Research results were shown in two tables 1 and 2.

Mites were sampled over 32 different months from August 2002 till October 2005, from 268 different samples in 430 test tubes. We believe that this sample number was sufficient to exclude a whole range of unknown factors influencing research results, such as control measures applied on farms, specific features of field population, sampling time and place, and transport.

Although basically we tried to simulate the conditions in a facility after flock depopulation, we succeeded in this only partially. We were unable to avoid the stress caused by sampling, sample transport and changed storage conditions. Inspecting samples also caused additional stress to the mites.

Table 1 Overview of sampling dates, average and maximum survival periods over months and years, and the number of poultry farms from which the poultry red mites were sampled from 2002 to 2005.

	Sampling month/date	Number of poultry farms from which samples were taken	Survival period (weeks)	
			Average	Maximum
1.	08/2002	1	47	47
2.	09/2002	3	42	44
3.	10/2002	2	39	43
4.	03/2003	4	19	24
5.	05/2003	9	12	15
6.	06/2003	7	7	11
7.	07/2003	8	8	15
8.	08/2003	16	9	31
9.	09/2003	18	28	39
10.	10/2003	6	23	32
11.	11/2003	5	30	35
12.	12/2003	1	11	11
13.	01/2004	1	9	9
14.	02/2004	6	12	18
15.	03/2004	7	10	13
16.	04/2004	12	12	27
17.	05/2004	14	11	23
18.	06/2004	17	15	29

19.	07/2004	11	9	23
20.	08/2004	3	14	21
21.	09/2004	8	8	17
22.	10/2004	9	7	19
23.	11/2004	4	12	20
24.	01/2005	4	9	11
25.	03/2005	4	13	20
26.	04/2005	29	alive	alive
27.	05/2005	13	alive	alive
28.	06/2005	9	alive	alive
29.	07/2005	19	alive	alive
30.	08/2005	3	alive	alive
31.	09/2005	12	alive	alive
32.	10/2005	3	alive	alive
	2002-2005	**268**	**16.64**	**23.88**

Test tubes limited the mites' movement, imposing inability to impact their environment as they normally would. We therefore deem that these circumstances somewhat reduced the survival capacity of sampled mites compared to those in field conditions.

Sample amounts (0.5-1 cm^3) took in consideration the life patterns of *D. gallinae* in large populations, and the possible unknown mutual influence between the number of units and development stages on the survival of the most resistant ones.

Table 2 Overview of samples surviving longer than 9 months

	Sample number	Sampling place	Sampling date	Death date / still alive	Starvation period
1.	20.	Valjevo	11/08/02	05/07/03	47
2.	22.	Križevac	28/09/02	05/07/03	40
3.	26.	Prokuplje	14/09/02	23/07/03	45
4.	27.	Kruševac	28/09/02	29/07/03	44
5.	55.	G. Matejevac	27/10/02	27/08/03	44
6.	209.	Nikšić	25/09/03	25/06/04	39
7.	347.	Dragocvet	06/04/05	still alive	55
8.	350.	Vranjska Banja	07/04/05	still alive	-
9.	353.	Valjevo	12/04/05	still alive	-
10	360.	Zrenjanin	26/04/05	still alive	-
11.	361.	Valjevo	26/04/05	still alive	-
12.	371.	Nikšić	13/05/05	still alive	-
13.	372.	Martinići	13/05/05	still alive	-
14.	374.	Nikšić	13/05/05	still alive	-
15.	378.	G. Milanovac	28/05/05	still alive	-
16.	379.	Valjevo	29/05/05	still alive	-
17.	380.	Valjevo	29/05/05	still alive	-
18.	384.	Ćuprija	01/06/05	still alive	-
19.	385.	Priboj	01/06/05	still alive	-
20.	392.	Novi Bečej	29/06/05	still alive	-
21.	393.	Krušćić	05/07/05	still alive	-
22.	396.	Inđija	08/07/05	still alive	-
23.	397.	Bošnjani	08/07/05	still alive	-
24.	398.	Valjevo	11/07/05	still alive	-
25.	403.	Inđija	19/07/05	still alive	-
26.	405.	Prokuplje	21/07/05	still alive	-
27.	406.	Kosančić	21/07/05	still alive	-
28.	411.	Melenci	30/07/05	still alive	-

We did not monitor the death rate of *D. gallinae* in its developing stages, focussing attention on the most vital population segment, whose survival is essential to the survival of the population on a given location.

We did not establish the death rate percentage in relation to starvation time, because we believe that species with such high multiplication ability as *D. gallinae* quickly substitutes for the loss of a higher number of units. We believe that the most resistant *D. gallinae* units are the key to population survival.

We did not establish the reproduction ability of the surviving mites.

Table 1 shows sampling dates, average and maximum survival periods over months/years, as well as the number of poultry farms from which poultry red mites were drawn from 2002 to 2005. The stated total, average values of average and maximum survival of *D. gallinae* did not include live samples, so that they are approximate and lower than the actual ones.

The sources quote various data for possible starvation of *D. gallinae*, ranging from 34 days to 9 months (Wall and Shearer, 1997, 2001; Saif 2003.; Babić et al. 1956; Simić and Živković 1958; Bowman 1980; Mullen and Durden 2002; Pavlović 2002; Merck Veterinary Manual 1998; Chauve 1998; Nordenfors 1999, 2000).

Nordenfors et al. (1999) point to starvation capability of *D. gallinae* for 9 months on 5°C. These authors, however, did not establish the discrepancy between their findings and the findings of Harrison (1962), recording starvation survival for 9 months on 25°C. Nordenfors et al. state in their research findings that *D. gallinae* can survive only 6 weeks on 25°C, pointing to air humidity as a possible reason. Humidity in their experiments was only 23%, while in Harrison the humidity of *D. gallinae* samples was 80% (Nordenfors et al. 1999).

Humidity and temperature in our research was not maintained, especially not constant. The reason for this is that *D. gallinae* in field conditions are not exposed to constant temperature and humidity conditions. Air humidity in the facilities falls after flock depopulation, while temperature varies depending on the time of the year. Being interested in the survival rates in unheated, vacant facilities, we kept the samples in such spaces exposing mite samples to seasonal temperature fluctuations in accordance with annual weather changes of continental climate. Not knowing the other authors' work methods, we cannot clearly compare our results to theirs.

The longest survival period of *D. gallinae* field samples was 55 weeks, i.e. 13 months. This sample was taken in Dragocvet, near Jagodina. The most recent inspection was carried out on April 28, 2006, when we found that the sample still contained live mites.

We do not regard the results stated here as final. The surviving *D. gallinae* samples will be observed further, and new samples will be included into research.We did not observe patterns of impact of season dynamics on the mite vitality in test conditions without food.

Based on the author's field experience, *D. gallinae* can be found in farming facilities 1.5 year after flock depopulation. We therefore believe the obtained research results to be only a step toward the real answer to the question how long poultry red mites can actually starve. In addition, an infinite pause period in the facility and equipment operation is not necessarily fatal for *D. gallinae* population if they find an alternative food source.

Returning to the current situation and over 80% *D. gallinae* infestation rate of poultry farms in northern Europe (Van Emous, 2005), the invasiveness of *D. gallinae* is not only due to their biological features, but also to the inappropriate approach to their control. Thus the misestimation of infestation of poultry facilities of poultry farms and equipment after flock depopulation in a way contributed to the expansion of this parasitosis.

We believe that in individual cases it is better to assess the infestation of the facility and equipment, and apply mite control measures even if they are not infested, than to create conditions for the expansion of *D. gallinae.* Therefore, based on the obtained research results, we

propose an approach with additional safety period for practical assessment subsequent to facility operation pause.

We propose the following assessment: the facilities and equipment should be regarded as infested with *D. gallinae* for 1.5 years after flock depopulation, and mite control measures should be taken. After 1.5-2 years of operation pause, we believe that the facilities and equipment should be regarded as suspicious and mite control measures should be applied. Only a pause of more than 2 years should be enough for the present *D. gallinae* population to die out. Only in this case, and without alternative food sources, can mite control measures regarded as unnecessary.

Conclusion

Data obtained in our research indicate a much higher starvation capability of *D. gallinae* than recorded earlier. The longest survival period of *D. gallinae* amounted to 55 weeks, i.e. 13 months. The presented results of starvation capability studies of *D. gallinae* are to be regarded as preliminary publication. According to the authors' field experience, poultry red mites can sometimes be found one and a half years subsequent to facility depopulation. We therefore believe the results obtained to be only a step toward the real answer to the question how long poultry red mites can actually starve.

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Preliminary data concerning the monitoring of the infections, parasitary diseases and welfare of horses population from Danube delta.

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The objectives of the study was to diagnosis the new infection and main parasitic diseases of horse's population from some area of Danube Delta Reservation (ARBDD). The study reveals differences between helminthic population, according with the horse's areal origin. The second objective of this study was focused in the new diagnosis infection horse diseases in Romania - West Nile. According with this study, made in 42 locality from Tulcea county, from 666 studied horses, 214 (32,1%) was positive for these infection disease. The distribution of West Nile disease in the studied area, was monitories by creation a satellite maps of spreading diseases. The study of these maps helps us to elaborate few epidemiological hypotheses, which is very useful in order to supervise the distribution of this disease.

During these studies, we also investigated the welfare of the horses from the Tulcea county, the majority of the animals live in improper condition, especial during summer season. In that period the free cohabitation of a large number of horses attract the main parasitic vectors, especial insects (mosquito) which represent an important risk factor for spreading infection and parasitic contagious diseases.

Key words: horse, parasitary diseases and welfare, Danube delta

Researches concerning microbiological condition of some natural water sources for animals consumption

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The microbiological investigation of 110 samples from the local water sources and 56 samples from the surface waters for animals consumtion was performed.

According to the microbiological conditions of Law 458 / 2002 , Law 311 / 2004 and G.D 100 / 2002, 64,54 % of the local water source samples and 100% of surface waters samples were inadequate.

In warm seson, the water`s microbiological indicators were more increased because the temperature was favourable for bacterias replication.

Key words: water quality, bacteriological exams

The quality of water that is consumed by animals influences their health condition as well as the reproduction efficiency and the quality products. Starting with those ideas, we intended, in the present work, to analyse from hygienico-sanitary point of view some natural sources of water (non-treated waters), near Iasi county, taking into consideration that, in the rural area, those sources of water takes an important place to the prejudice of the treated waters supplied by centralized networks.

The level of bacteriological contamination of water was appreciated by determining the total number of embryos at 22^{o}C and 37^{o}C and of some qualitative micro-biological indicators (total coliforms, faecal coliforms).

Materials and methods

There has been examined 166 samples of water, from which 110 samples (66,26%) from local sources, (fountains and springs) and 56 samples (33,73%) from surface sources of water (rivers, small rivers, piscicol areas, and other types of surface water basines) taken as closer as we could from the area in which animals are drinking water. (table no 1).

The samples were taken both in cold season (december, january, february, march, october and november months) and also in hot season (aprilie, may, june, july, august and september months).

There had been used for that purpose recipients that are resistant to sterilized temperatures (160-180ºC) and which do not give toxic substances with bacteriostatic or bactericide effect.

Table 1

Samples of water analysed in each season and types of water sources

Season	Type of water sources	2003	%	2004	%	Total	%
Cold season	Water from local sources	26	**52**	24	**48**	50	**30,12**
	Water form surface sources	8	**50**	8	**50**	16	**9,63**
Hot season	Water from local sources	30	**50**	30	**50**	60	**36,14**
	Water form surface sources	20	**50**	20	**50**	40	**24,09**
Total		**84**	**50,60**	**82**	**49,40**	**166**	**100**

Determination of the number of native bacterias (saprofites) at 22ºC and of the number of mesofiles bacterias (37^{o}C) was made according to the method STAS 3001/91.

The number of colonies developed on the surface and on the ground mass was established with the help of the colonies counter (figure no. 1) not taking into consideration the parts with more than 300 colonies.

Figure no. 1 Colonies counter

Determination of the probable number of coliform bacterias was made with the help of indicated technique STAS 3001/91, but international standardized methods (ISO 9308 – 2 – 1990).

Work method to determine the probable number of thermo-tolerant bacterias was of MPN determination according to STAS 3001/91, ISO 9308 – 3/1990, şi ISO 9308 – 3 – 1998 .

Caracterization of water sources from a bacteriological point of view was made according to the present normatives:

Law 458 / 2002 and Law 311 / 2004 , concerning drinking water quality, for waters coming from local sources (fountains and springs)

HG 100 / 2002, for classification of surface waters used for drinking (table 2).

Table 2

Maximum admited limits for microbiological indicators for each cathegory of surface waters (HG 100/2002)

Bacteriological indicator	**Quality cathegory Maximum admited limit**			
//	A1	A2	A3	Degradated waters
MPN total of coliforms	50	5000	50000	Above maximum admited limits for A3 cathegory
MPN faecal coliforms	20	2000	20000	Above maximum admited limits for A3 cathegory

Results and debates

According to the microbacteriological contamination, established based on the obtained results at qualitative and quantitative indicators, the analysed sample waters were grouped into two qualitative cathegories: conform samples and non-conform samples with norms foreseen by the present legislation (tables 3 and 4).

Table 3

Water quality from local sources

Season /year	No of analysed samples	Bacteriological concordant samples	%	Bacteriological nonconcordant samples	%
Cold 2003	26	8	**30,99**	18	**69,23**
Hot 2003	30	9	**30,0**	21	**70,0**
total	56	17	**30,35**	39	**69,64**
Cold 2004	24	8	**33.33**	16	**66,66**
Hot 2004	30	14	**46.66**	16	**53,33**
Total	54	22	**40.74**	32	**59,25**
Total gen.	110	39	**35,45**	71	**64,54**

Table 4

Quality of surface waters used to feed animals with water

Year	Season	No of samples	A1	%	A2	%	A3	%	Degradated waters	%
2003	Cold	8	3	**37,5**	3	**37,5**	2	**25**	2	**0**
	Hot	20	8	**40,0**	8	**40**	2	**10**	2	**10**
2004	Cold	8	3	**37,5**	3	**37,5**	2	**25**	0	**0**
	Hot	20	10	**50**	5	**25**	5	**25**	0	**0**
total		56	24	**42,80**	19	**33,90**	11	**19,64**	2	**3,57**

Analysing the data from table 3 we notice that, no matter the season, the presence of bacteriological nonconcordant samples is higher than the ones concordant from a bacteriological point of view (64,54%, respectivelly 35,45%).

In 2004, the percentage of the nonconcordant samples was reduced comparing with 2003 (from 69,64% to 59,25%), a fact that means an improval of water quality.

Analysing the percentage of quality cathegories at surface waters (table 4) we notice that fact that, in 2004, it was registered an improval of the quality of those sources of water, meaning that the percentage of waters from A1 cathegory was increased (clear waters) in hot season (from 40 % in 2003, to 50% in 2004).

We think that the decrease of the microbiological condition of analysed waters is due to the introduction of the measures for environment protection.

Another aspect, aparently in contradiction, that we notice from analysing the above mentioned tables, is the fact that the percentage of waters from A1 cathegory increases in hot season: in 2003, from 37,5 % to 40 %, and in 2004, from 37,5 % to 50 %.

The surface waters are used to feed animals since the end of march – the begining of april, when the snow starts to melt and the waters that wash the contaminated soils collects in the riverbeds of the rivers and in the surface basins.

In hot season, a stratification and sedimantation of mass water is produced, which determines a decrease of the number of bacterias in the waters aproximativelly clean and with no other polluting sources. In waters already bacteriological polluted, in the same time with the increase of temperature, and in the presence of trofic substances, some bacterial species are increased.

Thus, we can explain the fact that the medium of the number of mezofiles bacterias shows a decrease in hot season comparing with cold season, in clean waters, from 2375 to 1538 in 2003, and from 2362 to 1937,14 in 2004, while in polluted waters, those values increase from 15275 in cold season to 67023 in hot season in 2003 and from 12950 to 24783,3 in 2004 (table 5).

According to the values of the parameter, the total number of mezofiles bacterias, none of the water samples analysed in cold season of the years 2003 and 2004 was not framed in A cathegory (under 1000 colonies / ml), 56,25 % were framed in B cathegory (1000 – 3000 colonies / ml) and 43,75%, in C cathegories (over 3000 colonies / ml). In hot season, 10% from the analysed samples were framed in A cathegory, 30% belonged to B cathegory , and 60% to C cathegory.

The evolution of the MPN averages of termo-tolerant coliforms and E. coli / 100 ml of water indicates significant increases from the cold season to the hot one, both in local sources and also in surface waters (diagrams 1 and 2).

From the analyses we have made, results the fact that the surface waters are more frequent and more intense contaminated termo-tolerant coliforms and E. coli, comparing with waters coming from local sources.

That fact makes us wonder about the hygiene of the animals feeding from that kind of water sources, taking into consideration that those indicative bacterias may be anytime accompanied by pathogen embryos for animals.

CONCLUSIONS

1. There have been analysed from a micro-biological point of view 166 water samples coming from natural sources (non-treated) for animals to drink water.
2. 64,54 % from the samples taken from local sources of drinking (fountains and springs) did not meet drinking standards and 100% from the samples taken from the surface waters.
3. The repartition of the samples non-concordant from a microbiological point of view, on each season, shows a higher percentage of it in hot season, comparing with cold season.
4. In hot season, water has bacteriological indicators that have higher values comparing with cold season, due to the multiple posibilities of contamination, but to the posibilities of multiplication of bacterias in adequate conditions.
5. The use of the surface waters to feed animals with water presents the risk to take hidruce infections.

The average of MPN CTT and MPN E. coli values in water samples taken from surfaces sources used to feed animals with water

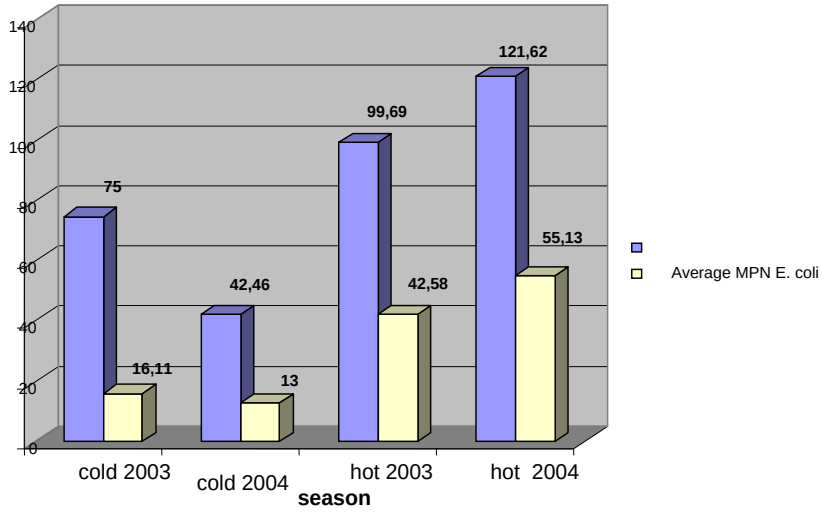

Diagram no.1

Variation of the averages of MPN CTT and MPN E. coli in water samples taken from surface sources to feed animals with water

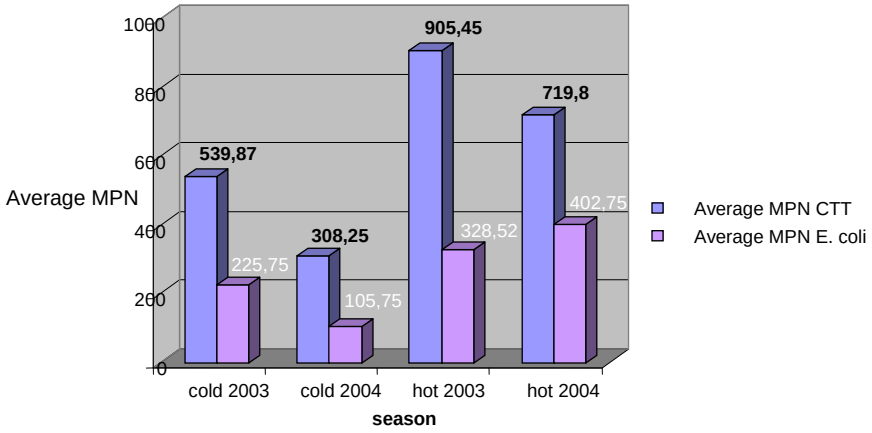

Diagram no. 2

Table nr. 5

Variation of the number of mezofile and native bacterias (37 şi 22°C) on each surface water cathegory and on each season, in 2003 and 2004

Year Season	Water Sample\ cathegory **A/ %**	Min. no Max. no **Average UFC 37°C**	**Average UFC 22°C**	22°C/ 37°C	water sample Cathegory. Analiz **B/%**	Min. no Max. no **Average UFC 37°C**	**Average UFC 22°C**	22°C/ 37°C	Water Sample\ cathegory **C/%**	Min. no Max. no **Average UFC 37°C**	**Average UFC 22°C**	22°C/ 37°C
2003 cold	0/0	**/**	/	/	4/50	2200-2500 **2375**	**6425**	2,70	4/50	5500-35000 **15275**	**39400**	2,57
2004 cold	0/0	**/**	/	/	5/62,5	1580-2660 **2362**	**6180**	2,61	3/37,5	5300-21000 **12950**	**34500**	2,66
T=16	**0/0**	**/**	**/**	**/**	**9/56,25**				**7/43,75**			
2003 Hot	2/10	880 -900 **890**	**2415**	2,71	5/25	1200-2500 **1528**	**4384**	2,86	13/65	5200-248000 **67023**	**157044,6**	2,34
2004 Hot	2/10	440-850 **645**	**1790**	2,77	7/35	1220-2200 **1937,14**	**5257**	2,71	11/55	3220-71000 **24783,3**	**71056,6**	2,86
T=40	**4/10**				**12/30**				**24/60**			

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Study of an outbreak of IBR-IPV in Romania

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Epidemiological investigations made in a farm of diary cows, initiated by a clinic suspicion lead us to the serologic diagnosis of an infection with IBR-IPV virus. Specific antibodies anti –IBR were detected using blocking-ELISA (IDEEX product) at 17/26 cows, 1/16 heifers and 4/18 calves, summing up 22 positives responses from 60 examined serums (36,66 %). A vaccination program using Bovilis IBR Marker (Intervet product) associate with a symptomatic and hygienic treatment was set up. The situation ameliorated and the herd was submitted to systematic surveillance.

Key words: IBR-IPV, serologic diagnosis, vaccination, surveillance

Serological investigation of WNV in horses from the south-east of Romania

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The WNV infection in human and animals is an actual subject in Romania. In the cadre of a research project we have done a supervision investigation of WNV in the horse population from 6 districts placed in the south –east of Romania (the inferior area of Danube).

A number of 167 samples of serum ingathered from 25 localities placed at the Danube river side or other adjacent rivers were examinee using ELISA – West Nile Indirect Kit (made by ID Vet) and Immuno Comb – Equine West Nile Virus Antibody Test Kit (made by Biogal Galed Laboratories).

Of all serums tested, 55 samples were found positive, meaning a 33% prevalence. The investigations are continuing.

Key words: West Nile Virus, horse, seroprevalence investigation

Researches concerning the technological density influence on Ross 308 hybrid broilers production performances

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Technological density assured in poultry halls setup is one of the most important factors to be managed in broilers rearing on permanent litter systems. The paper presents the studies on the yield performances of the Ross-308 broilers, reared within different technological densities conditions, as specified below: C group – 15 chicks/m^2; E1 group – 16 chicks /m^2 and E2 group – 14 chicks /m^2. Some specific indexes were observed, such as: microclimate factors (temperature, air moisture, ventilation), weight gain dynamics, daily feed intake, feed conversion ratio, flock looses and European Efficiency Factor. Best yields were observed at the broilers reared within 14-15 chicks/m^2 density values. Thus, the chickens from C and E2 groups had final live weights 11.49% and 10.85% higher than those belonging to E1 group. European Efficiency Factor (EEF) was found as 16.75% for C group, as compared to E1 group, while the boilers from E2 group showed 12.18% higher values than those in the E1 group.

Key words: setup technological density, broiler, Ross-308

The optimal technological density on series setup (chickens/m^2) is one of the important concerning broiler chickens rearing on permanent litter system. This is usually established according to the hybrid, to the final live weight to be achieved, to some other factors (slaughter age and weight, microclimate conditions, rearing season, shelter type etc.).

If the on setup density is exacerbated, some specific symptoms could be observed: growing rate decreasing especially before slaughtering; chickens non uniformity becomes frequent, mortality increases. Moreover, the litter get worse and illness incidence increases; legs skeleton deficiencies occur; meat quality (color, texture, scent, taste, flavor) and skin quality (scratches, texture) decreases, while feathering becomes low (*Vacaru-Opriş I. et al., 2002, 2005; Ross-308 Management Guide, 2002*).

Considering these, we proposed to assess the yield level of the chickens reared at different on setup differences.

Material and method

The experiment was setup within the S.C. AVIS 3000 S.A. company, Mintia place, Hunedoara county, on a flock of 48.600 day old chicken broilers, „Ross – 308" hybrid, allocated into four groups: control group (C), including 12.150 broilers, and two experimental groups (E1 and E2), having different size: E1 – 12960 broilers and E2 – 11.340 broilers (*tab. 1*).

The on setup density had different values for each group: de 15 chickens/m^2 in C group, 16 chickens/m^2 in E1 group and 14 chickens/m^2 in E2 group.

Some control representative groups were elected from the whole experimental groups, considering that some studied indices could not be assessed on such a huge flock. The representative groups were named control group no. 1, no.2 and no. 3, each of them including 200 chickens/group. All the control chickens were marked. If there were looses from these chicks,

they were replaced with other unmarked chickens in the hall, which had closer values to the average weight of the representative ones.

A permanent litter system was used for chicks rearing, till the age of 42 days, when they were slaughtered. All the shelters were endorsed with Big Dutchman technological equipments.

Table 1

Experimental design

Experimental group	C	L2exp	L3exp
Day old chickens (heads)	12.150	12.960	11.340
Control group	No. 1	No. 2	No. 3
Chickens within groups (heads)	200	200	200
Rearing period		0 – 42 days	
Onset technological density (chickens/m^2)	15	16	14

Studied indices:

- shelters microclimate: environment temperature (tº); air moisture (%); ventilation (m^3 air/hour/kg body weight);

- weight gain dynamics:

- live weight, after weekly weighting;
- average daily weight gain, weekly and cumulated;

- feed intake: g m.f./chick/day; feed conversion ratio (kg m.f./kg gain);

- flock looses and their reasons;

- European Efficiency Factor.

The recipes used in broiler feeding met characteristics closely similar to those proposed by „Ross Breeders" UK company, for their hybrid - „Ross-308" (*tab. 2*).

Table 2

Mixed fodders recipes used in experiments

Raw materials (%)	*Starter*	*Grower*	*Finisher*
Maize	46.46	40.21	41.46
Soymeal	39.81	26.10	12.02
Full fat soybean	-	18.36	24.78
Maize gluten	3.00	6.00	8.00
Barley	-	5.00	10.00
Fishmeal	7.00	-	-
Soybean oil	-	-	-
Monocalcium phosphate	0.90	1.22	0.98
Calcium carbonate	1.35	1.43	1.45
Premix	0.50	0.50	0.50
Rhodiment-Methionine	0.20	0.20	0.10
L-Lizine HCl ADM	0.10	0.15	0.11
Salt	-	0.15	0.09
Coline	0.12	0.10	0.08
Bioplus 2B	0.10	0.10	-
Sodium bicarbonate	0.11	0.23	0.28

AVATEC (lasalocid)	0.10	0.10	-
KEMZYME MS dry	0.05	0.05	0.05
MYCOSORB	0.10	0.10	0.10
L-Threonine ADM	0.10	-	-
TOTAL	100.00	100.00	100.00
Nutritional features			
EM kcal/kg	3017	3175	3225
CP %	24.50	22.50	21.00
CF %	6.59	7.87	7.84
GF %	4.09	4.33	4.09
Ca %	1.05	0.90	0.85
Available P %	0.50	0.45	0.42
Na %	0.17	0.16	0.16
Cl %	0.20	0.22	0.22
Lysine %	1.50	1.28	1.10
Digestible lysine for poultry %	1.35	1.10	0.92
Methionine %	0.68	0.62	0.53
Digestible methionine for poultry %	0.64	0.57	0.48
Methionine+Cystine %	1.09	1.05	0.95
Methionine+Cystine for poultry %	0.96	0.91	0.80
Threonine %	1.12	0.96	0.93
Triptophan %	0.27	0.24	0.21

Results and discussions

a) Microclimate from the shelters

Environment temperature

Halls temperature was assessed at litter level, knowing chickens behavior is a good indicator of this factor status.

The temperatures within the shelters accommodated the 36450 „Ross 308" broilers, were found as slightly superior to those specific from the standard dynamics in the management guide.

Thus, during day 2, the temperature in the experimental halls was 1.80÷2.41% higher than the guide standard, of +31ºC (31.56ºC for C group; 31.68ºC for E1 group and 31.60ºC for E2 group).

Between days 3 and 7, the temperature in hall no 1 (C group) was found of +31.19 ÷ +30.29ºC, while into the halls 2 (E1) and 3 (E2), it was found between +31.00 ÷ +30.50ºC limits; those temperatures was also higher than the company recommendations for the same period (+29 ÷ +27ºC). The differences remained similar over the entire rearing period. Thus, for the last week of life, the standard specifies +19ºC, while the temperature into the experimental halls measured +21.90 ÷ +20.28ºC.

Very high external temperatures were incriminated onto the high internal temperature levels, which passed over the limits indicated by the broiler management guide.

Air relative moisture

The assessments of this parameter showed good values, which were found quite similar to the requirements of the „Ross 308" hybrid technical specifications.

Thus, during first experimental week, the air moisture in the halls was measured and found very close to the values recommended by the „Ross Breeders" companies (58.81÷62.67% for C group;

57.23÷60.14% for E1 group and 57.54÷61.34% for E2 group, as compared to 55-60% values, as specified by the hybrid standards).

Similar dynamics was observed till the chickens slaughtering age (42 days), relative air moisture into the control group hall reaching 67.91±7.16%, 65.26±4.16% for E1 group and 65.23±5.34% for E2 group, as compared to 65% (technological requirements).

Ventilation

The ventilation ratio (admitted airflow) into the three halls, as measured till 21 days old, was of 0.8-1 m^3/kg live weight/hour. The air velocity did not pass over 0.1 m/s in any of the studied halls.

From the 22nd day, the ventilation ratio proportionally increased, according to the weight gain values. Thus, values of 1-3.8 m^3/kg live weight/hour were observed for the C group, while other values, of 0.8-4 m^3/kg live weight/hour were found for the experimental groups (E1 and E2).

It could be stated that the ventilation ratio, within the three studied halls, was found between normal limits, also specified by the „Ross 308" hybrid management guide.

b) Chickens weight gain dynamics

Individual weightings were made in order to asses the body weight of the all marked chickens.

At the experiment setup, very close values for the body weight were observed between the three experimental groups, from 40.52±0.49 g at E1 group, to 40.71±0.34 g at C group (*tab. 3*).

Table 3

Live weight of the chickens

Chickens age (days)	*Experimental groups*	*n*	$\bar{X} \pm s_{\bar{X}}$ *(g)*	*V%*
1	C	200	40.71±0.34	8,07
	E1	200	40.49±0.49	8,14
	E2	200	40.52±0.49	7,61
	Fisher Test	$\hat{F} = 2.47 < F_{0.05}$ (2;598) 2.60. *There were not found significant differences.*		
42	C	200	2370.80±25.14	17,83
	E1	200	2126.46±23.08	18,64
	E2	200	2357.34±22.79	19,37
	Fisher Test	$F_{0.05}$ (2;598)=2.60; $F_{0.01}$(2;598)=4.60; $F_{0.001}$(2;598)=5.42 $\hat{F} = 5.59 > F_{0.001}$(2;598)=5.42 *(***).*		
	Tukey Test	C-E1		**
		C-E2		n.s.
		E2-E1		**

Notice: Fisher Test- *** - very significant;
Tukey Test - ** - significant; n.s. – not significant.

Best performance at last weighting (the 7th - 42 days old chicks) was achieved by the C group – 2370.80 g, respectively 0.56-10.3% higher than the average live weight observed in experimental groups.

Variation coefficient was found as lower than 20%, indicating a middle area homogeneity (V%=17.83-19.37).

Statistically significant differences were found between groups C-E1; E2-E1.

The lowest performance was achieved by the E1 group, which had an onset density value of 16 chickens/m^2.

When the average live weights obtained by the chickens in the experiment were compared to those specified into the „Ross 308" management guide, there were found negative differences at

the end of the 42 days rearing period. During rearing period, some weighting did show higher values of the experimental groups as compared to those considered as standard. Thus, at age of 7 days, chickens from E1 and E2 groups achieved live weights 1.33-1.44% higher than the standard ones; at age of 21 days, all groups did show 1.17-6.46% higher values. The same situation was observed at 28 days old, the chickens achieving average weight higher with 2.52-3.50% as compared to standard. At 35 days old, broilers from experimental groups were 4.82-7.16% weightier than the standard specifications.

Despite this, at the end of the rearing period, the chickens from experimental groups did not pass over the standard weight value for this hybrid (2400 g), although two groups realized values closer to the standard ones (C – 2370.80 g and E2 – 2357.34 g).

Average daily gain, expressed weekly and cumulated

The average daily gain (ADG) and the cumulated weight gain were calculated at the end of each week. Thus, after the first 7 days of life, the chickens belonging to E1 and E2 experimental groups did show higher weight gains (2.89%, respectively 2.72%) as compared to the reference treatment (*tab. 4*).

Table 4

Average daily, weekly and cumulated weight gain of the chickens

Experimental groups	*Average body weight at the experiment onset (g)*	*Average body weight at the end of the experiment (g)*	*Average weekly value of the ADG (g)*	*Cumulated gain (g)*	*± % as compared to C group*
	7 days old				
C	40.71	161.08	17.19	120.37	-
E1	40.48	164.34	17.69	123.86	+2.89
E2	40.52	164.17	17.66	123.65	+2.72
	14 days old				
C	161.08	414.91	32.26	374.20	-
E1	164.34	420.70	36.62	380.22	+1.60
E2	164.17	432.19	38.28	391.67	+4.66
	21 days old				
C	414.91	804.35	55.63	763.64	-
E1	420.70	805.42	54.96	764.94	+0.17
E2	432.19	846.37	59.16	805.85	+5.52
	28 days old				
C	804.35	1311.24	72.41	1270.53	-
E1	805.42	1313.19	72.53	1272.71	+0.71
E2	846.37	1323.84	68.21	1283.32	+1.00
	35 days old				
C	1311.24	1920.64	87.05	1879.93	-
E1	1313.19	1914.18	85.85	1873.70	-0.33
E2	1323.84	1956.87	90.43	1916.35	+1.93
	42 days old				
C	1920.64	2370.80	64.30	2330.09	-
E1	1914.18	2126.46	30.32	2085.98	-10.47
E2	1956.87	2357.34	57.21	2316.82	-0.56

At the other three weightings, the broiler from the experimental groups achieved 0.17-5.52% higher weight gains than those obtained by the chickens in the control (C) group.

During weeks 5 and 7, the chickens in the control group obtained 0.11-10.47% higher weight gains, as compared to experimental groups, mainly to the E1 group, which had the highest density value – 16 chickens/m^2.

Concerning the average daily gain, as expressed for the entire rearing period, it was found of 55.47 g at C group, of 49.66 g at E1 group and of 55.16 g at E2 group.

Par consequence, it could be stated that feeding chickens feed without fodder additives did not produce beneficial effects onto the average daily gain, while assuring densities higher than 15 chickens/m^2 leaded, also, to lower weight gains.

c) Feed intake

Feed intake intensity evolved in accordance with the achieved body weight. As it is well known, there is a straight relationship between growing speed and feed conversion ratio – animals with better growing performance consume less food and conversely.

Thus the daily and cumulated feed intakes were higher in E2 group, as compared to other groups; par consequence, at the age of 14 days, cumulated feed intake reached 483 g in C group, 462 g in E1 group, respectively 499 g in E2 group.

At 28 days old, C group had a cumulated feed intake of 1903 g, while this parameter was found as 6.04% lower in E1 group and 3.78% higher in E2 group.

The dynamics was the same after 28 days old. Thus, at age 42, the cumulated feed intake was higher with 2.94%-6.30% at C group, as compared to the E2 and E1 group.

Feed conversion ratio was calculated at the end of the 42 days rearing period. The best value was found for C group, of 1.809 kg feed/kg gain, while the experimental groups consumed 3.59-4.69% feed for the achieved gain (*tab. 5*).

Table5

Feed conversion ratio

Experimental groups	Overall gain (kg/chick)	Average feed intake (kg/chick)	FCR (kg feed/kg gain)	±% as compared to C group
C	2.330	4.217	1.809	-
E1	2.085	3.951	1.894	+4.69
E2	2.316	4.341	1.874	+3.59

d) Flock looses and their reasons

During entire experimental period, flock looses din not pass 3.5% for any experimental group (*tab. 6*).

Table 6

Flock looses dynamics

Experimental groups	*Flock size at the experiment onset (chicks)*	*Flock size at the end of the experiment (chicks)*	*Looses (heads)*	*%*
C	12150	11747	403	3.31
E1	12960	12533	427	3.29
E2	11340	10974	366	3.22

Most of looses were mainly observed during the 1st week of life, being caused by some accidents and also by enteritis and coccidiosis. Other specific illness was not observed during experimental period.

e) European Efficiency Factor

When chickens reached 42 days old, the European Efficiency Factor was calculated, in order to asses the economical efficiency of poultry raising.

As computation basis for the EEF, some elements were used, such as: chickens age prior to slaughter moment; average live weight at slaughtering age/group (kg); viability/group (%) and feed conversion ratio (FCR kg feed/kg weight gain). Computation elements and the EEF values, as resulted from the mathematical relation below, are presented in *table 7*:

$$EEF = \frac{\text{viability (\%)} \times \text{body weight (kg)}}{\text{age (days)} \times \text{IC (kg feed/kg gain)}} \times 100.$$

Table 7

European Efficiency Factor

Experimental groups	*Age (days)*	*Av. living weight (kg)*	*Mortality (%)*	*FCR (kg feed/kg feed)*	*EEF*
C	42	2.370	3.31	1.809	301.63
E1	42	2.126	3.29	1.894	258.35
E2	42	2.357	3.22	1.874	289.84

Considering the calculated values for EEF, it could be observed some 3.90-14.34% higher values for the control group, as compared to the experimental groups, due to higher body live weight of the chickens in the reference group at slaughtering moment (+0,54…+10,29%).

Conclusions

1. Microclimate factors from the shelters accommodated chickens did not show very significant variations between halls. Thus, *the temperature* was a little bit higher than that recommended by „Ross Breeders" company for the „Ross 308" hybrid. Higher temperature into the shelters environment dew to higher environment temperatures outside the halls, those being not perfectly managed as climate. *Air relative moisture* varied between closer limits, around the standard values recommended by the hybrid producer.
2. When the live weight dynamics was assessed, it could be observed several fluctuations, as compared to the broiler management guide: at 7 days old, the chickens within the E1 and E2 groups achieved 1.33-1.44% higher values than the standard; at age 21, all groups shown 1.17-6.46% higher values, while at the end of the experiment the body weight values passed under the standard recommendations. The situation has been due to the heat stress during the last day of life. The average daily gain (ADG) was correlated to the growing speed values, being 0.56-10.47% lower than the values found in control group.
3. Cumulated feed intake (g/chicken) was of 4341 g at E2 group, respectively 2.83-8.98% higher than these observed in experimental groups. Concerning the feed conversion ratio (g feed/g

weight gain), this was found as 3.59-4.69% higher in experimental groups, as compared to the reference one.

4. Overall flock looses represented 3.31% at control group; 3.29% at E1 group and 3.22% at E2 group. Highest loss rate was observed during the first week, mainly dew to accidental reasons. Other reasons consisted in enteritis and coccidiosis. No other illness symptoms were observed during reraring period.
5. Every experimental group passed over the 250 limit of the EEF (301.63 at C group; 258.35 at E1 group and 289.84 at E2 group). The control group, which had best body development and the lowest feed intake, achieved the highest EEF value, 3.90-14.34% higher than the values obtained within experimental groups.

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Unii indici biochimici ai sângelui şi nivelul titrelor de anticorpi la puii vaccinaţi contra Bursitei infecţioase în combinaţie cu biomasa din streptomicete

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The article included authors dates of serological immune response and any biochemical modifications parameters of blood of one day old chickens vaccinated with PA and Winterfield strains separate and in combination with biomes of Streptomicetes. The biomes was administrated in the feed . In results was established that vaccination one day old chickens against Gumboro disease didn't stimulated the high level of antibodies which can protected the chickens against disease. More high level of antibodies was in the group of the chickens were the vaccines strains ware administrated with biomes of Streptomicetes. In the same groups the chickens had more high level of total proteins, albumins and globulins.

Key Words: Gumboro disease, immune response

Bursita infecţioasă aviară (BIA) este cunoscută pe tot globul pământesc provocând pagube economice considerabile în special ţărilor cu creşterea intensivă a păsărilor. Boala este una dintre cele mai periculoase boli infecţioase la puii de găină care se caracterizează cu scăderea masei corporale, mortalitate de la 15 până la 45 – 60%, rată crescută a conversiei furajelor.

O receptivitate mai înaltă o au puii cu vârsta de 3 – 6 săptămâni.

Datorită faptului că virusul bursitei infecţioase afectează ţesutul limfoid, în special celula limfocitară „B” boala este considerată „imunosupresoare” şi este grupată împreună cu alte boli cu acţiune de acest gen cum sunt complexele leucoze – sarcom, leucemie, grupa bolilor SIDA-like).

Imunosupresia produsă de distugerea limfocitelor „B” afectează imunitatea mediată umoral şi cea celulară. Ea reprezintă principala cauză în moartea puilor. Păsările contaminate cu virusul BIA prezintă o scădere a titrelor de anticorpi în imunizările contra pseudopestei aviare, bronşitei infecţioase, sunt mai sensibile la infecţiile colibacilare, micoplasmoză, salmoneloză etc.

Studiul efectuat de către noi a avut scopul aprecierii eficacităţii imunologice a puilor vaccinaţi contra BIA la vârsta de o zi şi acţiunea biomasei din streptomicite la unii indici biochimici ai sângelui puilor vaccinaţi în combinaţie cu vaccinările din tulpinile PA şi Vinterfield.

Material şi metodă

Investigaţiile au fost efectuate pe 6 grupe de pui cu vârsta de o zi, separaţi a câte 20 capete fiecare, care se întreţineau în condiţii analogice.

I grupă – lot martor, a II – grup – puii care au primit numai biomasa de streptomicete, a III – grupă – puii vaccinaţi cu tulpina PA + biomasa de streptomicete, a IV grupă – puii vaccinaţi cu tulpina Vinterfield + biomasa de streptomicete, a V grupă – puii vaccinaţi numai cu tulpina PA, şi a VI grupă – puii vaccinaţi numai cu tulpina Vinterfield.

Vaccinarea puilor s-a efectuat la vârsta de o zi cu apa de băut. Biomasa de streptomicete s–a administrat în combifuraje în raportul 1 g/ 1 rg combifuraj la puii cu vârsta până la 21 de zile şi 2 gr / 1 kg combifuraj la puii cu vârsta 21 – 45 zile.

La vârsta 15, 30, 45 de zile din fiecare grupă sau sacrificat câte 5 capete puii la care se examina: greutatea corporală şi a bursitei fabricius, se recoltă sânge şi ser sanguin pentru examenul biochimic şi a nivelului titrelor de anticorpi contra virusului bursitei infecțioase aviare (BIA).

Rezultate şi discuţii

Rezultatele investigațiilor serologice sunt prezentate în tabela № 1 de unde observăm că la puii din grupele lot martor titre de anticorpi postvaccinali nu au fost depistați nici la una din examinările efectuate.

La a 15 zi după vaccinare titre de anticorpi au fost depistate la puii vaccinați cu tulpina PA şi Vinterfield în combinație cu biomasa de streptomicete numai în diluția serului 1:50 care au constituit două simboluri „+” din patru posibile. Titre de anticorpi cu nivelul de un simbol „+” au fost depistate şi la puii din grupele vaccinate numai cu tulpinile vaccinale PA şi Vinterfield. La a 30 zi după administrarea vaccinului titre de anticorpi la nivel de un simbol „+” au fost depistate la puii din grupele vaccinate cu vaccinurile PA şi Vionterfield în combinație cu biomasa de streptomicete. La puii din celelalte grupe titre de anticorpi postvaccinali nu s-au depistat.

De asemenea, la a 45-a zi după vaccinare nici la o grupă din puii vaccinați nu s-au depistat titre de anticorpi postvaccinali.

În tabelul № 2 sunt prezentate unii indici biochimici ai sângelui la puii vaccinați cu tulpinile PA şi Vinterfield aparte şi în combinație cu biomasa de streptomocete.

La 15 zile după vaccinare, la puii din grupa lot martor proteinele totale au constituit 14,6 g/l dintre care 11,4 g/l albuminele şi 3,2 g/l globulinele. Cel mai înalt nivel de proteine s – a stabilit la puii din drupa lot martor la care în rație li s-a administrat biomasa de streptomicete ce a constituit respectiv proteinele totale – 22,6g/l corespunzător.

Mai înalt nivelul de proteine a fost şi în grupa puilor vaccinați cu tulpina PA în combinație cu biomasa de streptomicete fiind de 20,9 g/l. În celelalte grupe de pui acest indice a variat de la 11g/l până la 16,5 g/l.

La a 30-a zi după vaccninare la puii din lotul martor proteinele totale au constituit 15,1 g/l. La puii grupelor experimentale acest indice a fost mai înalt şi a constituit în grupele puilor vaccinați cu vaccinările PA şi Vinterfield cu adaos de biomasă a streptomicetelor 18,6 şi 21,6 g/l corespunzător şi 17 şi 16 g/l la puii vaccinați numai cu vaccinările nivelul albuminelor şi globulinelor.

La a 45-a zi după vaccinare la puii din grupa lot martor nivelul proteinelor a constituit 16,3 g/l, pe când la puii din grupele vaccinați cu tulpinile PA şi Vinterfield în combinație cu biomasa de streptomicete a constituit respectiv 18,2 şi 18,6 g/l. Puțin mai jos acest indice a fost în grupa puilor unde li s-au administrat numai vaccinările constituind 17,6 şi 15,1 g/l. Corespunzător şi nivelul albuminelor, globulinelor a fost mai înalt în grupele puilor unde vaccinările au fost administrate în combinație cu biomasa de streptomicete.

Tabela № 1

Nivelul titrelor de anticorpi la puii vaccinaţi contra bursitei infecţioase în combinaţie cu biomasa de streptomicete

Gr.	№ de capete	Vârsta puilor vaccinaţi (zile)	Tulpina vaccinală / Biomasa de streptomicete	Perioada de adm.a biomasei de streptomicete (zile)	Diluţia serului 50/100	Perioada de examinare după vaccinare		
						15 zile	30 zile	45 zile
I	20	1	L / M	-	50 / 100	- / -	- / -	- / -
II	20	1	L / M / BM	45	50 / 100	- / -	- / -	- / -
III	20	1	PA / BM	45	50 / 100	+ + / -	+ / -	- / -
IV	20	1	Vinterfvield / BM	45	50 / 100	+ + / -	+ / -	- / -
V	20	1	PA / -	45	50 / 100	+ / -	- / -	- / -
VI	20	1	Vinterfield / -	45	50 / 100	+ / -	- / -	- / -

Tabela № 2

Unii indici biochimici ai sângelui la puii vaccinaţi contra Bursitei infecţioase în combinaţie cu biomasa de streptomicete

Gr	№ de capete	Tulpina vaccinală / Biomasa de streptomicete	Indicii biochimici ai sângelui după vaccinare														
			15 zile					30 zile					45 zile				
			Proteine	Albumine		Globuline		Proteine g / l	Albumine		Globuline		Proteine	Albumine		Globuline	
			g / l	g / l	%	g / l	%		g / l	%	g / l	%	g / l	g / l	%	g / l	%
I	20	LM	14,6	11,4	78,0	3,2	21,9	15,1	10,2	67,5	4,9	32,4	16,3	10,2	62,5	6,1	37,4
II	20	LM / BM	22,6	13,4	59,2	9,2	40,7	16,3	12,2	74,8	4,1	25,1	18,4	12,0	65,2	6,4	34,7
III	20	PA / BM	20,9	13,2	63,1	7,7	36,8	18,6	12,5	67,2	6,1	32,7	18,2	10,7	58,7	7,5	41,2
IV	20	Vinter/BM	12,5	9,4	75,2	3,1	24,8	21,6	15,8	73,14	5,8	36,8	18,6	10,5	56,4	8,1	43,5
V	20	PA / -	16,2	12,1	74,6	4,1	25,3	17,0	10,5	61,7	6,5	38,2	17,6	12,4	70,4	5,2	29,5
VI	20	Vinter / -	11,0	6,8	61,8	4,2	38,1	16,0	9,7	60,6	6,3	39,3	15,1	8,7	57,6	6,4	42,3

Concluzii

1. Administrarea vaccinărilor contra bursitei infecţioase aviare la puii cu vârsta de o zi nu stimulează formarea nivelului de anticorpi care ar putea proteja puii de boală.
2. Utilizarea vaccinurilor contra bursitei infecţioase aviare în combinaţie cu biomasa de streptomicete stimulează formarea titrelor de anticorpi şi influenţează pozitiv la indicii biochimici ai sângelui manifestându-se prin majorarea nivelului proteinei totale şi respectiv a albuminelor şi globulinelor.

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Researches regarding Gumboro disease (Infectious Bursal Disease)

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Gumboro disease is a worldwide spread disease appeared in the last decades and probably is the most important avian infectious disease.

Infectious bursal disease virus affects Harder gland responsible for anterior respiratory tract immunity. In this way favors other respiratory infections as infectious bronchitis, laryngitis and tracheitis. In the last period were emitted, hypothesis regarding the disease dynamics. Mainly this viral infection is considered an immune complexes disease. This explain the hemorrhagic lesions and immune disturbances.

The following paper wishes to present few epidemiological investigations in a fowl farm.

Key Words: Gumboro disease, immune response

MATERIAL ŞI METODE

În perioada analizată 2004-2005 a fost urmărită evoluția bursitei infecțioase aviare, într-o unitate avicolă pentru creşterea puilor de găină broiler. Fluxul tehnologic în acest interval de timp a respectat principiul „totul plin-totul gol", pe fermă, cu perioade de vid sanitar de cel puțin de 2-3 săptămâni.

Investigațiile epidemiologice efectuate au urmărit pierderile pe săptămână, dar şi totale, vârsta declanşării bursitei aviare, mortalitatea cauzată de BIA (număr şi procente).

REZULTATE ŞI DISCUŢII

Din investgațile epidemiologice rezultă că la unitatea „F", bursita infecțioasă aviară evoluează începând din 1990 şi nici până astăzi nu a putut fi eradicată în totalitate.

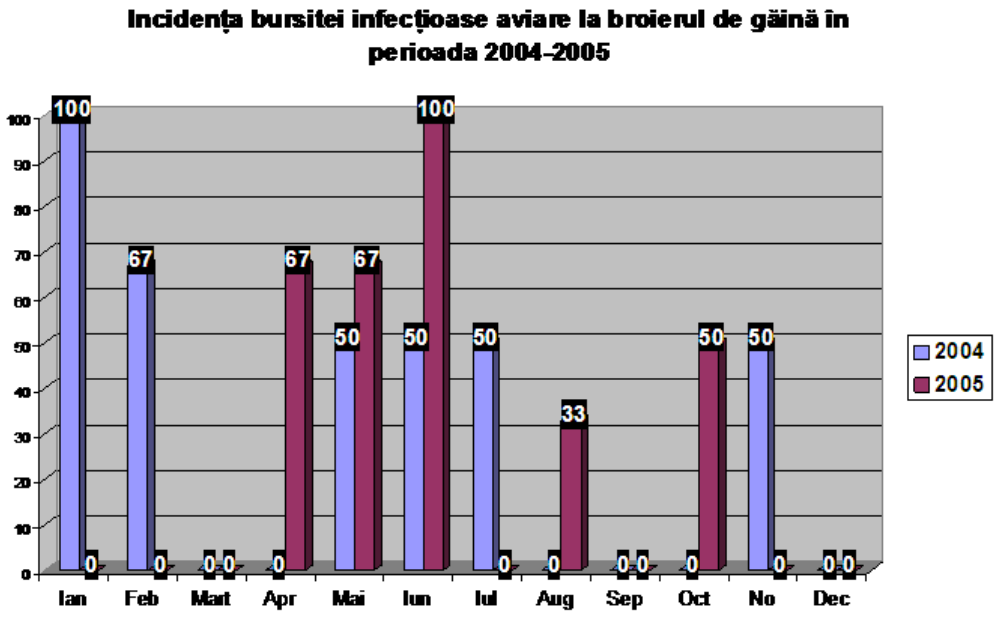
Apariția şi evoluția bursitei infecțioase este favorizată de mai mulți factori, printre care un rol important îl are vârsta, sistemul de creştere şi starea de sănătate.

În tabelul nr.1 şi graficul nr.1, este redată incidența lunară şi pe ani (2004-2005) a bursitei infecțioase aviare la puii de carne, exprimată prin raportarea numărului episoadelor (focarelor) de boală la numărul de serii de pui susceptibili, receptivi la infecția cu virus BIA în perioada respectivă.

Tabel nr. 1

Incidența bursitei infecțioase aviare la broierul de găină în perioada 2004-2005

Anul	Sp.	Episoade de bursită infecțioasă/serii de pui susceptibili												
		Ian	Feb	Mart	Apr	Mai	Iun	Iul	Aug	Sep	Oct	Nov	Dec	Total
2004	Nr.	1/1	2/3	0/1	0/2	1/2	1/2	1/2	0/2	0/1	0/3	1/2	0/2	7/23
	%	100	67	-	-	50	50	50	-	-	-	50	-	30
2005	Nr.	0/1	0/2	0/1	2/3	2/3	1/1	0/1	1/3	0/2	1/2	0/2	0/3	7/24
	%	-	-	-	67	67	100	-	33	-	50	-	-	29



Grafic nr. 1

În perioada 2004-2005 s-a înregistrat evoluție variabilă a bursitei infecțioase aviare în aceeaşi fermă, atât în cursul fiecărui an cât şi în toată perioada analizată. Astfel, boala a avut apariții sporadice în general şi doar în câteva cazuri a evoluat pe câte două serii succesive de pui în aceeaşi fermă.

Anul 2004, a debutat în condiții de presiune infecțioasă ridicată datorită evoluției bursitei infecțioase la puii de carne în fermă în ultimul trimestru al anului 2003. În luna ianuarie 2004 boala a evoluat la singurul efectiv de pui receptivi (100%), iar în februarie la două din trei efective (67%). După o perioadă de acalmie de două luni, boala a reapărut la câte unul din două efective receptive (50%) în lunile mai, iunie şi iulie, după care a urmat o perioadă de acalmie de 3 luni şi din nou un episod de boală la unul din două efective de pui în luna noiembrie (50%).

Boala a evoluat la 7 din cele 23 serii de broiler găină populate în anul 2004 (30%), din care într-o singură fermă la două serii succesive de pui.

Anul 2005 a debutat fără boală, dar BIA a reapărut la câte două din trei efective receptive în lunile aprilie şi mai (67%) şi la singurul efectiv receptiv în luna iunie (100%). Apoi a evoluat la câte unul din trei (33%) respectiv două (50%) efective receptive în lunile august şi octombrie.

Boala a evoluat în total la 7 din cele 24 serii de pui populate în 2005 (29%), de asemenea într-o singură fermă la două serii succesive de pui.

Prevalența bursitei infecțioase aviare la broilerul de găină în perioada 2004-2005 a fost de 30% şi 29%.

Analizând din perspectiva evoluției sezoniere a bolii la broiler, se constată că în anul 2004 bursita infecțioasă a avut o incidență mai mare în lunile de iarnă: ianuarie - februarie, iar în anul 2005 în lunile de primăvară – vară (grafic nr. 1).

Este de notat faptul că în perioadele ianuarie - martie, mai - iulie şi septembrie-noiembrie ale fiecărui an se populează în aceeaşi fermă pui de o zi proveniți din reproducători tineri, concomitent cu puii de la reproducători adulți la jumătatea şi/sau la sfârşitul perioadei de

producție. Acest lucru se concretizează în existența unei foarte mari variabilități a protecției maternale față de BIA între grupurile de progeni, variabilitate accentuată de diferențele existente în interiorul fiecărui grup, progeni care vor avea expunere şi răspuns diferite, atât față de virusurile vaccinale cât şi față de cele patogene.

Evoluția episoadelor de bursită infecțioasă aviară la broilerul de găină în anul 2004 şi caracteristicele epidemiologice ale acestora sunt redate în tabelul nr. 2 şi graficul nr. 2.

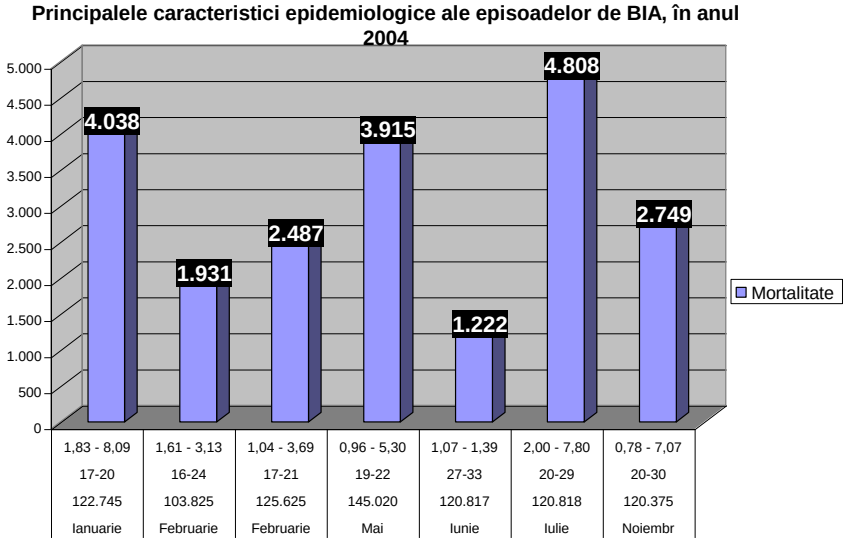
În 6 din cele 7 episoade (86%) debutul bolii s-a produs la vârsta de 16-24 zile, iar durata evoluției a variat între 5-9 zile (tabel nr. 2 si grafic nr. 2). Cele mai mici valori ale mortalității specifice s-au înregistrat în episodul 5 (1,02%) în care debutul bolii a avut loc la 27-33 zile. De asemenea în episoadele 6 şi 7 mortalitatea cea mai mică (2,00% respectiv 0,78%) au avut-o loturile cu vârsta cea mai mare la debutul bolii (29 respectiv 30 zile), dar relația nu se confirmă în toate episoadele. Limitele de mortalitate între loturi sunt foarte variabile (0,78-8,09%) şi sunt cu atât mari largi cu cât boala a afectat mai multe loturi în cadrul aceluiaşi episod (tabel nr.2).

În ferma în care boala a evoluat pe două serii succesive de pui, în primul episod mortalitatea BIA a fost de 1,98% cu limite de 1,04-3,69%, iar în cel de-al doilea a fost de 2,70% (0,96-5,30%) şi au fost afectate de fiecare dată 10 din 12 loturi, în acelaşi adăposturi (tabel 2). Debutul bolii s-a produs puțin mai târziu în al doilea episod, la 19-22 zile față de 17-21 zile în primul, dar a diferit şi schema de vaccinare între ele. Mortalitatea mai mare la al doilea efectiv poate fi consecința unei presiuni infecțioase ridicate datorate posibilei remanențe a virusului BIA în fermă după primul episod.

Tabel nr.2

Principalele caracteristici epidemiologice ale episoadelor de BIA, în anul 2004.

Nr. episoade Perioada evoluției	Nr. efectiv (pui)	Vârsta apariției BIA(ziua)	Durata bolii (zile)	Nr.loturi cu BIA	Limite de mortalitate între loturi %	Mortalitate BIA	
						Nr.	%
Ian.	122.745	17-20	6-9	6/12	1,83 - 8,09	4.038	3,29
Feb.	103.825	16-24	5-8	2/8	1,61 - 3,13	1.931	1,86
Mart.	125.625	17-21	5-8	5/12	1,04 - 3,69	2.487	1,98
Mai	145.020	19-22	6-8	5/12	0,96 - 5,30	3.915	2,70
Iunie	120.817	27-33	6-7	1/4	1,07 - 1,39	1.222	1,02
Iulie	120.818	20-29	6-8	6/12	2,00 - 7,80	4.808	3,98
Nov.	120.375	20-30	5-7	6/12	0,78 - 7,07	2.749	2,45
TOTAL	859.215	16-33	5-9	31/72	0,78 - 8,09	21.150	2,69



Grafic nr. 2

Dinamica mortalităţii săptămânale comparative între efectivele de pui care au trecut prin bursită infecţioasă aviară şi cele fără boală este redată în tabelul 3 şi grafic nr. 3.

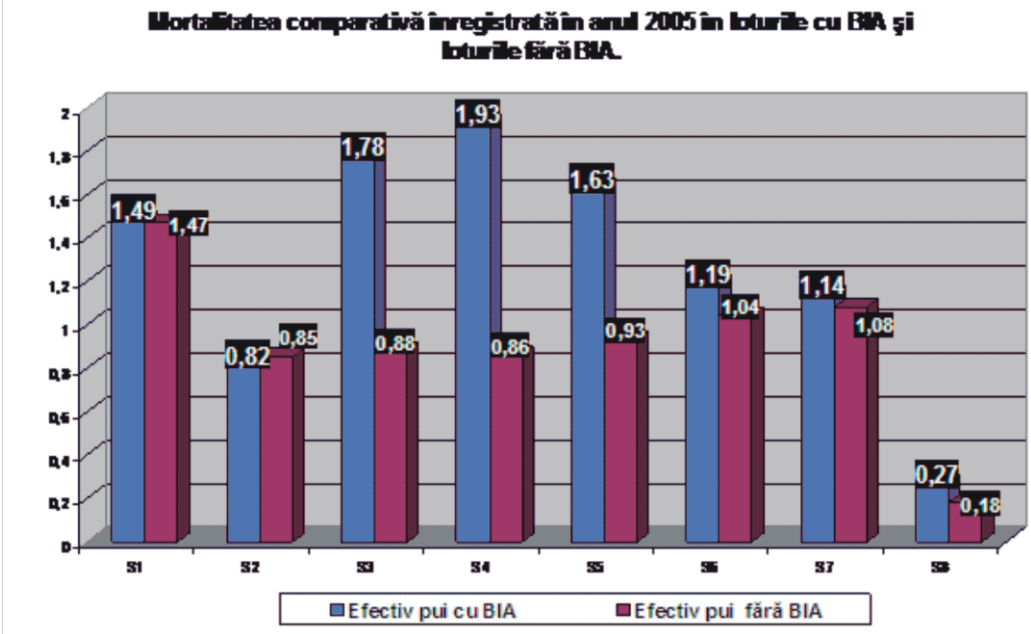
Din analiza datelor înregistrate, reiese că puii au avut în primele două săptămâni de viaţă o evoluţie aproximativ identică (pierderi 2,31% respectiv 2,37%), după care mortalitatea la puii trecuţi prin boală este aproape dublă în săptămânile 3-5 şi la nivele superioare în ultimele săptămâni de viaţă faţă de cea înregistrată la puii fără BIA; în săptămâna a 8-a procentul de mortalitate este influenţat de câte zile s-au scurs până la sacrificare.

Deşi evoluţia curbei mortalităţii fiecărui episod de boală a avut o dinamică specifică BIA pe durata a 5-9 zile, exprimarea grafică este rezultanta debutului variabil, între 16 şi 33 de zile de viaţă a celor 7 episoade de boală.

Tabel nr. 3.

Mortalitatea comparativă înregistrată în anul 2005 în loturilecu BIA şi loturile fără BIA.

Specif.	Nr. efectiv	Mortalitate săptămânală (%).								Mortaltate totală %	Motlitate BIA %
		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8		
Efectiv cu BIA	859.215	1,49	0,82	1,78	1,93	1,63	1,19	1,14	0,27	10,27	2,69
Efectiv fără BIA	1.43512	1,47	0,85	0,88	0,86	0,93	1,04	1,08	0,18	7,29	-



Grafic nr. 3

Mortalitatea totală (tabel nr.3) a efectivelor cu BIA a fost de 10,27% (7,19-12,18%) în timp ce la puii fără boală a avut valoarea medie de 7,29%, diferenţă exprimată în graficul 3 care scoate în evidenţă dimensiunea pierderilor economice indusă de mortalitatea determinată direct sau indirect de trecerea prin boală.

În primele 3 episoade boala a debutat la 20-22 de zile, a avut evoluţie de 4-7 zile şi mortalitate specifică cuprinsă între 1,22 şi 2,01% (tabel nr.4).

În celelalte 4 episoade în care debutul bolii s-a produs între 14 şi 17 zile, evoluţia a fost de 4-9 zile şi mortalitate totală BIA de 1,02-4,24% (tabel nr. 13 şi 14). Boala a evoluat în toate loturile receptive numai în 3 din cele 7 episoade (43%). Limitele de mortalitate au fost foarte largi (0,31 – 10,68%) nivelele cele mai mici fiind în majoritatea cazurilor în loturile cu vârsta mai mare la apariţia bolii şi cu evoluţia cea mai scurtă (4-5 zile).

În episoadele 3 ş1 6 bursita infecţioasă clinică a fost urmată de coccidioză intestinală sau cecală şi sindroame respiratorii cronice complicate, situaţii în care mortalitatea totală a fost de 11,63 respectiv 15,32%.

CONCLUZII

Din analiza epidemiologică a episoadelor de bursită infecțioasă se desprind următoarele concluzii:

1. Boala a evoluat în toate fermele de pui de carne, sub formă de episoade cu gravități diferite în cursul anilor2004-2005.
2. Toate episoadele înregistrate au evoluat pe pui vaccinați, cu diferite tulpini vaccinale de virus BIA.
3. Episoadele de bursită infecțioasă s-au caracterizat printr-o mare variabilitate a dinamicii în ceea ce priveşte seriile de pui şi în cadrul fiecărei serii.

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Lesions and clinic findings in Gumboro disease (Infectious Bursitis)

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After a serious outbreak in 1990, this viral disease spread in all fowl farm.

The new evolution form of the disease, lead to higher peaks of the morbidity and mortality. The virus immunosuppressant effect lead to longer outbreaks and chick's receptivity get extended.

Gumboro disease evolution had various pathogenicity levels, sometimes because of the direct losses sometimes because of the immunosuppressant effect.

In this paper are presented researches regarding clinical inquires in a fowl farm.

Key Words: Gumboro disease, lesions, clinic findings

Începând din 1994 au apărut episoade noi de bursită infecțioasă la pui broiler în România, la început rare, apoi odată cu creşterea presiunii infecțioase în teritoriu acestea au devenit mai frecvente.

Tabloul evoluției bursitei infecțioase aviare a cunoscut intensități diferite cu efecte la unele serii deosebit de grave atât prin volumul pierderilor directe, cât şi a celor datorate imunosupresiei instalate.

MATERIAL ŞI METODE

La un număr de 100 de pui broiler din hala 3 şi 250 pui broiler din hala 1, unde s-au observat o mortalitate mai crescută, s-au efectuat examene clinice, utilizându-se metodele generale şi înscrierea datelor în foi de observație.

Leziunile macroscopice s-au evidențiat prin necropsii efectuate pe un număr de 175 de pui, care în timpul vieții au prezentat semne clinice de bursită infecțioasă .

REZULTATE ŞI DISCUŢII

În urma examenului clinic efectuat în unitatea „F" s-a constatat un debut brusc cu apatie, piuit, horiplumație, aripi lăsate, aglomerare în grupuri uneori sub sursa de căldură, inițial polidipsie apoi anorexie, descărcări diareice de culoare albă, albă-gălbuie până la cărămiziu însoțite uneori de prurit pericloacal sindrom de imobilitate, mioclonii, decubit sterno-abdominal sau lateral cu picioarele în extensie (fig. nr. 1 şi fig. nr. 2).

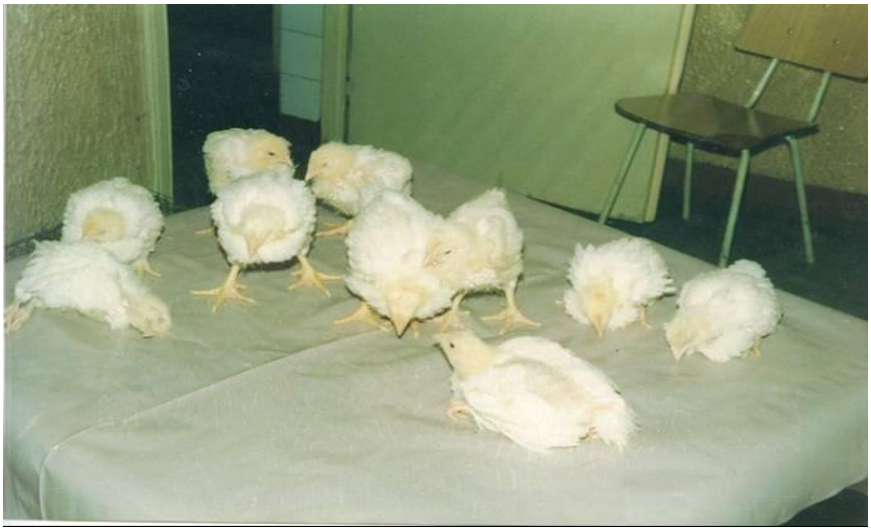

Fig nr. 1
Aspect clinic - sindrom de imobilitate

Fig nr. 2
Aspect clinic - decubit cu picioarele în extensie

Astfel, examenul clinic efectuat la un număr de 250 pui de găină din hala nr.3 şi 100 pui de găină din hala nr 1, a evidenţiat următoarele manifestări (tabel nr.1):

- apatie, incoordonări în mers, la un număr de 20 pui din hala nr.3, ceea ce reprezintă 20% şi la 50 (50%) de pui din hala nr.1;
- diaree apoasă, de culoare albicioasă care murdăreşte şi aglutinează penele din jurul orificiului cloacal, observată la un număr de 100 (%) pui din hala nr.1;
- moarte subită au reprezentat un număr de 15 pui (15%) pui din hala nr.3 şi 62 (25%) pui din hala nr.1 (tabel nr.1).

Tabel nr.1

Semnele clinice în bursita infecțioasă

Nr.crt.	Hala	Nr. pui examinați clinic	Semnele clinice	Nr.pui cu semne clinice	%
1	1	250	apatie, incoordonări în mers; diaree apoasă culoare albicioasă; moarte subită.	50 100 62	20,0 40,0 25,0
2	3	100	apatie, incoordonări în mers; diaree apoasă, culoare albicioasă; moarte subită.	20 50 15	20,0 50,0 15,0

La examenul necropsic a unui număr de 175 pui, care în timpul vieții au prezentat semne clinice de boală, s-a constatat: cadavrele deshidratate, cu penele din zona pericloacală murdărite cu dejecții alb-cretacee. Rigiditatea cadaverică s-a instalat rapede.

La deschiderea cadavrelor s-a observat prezența peteşiilor şi sufuziunilor în musculatura pectorală la 18% din cadavre, în timp ce în musculatura membrelor (fig. nr. 3, fig. nr. 4), îndeosebi în jurul articulațiilor femurotibiale la peste 60% din cadavre (tabel nr. 2).

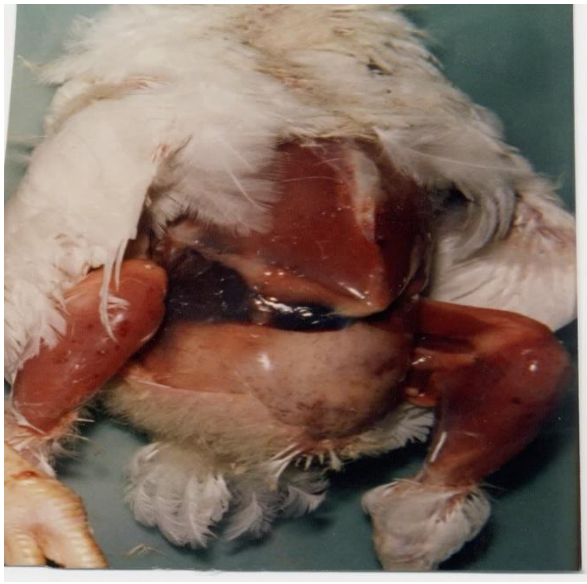

Fig nr. 3 Hemoragii musculare – piept şi membre

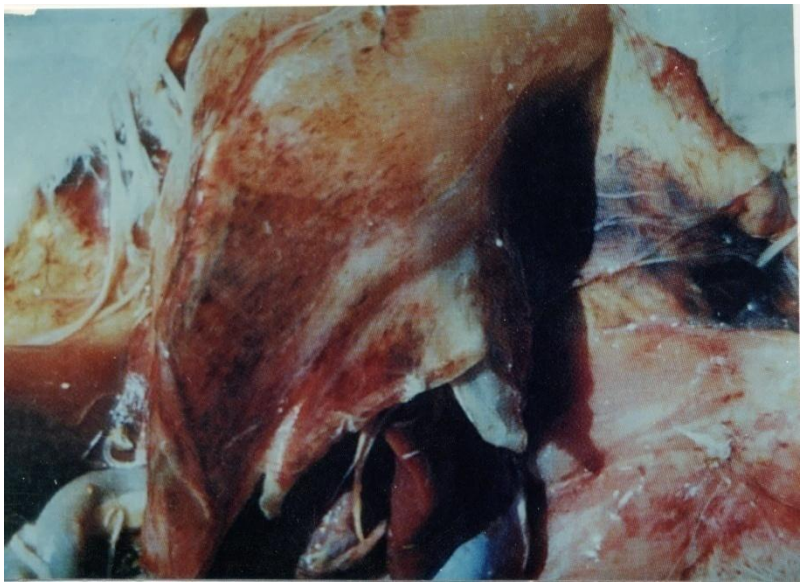

Fig. nr. 4
Hemoragii punctiforme generalizate în musculatură

Tabel nr. 2

Leziunile macroscopice în bursita infecțioasă.

Nr.crt.	Nr.pui necropsiați	Organul	Leziunea	Nr. pui	%.
1	175	Bursa Fabricius	edemațiată, hiperemiată, mărită în vulum.	175	100,00
			culoare gălbuie, conținut cu aspect gelatinos şi un depozit cazeos	141	81,00
			aspect hemoragic, cu striuri sau coaguli de sânge, aspect de vişină putredă	34	19,00
2	175	Musculatură	Hemoragii punctiforme sau difuze la gambă şi piept	33 87	19,00 50,28
			aspect de carne fiartă.	38	21,42
3	175	Rinichi	măriți în volum şi distrofici	67	38,05
			aspect hemoragic	47	26,83
			culoare închisă (gri-pal la brun închis)	86	43,42
			ureterele pline cu urați	37	21,13
4	175	Tub digestiv	proventriculită hemoragică-necrotică		17,23
			enterită cataralâ	175	100,00
			inel hemoragic		16,59
			hematoame în pretele proventricolului	18	11,00
5	175	Ficat	congestionat distrofic	12	6,82
			ectazie biliară	11	5,72
6	175	Splina	hiperplaziată şi congestivă	21	12,56
			focare mici cenuşii-gălbui, subcapsulare	37	21,13

Ficatul congestionat sau distrofic, cu ectazie biliară. Rinichii, în peste 80%, distrofici, cu aspect marmorat şi cu ureterele pline cu urați, motiv pentru care boala a fost denumită „nefrozo-nefrită infecțioasă”, (fig. nr. 5).

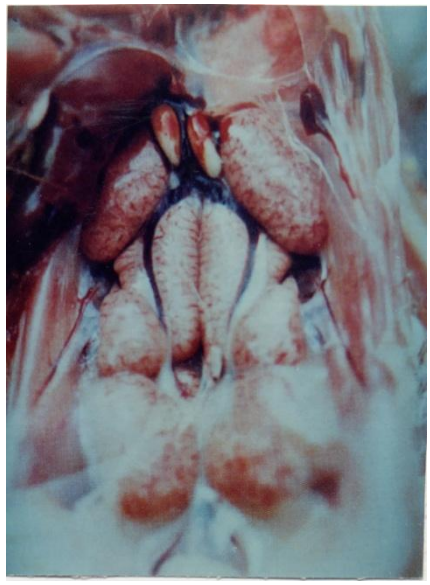

Fig. nr. 5 Rinichi măriți în volum - aspect hemoragic

La peste 10% din cazuri s-a observat proventriculita hemoragico-necrotică difuză şi sub formă de inel hemoragico-necrotic în 7% din cazuri. În rare cazuri (2%) au fost prezente hematoame în peretele proventricolului, cu dimensiuni de 4-6 mm, vizibile prin traversul mucoase. În toate cazurile (100%) a fost prezentă enterita catarală. In schimb, în evoluțiile mai grave, la 11% din cazuri s-a constatat leziunea de enterită hemoragică cu un conținut serosanguinolent şi eroziuni subcuticulare în stomactul muscular.

Splina uşor hiperplaziată şi congestivă cu cu prezența unor focare mici alb-cenuşii sau gălbui, localizate subcapsulare şi unifotrm distribuite în prima fază, apoi de culoare palidă, anemică în a doua fază.

Bursa Fabricius a fost afectată în toate cazurile (100%), cu modificări diferite în funcție de faza de evoluție a bolii (fig. 6, 7).

În primele 3-4 zile de boală, bursa era mărită în volum cu pînă la dublare şi cu prezența la exterior a unui transudat gelatinos. Pe secțiune, pliurile erau evidente, de culoare crem iar lumenul bursal conținea un depozit cazeos. În peste 9% din cazuri bursa Fabricius prezenta aspectul unei vişine putrede, tumefiată, roşu-negricioasă, cu coagul de sânge, iar pe secțiune prezenta necroze. În 13% din cazuri, s-a observat prezența unor hemoragii punctiforme sau striuri hemoragice interfoliculare. Incidența cea mai mare a leziunilor hemoragice s-a întâlnit în momentul mortalității maxime. Începând din ziua a 5-a de boală bursa s-a redus în volum până la mai puțin de jumătate din greutatea normală după 8 zile de evoluție în majoritatea cazurilor.

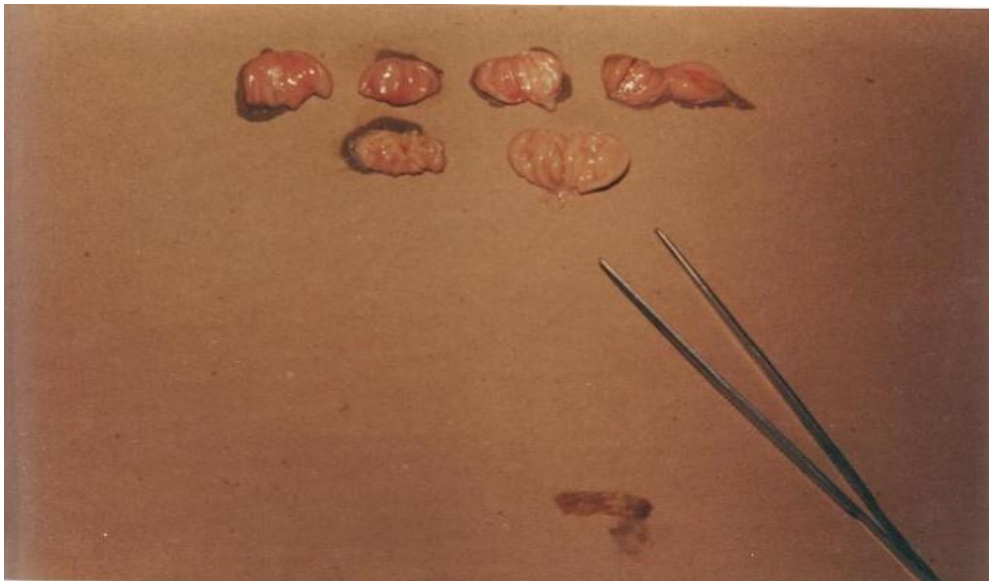

Fig. nr. 6 Leziuni anatomopatologice ale bursei lui Fabricius

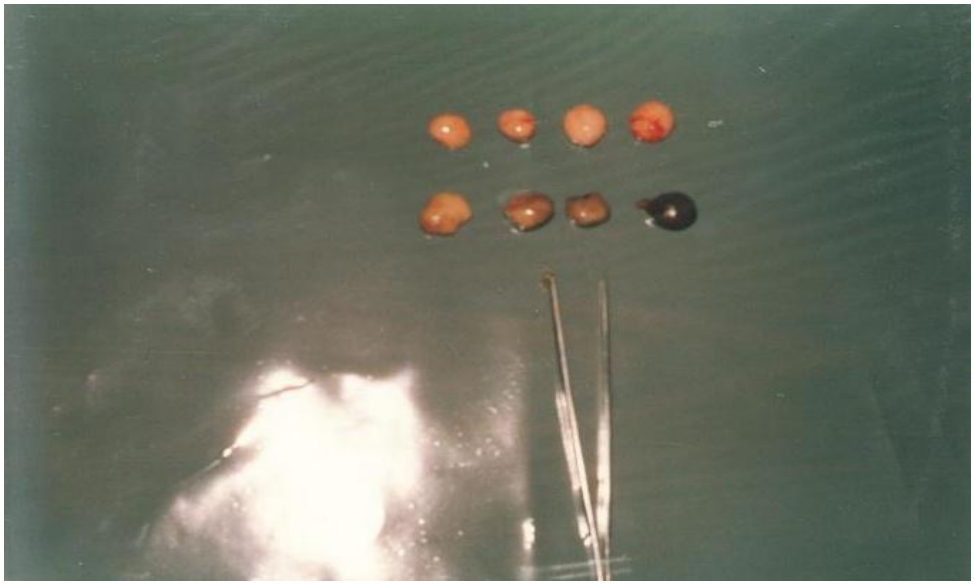

Fig. nr. 7 Aspect anatomopatologic al bursei lui Fabricius

CONCLUZII

După efectuarea examenului clinic şi a examenului necropsic s-au stabilit următoarele concluzii:

1. Marea majoritatea a episoadelor (88%) de bursită infecțioasă a debutat la puii în vârstă de 14-22 zile şi numai în trei cazuri (12%) debutul a avut loc la pui de peste 26-28 zile.
2. Durata de evoluție a episoadelor a variat între 4-9 zile, fiind cu atât mai scurtă cu cât numărul loturilor afectate în cadrul unei serii de pui a fost mai mic. În episoade succesive a scăzut vârsta apariției bolii.
3. Mortalitatea relativă anuală atribuită bursitei infecțioase în perioada 2004-2005, în baza semnelor clinice şi a examenului necropsic a fost cuprinsă între 0,21-5,78% (valoare apreciată pe serii de pui).

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Internal quality control – an important tool of quality assurance management in veterinary clinical chemistry laboratory

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The concept of quality is defined by the International Organization for Standardization (ISO) as the totality of features and characteristics of a product or service that bear on its ability ti satisfy stated or implied needs.

A demonstrable quality of veterinary laboratory services contains two goals: implementation of quality management system (including a quality policy statement, identification of user needs, measurements procedures, reference measurements systems for providing traceabillity, control materials, proficiency testing with materials having reference-measurement-assigned values) and obtaining the recognition of competence by accreditation according to European Standard SR EN ISO/CEI 17025:2005 (4, 16).

The internal quality control in clinical chemistry concerns control of both systematic and random errors, which are interpretated using Shewart control charts and Westgard multi-rule quality control system (12,13).

This paper addresses the question of how to judge the rejecting or accepting an analitical run in the case of cholesterol analysis in blood serum, using a reference material.

Key words: quality assurance, internal control, Shewart charts, Westgard rules, cholesterol

The traditional approach to the quality control (QC) planing process in a clinical laboratory involves at lest few steps (9, 10, 11). First, the quality requirements, usually defined in terms of total allowable error (TE_a), must be specified. Results that contain analitycal errors that exceed TE_a are considered to be of unacceptable quality. The second step is the evaluation of accuracy and precision of the method. Third, critical size errors are calculated on the basis of the quality requirement and the assay accuracy and precision. The performance of alternative QC rules is assessed in terms of their probability of rejecting an analytical run when an out-of- control error condition exist. Finally, it is necessary to select control rules and the number of control samples per run to give a lowest false-rejection rate and a high error detection rate for critical size errors.

Westgard and colab. shows (Fig.1.) the relationships between the various types of quality goals, requirements, and specifications.

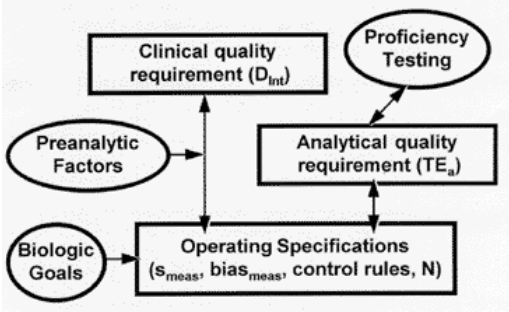

Fig. 1. Quality goals, requirements and specification (10)

Clinical quality requirements in the form of medically important changes or decision intervals (D_{int}) can be converted to laboratory operating specifications for imprecision (s_{meas}), inaccuracy ($bias_{meas}$), and QC (control rules, N). The most common sources of these analytical requirements are the proficiency testing or external quality assessment programs that specify acceptability limits in the form of a target value plus/minus certain tolerances (10).

Materials and methods

The cholesterol assay from blood serum was performed using the Hospitex Diagnostics EOS Bravo Forte, a fully automatic clinical chemistry analyzer. The reference material was HD calibrator serum, lot no. 220 and human normal control serum provided by Hospitex Diagnostics, lot no. 06-045 with reference values for cholesterol: target value- 93,4 mg/dl and range: 79,3-107,5 mg/dl.

The reagent used was Cholesterol liquid MONO reagent, from Hospitex Diagnostics, based on an enzimatic-colorimetric method. Al cholesterol esters present in specimen are hydrolyzed quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, free cholesterol is then oxidized by cholesterol oxidase to cholesten-4-ene-3-one and H_2O_2. The H_2O_2 reacts with p-chlorophenol and 4- aminoantipyrine in the presence of peroxidase to form a quinoneimine dye. The intensity of color formed is proportional to the cholesterol concentration and can be measured photometrically between 480 and 520nm .

There were performed 20 runs from the reference material, and have been were calculated the mean, standard deviation (SD) and level of variation (CV). The results are interpretated using Shewart control charts and Westgard multi-rule quality control system (2, 14, 15).

Results and disscussions

The results were analized using Shewart control charts and Westgard rules. In fig.2 are presented some of the most important Westgard rules, frequently used in clinical laboratory for accepting or rejecting the internal quality control results. These rules are used when are performed single runs from reference materials at least at two levels, and are monitorized during a long period of time.

2a. ***2b.*** ***2c***

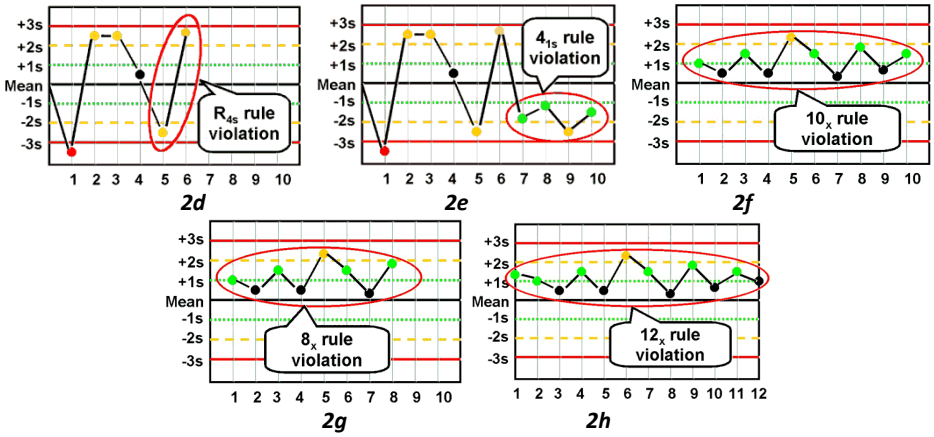

Fig. 2. Westgard rules: (2a) $\mathbf{1_{3s}}$ rule- the control run is rejected when a single control measurement exceeds the mean ±3SD limits; (2b) $\mathbf{1_{2s}}$ rule/ - is a warning rule to trigger careful inspection of the control when the measurement exceeds the mean ±2SD limits; (2c) $\mathbf{2_{2s}}$ - reject when 2 consecutive control measurements exceed the same mean ±2SD limits; (2d) $\mathbf{R_{4s}}$ – the control run is rejected when 1 control measurement in a group exceeds the mean +2SD and another exceeds the mean-2SD; (2e) $\mathbf{4_{1s}}$ - reject the control run when 4 consecutive control measurements exceed the same mean ± 1SD control limits; (2f) $\mathbf{10_x}$ - reject when 10 consecutive control measurements fall on one side of the mean (with modification for 8 or 12 control measurements 2g, 2h) (18)

In fig.3. are presented Westgard multirules. Multirule QC uses a combination of decision criteria, or control rules, to decide when an analytical run is in-control or out-of-control. The well-known Westgard multirule QC procedure uses 5 different control rules to judge the acceptability of an analytical run.

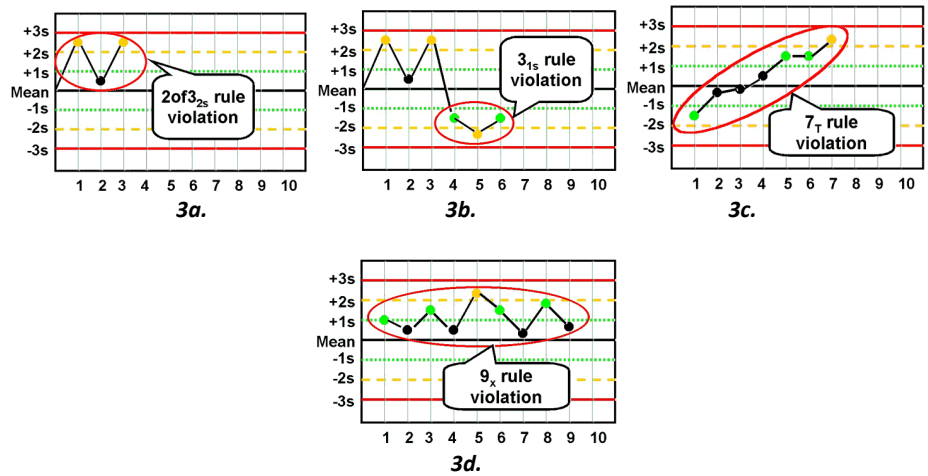

Fig.3. Westgard multirules: **(3a)** $2of3_{2s}$ - reject when 2 out of 3 control measurements exceed the same mean plus 2s or mean minus 2s control limit; **(3b)** 3_{1s} - reject when 3 consecutive control measurements exceed the same mean plus 1s or mean minus 1s control limit., **(3c)** 9_x - reject when 9 consecutive control measurements fall on one side of the mean. **(3d)** 7_T - reject when seven control measurements trend in the same direction, i.e., get progressively higher or progressively lower (18).

When the control values fall within the expected distribution, the run is classified to be **"in-control,"** accept the results, and report patient test results. When the control values fall outside the expected distribution, the run is classified as **"out-of-control,"** reject the test values, and do not report patient test results (Fig.4.).

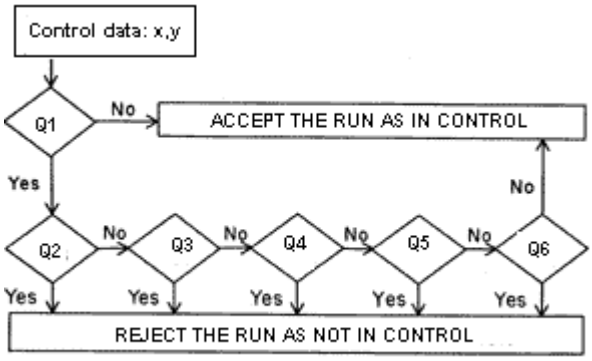

Fig. 4. Interpretation of the Westgard multi-rule used in this study (15)

Q1: Is either x or y outside the mean ±2SD interval?; **Q2**: Is either x or y outside the mean ±3SD; **Q3**: Are both x and y outside their respective interval mean ±2SD in the same direction of their mean?, **Q4**: Are both x and y outside their respective interval mean ±2SD in opposite direction of their mean? **Q5:** Are the last three control data outside of their respective interval, mean ±1SD, with all three in the same directions of their mean?; **Q6**: Are the last 10 measurements all on either the high side or the low side of their means?

The analytical data are presented in table 1. The Shewart charts was rised using the mean (M), M±1SD, M ±2SD and M ±3SD as decision limit for Westgard multi-rules aplication. The mean, standard deviation (SD) and coefficient of variation (CV) of the control materials were calculated. These parameters offer an image of quantitatively random errors, and the obtained values respect the following conditions: SD<1/12 and CV<1/8 from reference material interval (2,35 respectively 3,52) (1, 3, 5, 16, 17)

Table 1.
Analytcal data and random error parameters

Run no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Chol (mg/dl)	93,31	94,17	94,59	93,12	91,56	93,78	95,14	94,34	95,96	94,97	95,81	92,1	95,38	96,31	95,39	94,77	93,45	92,35	94,5	97,3
Mean (mg/dl)	**94,41**																			
SD (mg/dl)	**1,47**																			
CV (%)	**1,56**																			

False alarms were minimized by using the 1_{2s} rule as a warning rule (fig.5), then confirming any problem by application of more specific rules that have a low probability of false rejection.

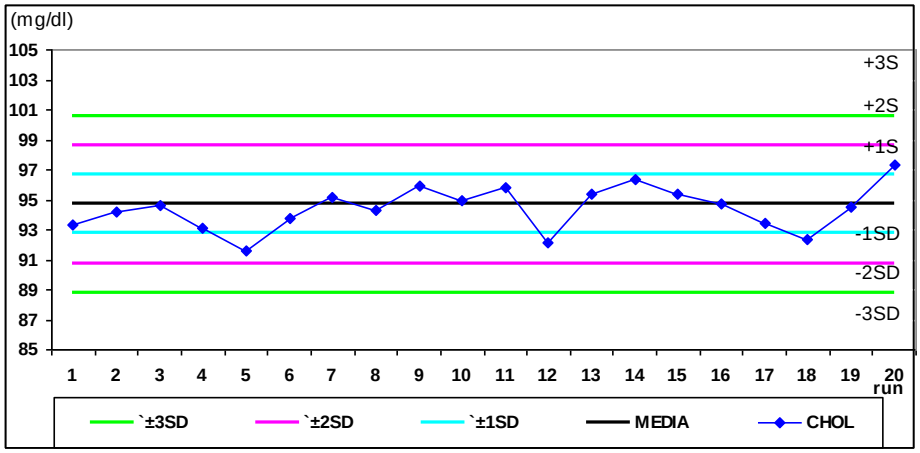

Fig. 5. Shewart chart for cholesterol values obtained from 20 multiple runs

The interpretation of quality control diagram, using Westgard multirule, is reduced in this particular case at only one question:

Q1: Are the results outside the mean ±2SD interval?	**NO**	→	**ACCEPT THE RUNS AS IN CONTROL**

Conclusions

- All 20 results haven`t exceeded the mean ±2SD interval, and according with Westgard multirules, all of them were accepted as run in control
- The quality parameters values (table 1) respect the following conditions: SD<1/12 and CV<1/8 from reference material interval (2,35 respectively 3,52) (1, 3, 5, 16, 17)
- Laboratory must know the imprecision, inaccuracy, and QC that are necessary to manage and assure the quality of the testing process.

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Silent killers - fungal volatile organic compounds as possible factors with impact on human health

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A basic understanding of the bioecology of fungi and how mycotoxins are produced are necessary to estimate the health effects of their metabolites on human health. Fungi are well known to produce many agents that can be toxic if exposure is long enough, like secondary products of fungal metabolism or fungal structural components. In the first category, there are antibiotics, mycotoxins, and volatile organic compounds (VOCs); the second category includes cellular membrane components such as ß-(1, 3)-D-glucans.

Mycotoxins are low volatile, so inhalation exposure is limited to the inhalation of airborne fungal particulates (spores) or fungic contaminated substrates that contain high concentrations of mycotoxins. A relevant issue is determining which cognitive functions make humans to attribute their health problems to environmental factors and how individuals with proved environmental health problems can perceive that as a biological stress indicator. Mycotoxins exposure have been linked to a variety of acute and chronic adverse health effects. Previously researchers have reported links between S. chartarum exposure and human health effects including upper and lower respiratory illnesses.

Generally, these effects can include acute symptoms such as pulmonary hemorrhages, dermatitis, recurring cold and flu-like symptoms, burning/sore throat, headaches, excessive fatigue and diarrhea. Chronic effects including carcinogenicity, mutagenicity, teratogenicity, central nervous system activity impairment, immune system damage, and specific effects on the heart, liver, kidneys and other organs.

Most epidemiological and toxicological databases now available are obtained from animal ingestion studies and case studies of occupational inhalation exposures among agricultural workers. This lack of strong information creates the need for serious researches to establish some standard limitations to eliminate or reduce the indoor human exposure on fungi and their metabolites.

In this review we summarized the effects of VOCs and mycotoxins against human beings health with emphasis on cognitive function impairment.

Key Words: indoor fungi, volatile organic compounds, mycotoxins

The complexity of the environmental microorganism charge can be in connection with diverse types of human and animal health problems (including infectious and noninfectious fungal diseases), modulating influences of fungal spore or volatile organic compounds on immunological status of each of us. (1) Moulds (or mold) are common in the indoor environment, and are ubiquitous in both indoor and outdoor environments being frequently spread by airborne spores that require moisture and a food source like cellulose or decaying food to grow. When mould spores mix with water and grow, they elongate, forming balloon-like protuberances (hyphae) which secrete digestive enzymes and mycotoxins. (2) The fungus then digests the food source to support its growth. (3, 4) About 100,000 fungal species have already been identified; in fact fungi are estimated to comprise an astounding 25% of the world's biomass. Various surveys of homes in North America and Europe have reported that visible mould and/or water damage are found in 23% to 98% of all homes. There are no official standards, at this time, for indoor airborne fungus concentrations. (5, 6) However, indoor fungal levels above a range of 150 to 1,000 colony-forming units per cubic meter of air (cfu/m3) are considered to be sufficient to cause serious human health problems. Numerous reports have documented that indoor air can often be contaminated with indoor fungal spore in excess levels that can rich at 1,000 cfu/m3. Relationships establish between incipient human-health changes due to fungal exposure, can be explained today through empirical epidemiological studies. The physical and chemical environment may interfere with cognitive functions in several ways. (7) The cognitive effects of physical environmental factors during or shortly after exposure are also thought to be a result of a change in the individual's general state (mood, stress, fatigue, arousal etc.) and resources available for performing a task. (8, 9) Research into environmental effects on cognitive functions takes three approaches: experimental laboratory studies, quasi-experimental field studies, and epidemiological investigations. (10, 11)

Experimental laboratory studies on humans are usually short-term studies of effects at moderate mycotoxins exposure levels. Animal studies are important when long-lasting behavioral neurotoxic effects are involved but they are hampered by the fact that many human cognitive functions cannot be studied since models are lacking.

Is necessary to understand better the relations between human wellbeing and aspects of the contemporary environment. (9, 12) Sensation-based perceptions deals with those adverse physico-chemical aspects of the environment (odor, fungal presence) capable of activating the senses and which, in so doing, may have negatively arousing properties associated with potentially adverse health effects.(13, 14) Like other so-called environmental syndromes such as chronic fatigue syndrome, multiple chemical sensitivity, or all together, the so-called sick building syndrome (SBS) is characterized by symptoms rather than by identified causative factors in the environment. Since a high proportion of somatic complaints in the context of environmental syndromes remain unexplained by conventional medical and psychiatric categories, the term "medically unexplained syndromes" is increasingly being used as a descriptor.

For SBS as for other environmental syndromes there are several identifiable gaps in our knowledge. (12, 15) Most molds, especially those with dry conidia, produce volatile odor constituents. In a few cases, these are fruity or flowery and may be adapted to attract arthropod dispersers (e.g. insects carrying the mold conidia to new growth sites) (16). Apart from experiencing such direct physiological irritation, humans and other vertebrates may be adapted to avoid such odors, and there may be a legitimate "psychological" objection to their presence in rooms. Mold growth in buildings may be accompanied by the growth of *Streptomyces* species, which usually have very strong earthy volatile odours. In addition, in very wet materials, copious bacteria may grow and may emit typical rotten or sour smelling odour molecules.(17, 18) Actively growing fungi produce a variety of volatile organic compounds (VOC,s), which may produce a distinctive musty, moldy odor. Fungal VOCs may include 3-methylbutan-1-ol, 3-methylbutan-2-ol,

fenchone, heptan-2-one, hexan-2-one, octan-3-one, octan-3-ol, pentan-2-ol, alpha-terpineol, and thujopsene. They emit these compounds into the indoor environment (the most prevalent compounds included xylene, toluene, 2-propanol, limonene, and heptane. Formaldehyde concentrations ranging from 1.7 to 13.3 microg/ m^3 and mean acetaldehyde levels ranging from <3.0 to 7.5 microg/m3. (19)

Larsen and Frivad studied in vitro production of fungal volatiles from 47 Penicillium taxa and detected alcohols, ketones, esters, small alkenes, monterpenes, sesquiterpenes, and aromates. (20, 21) However, aldehydes were not among the VOCs detected. Fiedler et al. in 2001 have studied VOC production by Aspergillus fumigatus, A. versicolor, A. niger, A. ochraceus, Trichoderma harzianum, T. pseudokoningii, Penicillium brevicompactum, P. chrysogenum, P. claviforme, P. expansum, Fusarium solani, and Mucor sp. More than 150 volatile substances derived from the fungal cultures have been analyzed by head-space solid-phase micro extraction (HS-SPME) (22). Each species had a defined VOC profile, which may be subject to considerable modification in response to external factors such as cultivation on different substrates. Cultivation on different substrate changes the number and concentration of VOCs (22). Wilkins et al. have studied the production of VOCs by mold species isolated from damp buildings witch were grown on sterile building materials and some synthetic media.

Patterns of the volatile organic compounds were very media dependent, but media, which favor terpene biosynthesis, may give patterns unique enough for identification of dominant indoor molds. (23) It was proposed that species-specific volatiles may serve as marker compounds for the selective detection of fungal species in indoor environments. Examination of VOCs from indoor air samples may become an important method in indoor air hygiene for the detection of type and intensity of masked contamination by molds. (22, 23) Additional fungal VOCs are compiled and listed by Ammann and Batterman. Almost all of the published information regarding fungal VOCs concerns species of Penicillium and Aspergillus. (24, 25) Some of the fungal VOCs have an unpleasant odor, the musty, moldy, and earthy odors are likely to come from 2- octen-1-ol and geosmin (1, 10-dimethyl-9 decalol). Ezeonu et al. identified ethanol, 2-ethyl hexanol, cyclohexane, and benzene from fiberglass air duct liners colonized by Aspergillus versicolor, Acremonium clavatum, and Cladosporium herbarum. (26, 27) Acetone and 2-butanone were only detected on agar plate samples of A. versicolor and A. obclavatum. The 2-ethyl hexanol and cyclohexane are eye and skin irritants, and benzene is a generally recognized hazardous chemical.

Studies of more than 1,600 patients suffering ill effects from fungus exposure were presented at 21st Annual Symposium of Man and His Environment in Dallas, Texas, in June 2003. (28,4) To cite a few studies: Lieberman examined 48 mould-exposed patients who had the following health problems: muscle and/or joint pain 71%, fatigue/weakness 70%, neurocognitive dysfunction 67%, sinusitis 65%, headache 65%, gastrointestinal problems 58%, shortness of breath 54%, anxiety/ depression/ irritability 54%, vision problems 42%, chest tightness 42%, insomnia 40%, dizziness 38%, numbness/ tingling 35%, laryngitis 35%, nausea 33%, skin rashes 27%, tremors 25%, and heart palpitations 21%.(4)

Fungi produce a wide variety of toxic chemicals called mycotoxins, and some common mycotoxins usually including : (28, 10, 29) aflatoxins - very potent carcinogens and hepatotoxins produced by some Aspergillus species; ochratoxins - nephrotoxic and carcinogenic - produced by some Aspergillus and Penicillium; sterigmatocystin – immunosuppressive and a liver carcinogen produced by Aspergillus species especially A. versicolour; and trichothecenes are produced primarily by Stachybotrys and Fusarium species, and have been reported to inhibit protein synthesis, cause hemorrhages and vomiting. Fungi also produce beta glucans which have immunological effects. (30,31)

Adverse human and animal effects from mycotoxins-contaminated foodstuffs have been well recognized since the early 20th century. (32) But the pathway of mycotoxin injury through inhalation is questionable. In the absence of ethical, controlled studies on human inhaled mycotoxin exposure, only animal controlled exposure and human epidemiology studies can be used. (1, 2, 33) Exposure to high indoor levels of Stachybotrys, Aspergillus and other fungi has been epidemiologically associated with infant lung haemorrhage. (34, 35) Although questions were raised after this association was discovered, it meets many epidemiologic criteria for causality. Acute infant pulmonary hemorrhages can be rapidly fatal; when the infant survives, lung blood vessel damage is present and deposits of haemosiderin will remain in the lung macrophages and can be seen in tissue obtained during bronchoscopy. (36) Stachybotrys fungi can produce a wide range of trichothecene mycotoxins (including satratoxins), several roridin epimers, verrucarin J and B and hemolysin. A hemorrhagic protein called stachylysin has been isolated from Stachybotrys collected from homes of infants with lung haemorrhage, and from serum of patients with residential Stachybotrys exposure. (37, 38) It is hypothesized that infants, with their rapidly growing lungs, are more susceptible to the toxic effects of Stachybotrys mycotoxins. Studies with Stachybotrys-exposed adults have noted a significantly higher incidence of health problems such as lower airway problems, wheezing, skin and eye irritation, flu-like symptoms and chronic fatigue. Indoor airborne mould exposure causes neurological dysfunction and cognitive deficits.

Some others clinical reports on large numbers of mould exposed patients found significant fatigue and weakness in 70% to 100% of cases, and neurocognitive dysfunction, including memory loss, irritability, anxiety and depression, in over 40% of the patients. These signs and symptoms constitute classic manifestations of neurotoxicity. (39) Quantitative electro-encephalogram studies have also noted significant longer nerve latencies in fungus-exposed patients. (24) A triple-headed SPECT brain scan revealed neurotoxic patterns in 26 of 30 (87%) mould exposed patients. (40) A study of autonomic nervous function in 60 mould-exposed patients found 95% had abnormal autonomic responses of the pupil. Visual contrast sensitivity studies were often abnormal in indoor mould-exposed patients. (23)

Additional studies have reported that mould exposed patients reactions are significantly poor on tests of attention, balance, reaction time, verbal recall, concentration, memory, finger tapping.(24, 39) Most of these patients also experienced many health problems including chronic fatigue, headaches, insomnia and decreased balance, concentration and attention. Studies of 10 indoor-mould-exposed children and 378 indoor-mould-exposed adults found significantly more neurophysiological abnormalities than in controls; this included abnormal EEGs and abnormal brainstem, visual and somatosensory evoked potentials as compared to 10 control children. (40, 41) The large number of observations, findings in neuropsychological symptomatic patients conduct to the explanations that exposure to indoor moulds can have adverse health effects. The development of good diagnostic tools for environmental syndromes in terms of “perceptual-cognitive factors” and person-situation interaction is another need, as is the development of physiological and sensory methods for future provoking tests.

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From concept to application of HACCP principles on the food safety systems

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The new European regulations require that each establishment develop and implement a system of preventive controls designed to improve the safety of their products, known as HACCP plan (Hazard Analysis and Critical Control Point). HACCP systems has been identified as a powerful tool for control of food safety issues. Hazard analysis serves as a basis for establishing critical control points (CCPs) in a process that must be controlled to ensure the safety of food. Critical limits are definite in that document the appropriate parameters that must be met for each CCP. Monitoring and verification are included in the system to ensure potential risks are control and documented in the HACCP plan. This paper will examine a model for implementation of a HACCP system provides a schema to ensure that product safety is continuously achieved.

Key Words: HACCP principles, food safety

INTRODUCTION

Food safety is a matter why concern for all parts of the food supply chain, including governments that develop food safety policy, food industries that must control potential hazards, and consumers who need to keep to the intended use of food. In the present, food safety policy may be set using the framework of risk analysis, part of which is the development of risk assessment studies [4]. This process has been catalyzed by international food trade requirements to base sanitary measures on sound scientific evidence and appropriate risk assessments. International organizations, such as the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), are in a position to provide countries with guidance, training, information resources, and technical assistance to develop or strengthen food safety infrastructure [2].

The seven HACCP principles was adopted by Codex Alimentarius Commission (FAO/WHO) and its applications is recommended in many steps. The objective of this review were to identify a structure of implementations HACCP principles in only seven steps, used in knowledge and program learning.

METHODS

In this review, we evaluated the scientific evidence available in the global literature for the benefits and impact of various communications referred to implementation HACCP principles. Evidence from the *Codex Alimentarius* was considered and information from some national administration was specifically evaluated. Sources for potentially eligible studies included journal articles, book chapters, technical reports, conference proceeding, and theses.

RESULTS AND DISCUSSIONS

The acronym HACCP stands for Hazard Analysis Critical Control Point, which is a prevention based food safety system [2]. HACCP is a conceptually simple system whereby food

establishments can identify and evaluate the food safety of their products, institute controls necessary to prevent those hazards from occurring or keeping them within acceptable limits, monitor the performance of control, and maintain records routinely.

HACCP was initially developed by the Pillsbury Company in USA, with the co-operation and participation for the space program (NASA). It comprised a systematic series of steps to check that foods destined to be consumed in Space, was 100% safe for consumption [1]. Every aspect of the Manufacturing processes was taken into consideration, to ensure the elimination of hazardous contamination, for food that was to be consumed, by the astronauts during space flight. HACCP replaced end product testing to provide food safety assurance and a preventive system for the production of safe food. HACCP represents an important food protection tool [2]. It is not limited to food franchises or chains.

The National Academy of Science recommended the use of the system in 1985. The International Commission on Microbiological Safety and Quality endorsed the use of HACCP system in food production, processing, and handling. In 1993, the Codex Alimentarius (FAO/WHO) adopted a HACCP document that now serves as a guide for countries to incorporate HACCP principles into their food industries [2].

HACCP takes on even more importance with globalization of the food supply and the need for a consistent system for assuring trading partners of thee safety of imported products. The seven principles HACCP has been universally accepted by government agencies, trade associations and the food industry around the world.

The HACCP concept was introduced by the Hygiene Rule 178/2004 in the production line of food in Europe [4]. The European Union adopted new food hygiene regulation [5] on 1 January-2006 that requires al food businesses within EU, except primary producers, to operate food safety management procedures based on seven HACCP principles. The concept can be applied by small individual food businesses, as well as National and regional companies. Significant flexibility has been included to allow small businesses to comply.

HACCP systems are not readily applicable to food businesses like retail caterers and the flexibility allows alternatives to HACCP that achieve the same outcome of safe food being produced. Today in the food industry, HACCP is used as a way of critically examining each stage of a process, and it's vulnerability to a hazard [3]. Particular attention is then given at that point, and once the potential hazard has been identified, measures are implemented to eliminate or minimize it. HACCP is not just for the larger operators, and should not be a sophisticated or complicated program. It is applicable to all elements of the industry, both small and large, independent or corporations if they are to provide safe food.

Every official establishment shall conduct, or have conducted for it, a hazard analysis to determine the food safety hazards reasonably likely to occur in the production process and identify the preventive measure the establishment can apply to control those hazards. Application of the seven principles of HACCP as mentioned in Codex Alimentarius [2] is essential for the production of safe foods.

The HACCP plan covering each product produced by that establishment whenever a hazard analysis reveals one or more food safety hazards that are reasonably likely to occur, based on the hazard analysis conducted [1, 3].

Activity 1: Identification Of Hazards And Control Measures

Assemble the HACCP team. Each team member should have been trained in HACCP and have a working knowledge of the process/product under study. This team will be the core group; other experts can be called in as required. This is essential for each HACCP study, even if it is performed in small or less developed businesses. In large, complicated or very sophisticated businesses, a multidisciplinary team is necessary to ensure that informed unbiased assessment are made.

Describing the product's characteristics. The start of any HACCP study is the collection and evaluation of data concerning the raw materials, the formulation of the product, the processing, storage, distribution, sales, preparation and use conditions. The team must examine the food product's characteristics, the processes that are actually applied and its expected use by the consumer. Important areas to consider are:

- formulation: the raw materials and ingredients to be used and the parameters which may influence the product's safety or stability;
- processing with process parameters and conditions which affect or may create the hazards;
- packaging: protection against contamination with chemical or recontamination and growth of microorganisms;
- storage / handling: the time and temperature conditions and handling in distribution centers, retail outlets and kitchens.

Describing expected use. It must described use by consumers, caterers or professional cooks, and target groups with disabilities (infants, adults, the elderly, immuno-compromised or sick people).

Producing a flow diagram. The diagram serve as a guide for the study. It should describe all the raw materials and the processing an packaging step. It should include the data needed for microbial, chemical and physical hazard analysis. The team should **confirm the flow diagram** by examination the production site of all stages of the manufacturing process, e.g. inspecting processing lines and storages facilities.

Determination of significant hazards. *Principle No. 1*: "Conduct a hazard analysis "/ Identify of hazards and assessment of their severity and probability of occurrence. A hazard analysis of each process must be carried out. The purpose of the analysis is to identify and list the food safety hazards reasonably likely to occur in the production process for a particular product and the preventive measures necessary to control the hazards. A food safety hazard is any biological, chemical or physical property that may cause a food to be unsafe for human consumption. For identify the hazards it must look each step in operation and identify what can go wrong. A listed hazard must be of such a nature that its prevention, elimination, or reduction to acceptable levels is essential to the production of a safe food.

Food safety hazards might be expected to arise from the following: natural toxins; microbiological contamination; chemical contamination; pesticides; drug residues; zoonotic diseases; decomposition; parasites; unapproved use of direct or indirect food or color additives; physical hazards.

For many agents of a biological or chemical nature, a potential hazard is not always a significant hazard with regard to the safety of the food. Many chemicals may only have n effect when ingested in a "high dose", ADIs and MRLs have been established for these. The concept of acceptable levels is crucial for HACCP, as is clear from the definitions of control measures and CCP. It is also inherent to the characterizing of hazard: the potential to cause an adverse health effect. Whether it is causing harm will, amongst other factors, depend on the level.

Consideration of control measures. Hazard can be controlled in many ways. Heating can kill micro-organisms and their growth can be prevented or limited by low or high temperatures, low water activity, by preservatives. Residues of veterinary drugs and pesticides can often be controlled by keeping a certain time between application and slaughter, milking or harvest which would reduce the residue to an acceptable level. Strict separation between raw materials and processed food is a control measure that prevents or limits cross-contamination with pathogens. Cross-contamination in processing lines with allergens can be eliminated through appropriate validated cleaning procedures and/or sensitive consumers can be informed by appropriate labeling. Visual inspection, sieving, metal detectors may be effective in controlling physical

hazards. The various options for control measures have to be considered for each significant hazard.

Activity 2: Determination of Critical Control Points

Principle No. 2: "Determine Critical Control Points (CCPs)" / Determination of critical control points required to control identified hazards. Once hazards have been identified, it must ensure they are adequately controlled. In general, the majority of hazards are controlled by ensuring that it is operating an effective prerequisite program, i.e. good hygiene practices. A critical control point is a point, step or procedure in a food process at which control can be applied and, as a result, a food safety hazard can be prevented, eliminated, or reduced to an acceptable level. All hazards identified during the hazard analysis should enable the establishment to identify which steps in their processes are CCP's. Identification of CCP's for controlling microbial hazards throughout the production process is particularly important because these hazards are the primary cause of foodborne illness. The establishment may find the CCP decision tree developed by the Codex Alimentarius useful in the CCP identification process.

At each process step, the team should consider the possible consequence of a deviation from the "normal" GMP procedure, whether such a consequence could be unacceptable with regard to food safety, and the probability that it occur.

A CCP may be a raw material, formulation, location, practice or process stage, but it must specific: acidification of a food to a specified pH; drying a food under conditions that prevent pathogen increase; the chlorination step of can cooling water or a product pasteurization step.

Activity 3: Specific of Critical Limits

Principle No. 3: "Establish critical limit(s)" / Specification of critical limits that assure that an operation is under control at a particular critical control point. The critical limits for preventive measures associated with each identified CCP must be established. A critical limit is the maximum or minimum value to which a process parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the identified physical, biological, or chemical hazard. The critical limit is the value that separates acceptability from unacceptability for each CCP. Critical limits are most often based on process parameters such as temperature, time, physical dimensions, humidity, moisture level, water activity, pH, titrable acidity, salt concentration, available chlorine, viscosity. Critical limits should be based on applicable legal regulations or guidelines. The tolerances and action levels must based by scientific and technical literature, surveys, experimental studies, or the recommendations of recognized experts in the industry, universities, or trade associations.

Activity 4: Establishment and Implementation of a Monitoring System

Principle No. 4: "Establish a system to monitor control of a CCP". When CCP's and critical limits have been identified it is important to have a way to monitor and record what is happening at each CCP. Monitoring is an integral part of HACCP and consists of observations or measurements taken to assess whether a CCP is within the established critical limit. Typically, monitoring will involve measuring parameters such as temperature and time, physical/chemical tests or observations. However, how monitor and how often will depend on the size and nature of business. Monitoring should in all cases be simple, clear and easy to use. Monitoring activities are necessary to ensure that the process is under control at each critical control point. Continuous monitoring is preferred, but when it is not feasible, monitoring frequencies must be sufficient to ensure that the CCP is under control.

Assignment of the responsibility for monitoring is an important consideration for each CCP. Personnel assigned the monitoring activities should be properly trained to accurately record all results, including any deviations, so that immediate corrective action may be taken.

If a monitoring result shows that an unacceptable deviation occurred, the product should not reach the consumer. The amount of product to be rejected, reworked or further investigated depends on the time passed since last monitoring result showed that the situation was under control. Full records must be kept of all monitoring data for management, audits, trend analysis and officials inspections

Activity 5: Establishment of Corrective Actions

Principle No. 5: "Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control" / Execution of corrective actions when critical limits are not met. The HACCP plan must include corrective action to be taken when monitoring indicates that there is a deviation from a critical limit at a critical control point. In such instances, corrective action plans must be in place to determine the disposition of thee potentially unsafe or noncompliant product and to identify and correct the cause of the deviation. Corrective actions are intended to ensure that no product injurious to health or otherwise adulterated as a result of the deviation enters commerce. The HACCP plan it self might require modification, perhaps in the form of a new critical limit, or of an additional CCP.

Monitoring data should be examined systematically to identify the points where controls should be improved or where other modifications are needed. In this way, the system can adapt to changes by constant fine-tuning.

Activity 6: Verification of the System

Principle No. 6: "Establish procedures for verification to confirm the HACCP system is working effectively". Verification is a very important element of HACCP and should always be included. It is intended to provide additional information to reassure the producer and the inspector that application of HACCP results in the production of safe foods. It comprises two distinct activities: validation and data gathering. It includes activities such as inspections and audits as well as the use of classical microbiological and chemical contaminant tests to confirm that the control measures operated as designed. Verification is different from monitoring.

HACCP systems must be systematically verified. After initial validation that the HACCP system can work correctly and effectively with respect to the hazards, the system must be verified periodically. Periodic verification involves the use of methods, procedures, or tests in addition to those used for monitoring, to determine whether the HACCP system is in compliance with the HACCP plan and/or whether the HACCP plan needs modification and revalidation to achieve its food safety objective. It ensures the HACCP plan is adequate, that is, working as intended. Verification procedures may include such activities as review of HACCP plans, CCP records, critical limits and microbial sampling and analysis.

Certification is a specific form of verification. It is performed by independent third parties; it deals with checking that a certain HACCP system, as described in a "HACCP standards", was applied. An auditor from a certification body will report on the business performance in relation to the standard, but will normally nor provide a judgment concerning the product's safety.

Validation ensures that the plants o what they were designed to do; that is, they are successful in ensuring the production of safe product. Plants will be required to validate their own HACCP plans.

Activity 7: Record Keeping

Principle No. 7: "Establish documentation concerning all procedures and records appropriate to these principles and their application". For the successful implementation of HACCP, appropriate documentation and records must be kept and be readily available. This ensure that information gathered during installation, modification and operation of the system would be readily accessible to everyone involved in the process as well as to outside auditors. It also helps to ensure the long/term continuity of the system.

Records should include explanations of how the CCPs have been defined, descriptions of control procedures and modifications to the system, monitoring and verification data, a file of deviations from normal practice and corrective actions.

The HACCP regulation requires that all plants maintain certain documents, including its hazard analysis and written HACCP plan, and records documenting the monitoring of critical control points, critical limits, verification activities, and the handling of processing deviations. One of the principal benefits of a HACCP process control system to both industry and regulatory officials is the availability of objective, relevant data.

HACCP plan must be appropriateness for every particular situation. This plan is a concise monography how describe the implemented system in more complex situations.

The contents of the HACCP plan shall, at a minimum: list the food safety hazards identified, which must be controlled for each process; list the critical control points for each of the identified food safety hazards, including, as appropriate: critical control points and attention points; list the critical limits that must be met at each of the critical control points; list of procedures, and the frequency with which those procedures will be performed, that will be used to monitor each of the critical control points to ensure compliance with the critical limits; include all corrective actions that have been developed to be followed in response to any deviations from a critical limit t a critical control point; provide for a recordkeeping system that documents the monitoring of the critical control points. The records shall contain the actual values and observations obtained during monitoring; list the verification procedures, and the frequency with which those procedures will be performed, that the establishment will use.

The HACCP plan shall be signed and dated by the responsible establishment individual. This signature shall signify that the establishment accepts and will implement the HACCP plan. The HACCP plan shall be dated and signed: upon initial acceptance; upon any modification and at least annually, upon reassessment.

The HACCP plan shall describe the corrective action to be taken, and assign responsibility for taking corrective action, to ensure: the cause of the deviation is identified and eliminated; the CCP will be under control after the corrective action is taken; measures to prevent recurrence are established; and no product that is injurious to health or otherwise adulterated as a result of the deviation enters commerce.

The official verification will verify the adequacy of the HACCP plan by determining: reviewing the HACCP plan; reviewing the CCP record; reviewing and determining the adequacy of corrective actions taken when a deviation occurs; reviewing the critical limits; reviewing other records pertaining to the HACCP plan or system; direct observation or measurement at a CCP; sample collection and analysis to determine the product meets all safety standards; and on site observations and record review.

CONCLUSIONS

HACCP is a systematic approach to identifying and controlling hazards (i.e. microbiological, chemical or physical) that could pose a danger to the preparation of safe foods. HACCP involves: identifying what can go wrong, planning to prevent it, making sure doing it. In simple terms, it involves controlling the safety of ingredients and supplies coming into a food business and that is done with them thereafter.

Application of the seven principles of HACCP as mentioned in Codex Alimentarius is essential for the production of safe foods.

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Principal determinants of meat sensorial quality

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Organoleptic evaluation of meat consists in describing the attribute of meat that can be perceived by the sense organs. These characteristics to be evaluated are: appearance, texture and consistency, smell and taste. The present paper will focus on the major determinants of meat sensorial quality, and will consider some intrinsic muscle and technological characteristics what influence these attributes.

Key Words: meat quality, Organoleptic evaluation

INTRODUCTION

The quality of meat and meat products is defined by the following criteria: palatability (typical texture and consistency, juiciness, good flavour); proportion of lean meat to fat; freshness and adequate conservability of the products; absence of harmful micro/organisms or substances; and appropriate (preferably minimal) use of additives and meat extenders [1, 2, 3].

The different criteria need different methods of quality control, such as: organoleptic evaluation; physical test methods; chemical analysis; microbial examination [4, 5]. Basic methods for quality control must involve sensory evaluation will be most important. The aim of this work was to give a brief review of factors affecting the eating quality of meat.

METHODS

This paper reviews current knowledge about factors of importance for sensorial meat qualities, with special emphasis on technological quality attributes. The scientific evidence available in the global literature for meat quality was specifically evaluated. It is insisted on the production and slaughter factors can be used to control technological quality traits. A brief overview of the last 10 years of research on the simples methods for evaluation the sensorial quality of meat demonstrates a diversity of sensory procedure was made.

RESULTS AND DISCUSSIONS

The muscle on the animal at slaughter is a living tissue with complex biochemical and physiological properties. It impose a series of treatments, changing its temperature, tension, and fluid and gaseous environment, and it changes from muscle to meat. These treatments can optimize or destroy meat quality. Eating quality of meat refers to the organoleptic factors influencing consumer acceptance and enjoyment of the product.

The main eating quality attributes of meat are: appearance, texture and consistency, smell and taste.

1. Appearance. The visual identification of quality meat is based on color, marbring and water holding capacity.

a) Colour is a major influence on the visual appeal of meat rather than on quality. The meat should have a normal color that is uniform through the entire cut. Visual appearance is very important in determining the likelihood of purchase. Meat color in red meats a bright red color is perceived by consumers as being indicative of freshness. The colour is primarily dependant on the concentration and chemical state of thee pigment myoglobin, which is responsible for moving oxygen through muscle. When meat is exposed to the oxygen in air, the purple-red myoglobin absorbs oxygen and is converted to bright pink oxymyoglobin. After prolonged exposure,

oxymioglobin is chemically oxidized to brown metmyoglobin, this process often termed “browning”.

There are several factors which affect the color of uncooked meat. Some of these factors are: species, ages and sex of the animal; cut of meat; water holding capacity of the meat; surface drying of the meat. In general, beef and lamb or mutton have more of a red color than do veal, fish, and poultry. Myoglobin concentration usually increases with age of the animal. Highly active muscles also have more myoglobin. The type of packaging used at retail and thus the amount of oxygen to which the meat is exposed, influences the meat’s color and appeal to the customer.

b) Marbling is small steaks of fat that are found within the muscle and can be seen in the meat cut. These visible depots within the muscle are known as marbling fat. Intermuscular fat is formed by depots of adipocytes situated between the muscles. Intramuscular fat is found within the muscles. Lean meat is the skeletal muscle of the carcasses, with all visible subcutaneuous and intermuscular fatty tissue removed. When lean meat is separated from fat and other tissues with a butchers knife then some traces of fat may remain with muscle. This type of lean meat can be referred to as marbring. Fat is a source of energy that is stored in muscle tissue. When fat is heated, it melts and lubricates the muscle fibers in the meat, helping to keep it moist. The marbling will increase also the juiciness, tenderness and the flavor in the product.

c) Water-holding capacity is an important physical characteristic affecting the quality and cooking yield of manufactured muscle foods. Water-holding capacity is the ability of meat to retain its water during application of external forces such as cutting, heating, grinding or pressing. It affects: color – raw, tenderness, juiciness, nutrient quality, cooking losses and weep and purge. It can be witnessed by looking at the package, if excess water is found in the bottom of the retail package, it may lead to dry cooked product.

In the muscle, water is bound (4-5%) with molecules polarized, associated with protein; immobilized, held by capillary forces and less organized; free, held by capillary forces. Net change effect depends by lactic acid causes pH decline. The **isoelectric point** is the pH value at which the number of + and – charged groups are equal. These groups tend to be attracted to each other and only those “left over” are available to attract water. In DFD meat, pH of 6.3 which results in higher water binding capacity has a pH far away from 5.1 to 5.2. In PSE meat, low water holding capacity due to pH approaching to 4. The **steric effect** is the lack of space for water molecules within the protein structure. It is in direct proportion to the breakdown of ATP postmortem. Water holding capacity is a contributing factor to the juiciness of meat.

2. Texture and consistency (tenderness and juiciness)

Juiciness depends on the amount of water retained in a cooked meat product. Juiciness increases flavor, helps soften meat – making it easier to chew, and stimulates saliva production in the mouth. Water retention and lipid content determine juiciness. Marbling and fat around edges helps hold in water. Water losses are from evaporation and drip losses. Meat aging can increase water retention and therefore increases juiciness. Using proper cooking methods, such as cooking slowly and/or with moist heat, can increase juiciness. Cooking past medium can also dry the meat. The best way to increase the juiciness of the meat being prepared is to learn the best cooking method.

Tenderness is one of the major and most variable eating quality attributes of meat. Tenderness can be attributed to a person’s perception of meat, such as: softness to tongue and cheek, resistance to tooth pressure, ease of fragmentation, meatiness, adhesion and residue after chewing. Tenderness has been linked to several factors, such as the muscle features, chilling process, electrical stimulation, handing and maturation.

a) Muscle features. The collagen component of muscles is fundamental in determining the structure of meat and is a major determinant of the eating quality, particularly tenderness of

meat. Collagen is a long, stiff protein that is the most prevalent protein in mammals. It's made up of three separate molecules composed of amino acid chains, twisted around each other to form a rope. This structure is what makes the collagen so strong; this strength is also what makes it more difficult to break down. The more collagen there is in a piece of meat, the tougher it is to cut and to chew. Skin is mostly collagen, and are the tendons that connect muscles to bones. For cuts that are high collagen, cooking with methods that use slow, moist heat, such as stewing or braising, are the best. Collagen is soluble in water and when it is cocked slowly with moist heat, it becomes gelatin. It can also make collagen less tough by slicing up meat into smaller pieces, which makes the fibers smaller and easier to break apart.

As an animal the mature connective tissue in the muscle gets thicker, therefore the products of older animals will not be as tender as the products from younger animals. Males tend to have more connective tissue than females and the muscle that are used more will also have more connective tissue. Young, rapid growing animals on a high quality diet will be the most tender. Cows and pigs have higher amounts of collagen in the legs, chest, and rump. Pork is generally more tender that beef because pigs are usually slaughtered art a younger age than cows, and so their muscles are less developed and have less collagen than o those of cows. Weight-bearing muscles and muscles that are constantly used contain higher amounts of collagen than muscles that aren't used for support.

b) Chilling process. Another factor is the state of the meat while being harvested has important effects on tenderness. The chilling process generally involves placing carcasses in conditions of 0 to 5^0C within one hour of slaughter. Contraction of the muscles during chilling can lead to increased toughness in meat. Carcasses are chilled rapidly soon after slaughter to prevent the growth of bacteria and to minimize weight loss during chilling. The muscle fibres tend to contract when a muscle is chilled rapidly to bellow 12^0C before the onset of rigor mortis. If temperature are too cold, the muscle fibers will shorten or shrink. This effect is called *cold shortening* and it make meat tough. This causes the sarcomeres within the muscle to shorten, which results in a very appreciable reduction in the tenderness of meat. Muscles shorten as they go into *rigor mortis* and pH of the muscle falls. The amount of muscle shortening affects the meat's tenderness. Cold shortening is a problem where rapid chilling systems are used, particularly for sheep carcasses where the low volume of meat means the muscle cools very rapidly. Similarly, if carcasses enter rigor mortis above 20^0C, *hot shortening* occurs. It is generally produce low to moderate shortening and reductions in meat tenderness, in comparison to cold shortening.

c) Electrical stimulation involves the application of a suitable electrical current to the carcass either immediately after slaughter, or at the end of the dressing line. This rapidly converts the muscle glycogen to lactate, lowering the pH and speeding up the onset of rigor mortis so that by time the muscle temperature is reduced, the fibers are unable to contract (cold shorten) and toughen. The degree of electrical stimulation must be controlled however, so that pH does not fall so rapidly that there is the danger of heat shortening. High voltage systems are particularly effective in lamb and pig carcass.

d) Hanging is the method of carcasses suspension influences the degree of tension which muscles are under rigor mortis occurs. Suspending lamb, beef and pig carcasses from the hip rather than by Achiles tendons, allows the commercially more important muscles of the carcasses to be stretched, thus improving tenderness. The trials show that for beef, correct hanging is more beneficial to meat tenderness than electrical stimulation.

e) Aging (maturation) is one important way to tenderize meat. Meat is aged by holding it at refrigeration temperatures (0 to 4^0C) for extended periods of time after slaughter and initial chill. There are two methods for again meat: dry aging and wet aging. *Dry aging* is much more expensive and takes than wet aging. Meat which is dry aged is hung in a very clean, temperature and humidity controlled cooler for a period of two to four weeks. During this time, enzymes

within the meat breakdown the muscle and connective tissue making it tender. *Wet aging* occurs when meat and its juices are vacuum packed in plastic and boxed for distribution. Because the plastic packaging does not allow loss of moisture, the meat absorb more moisture which results in an increase in juiciness and tenderness.

3**. Smell and taste (aroma and flavour).** Flavour and aroma are intertwined to create the sensation the consumer has during eating. These perceptions rely on the smell through the nose and on the sensation of salty, sweet, sour and bitter on the tongue. Meat flavor is affected by many different things. A major portion of flavor or believed to be due to the breakdown products of ATP or energy. This phenomenon leads to a stronger flavor in energy storing muscles than in other muscles. This is also part of the changes in flavor attributed to aging, where certain molecules in the product are being destroyed over time. Flavor is also attributed to many of the water soluble components of thee muscle. These components are held in by water retention during cooking.

Because it is a subjective property, is difficult to evaluate. Each species has its own characteristic flavour which can be traced to the fat within the muscle. Chemical reactions resulting in some 1000 compounds during cooking contribute to the individual meat's flavour. The flavour of meat can be influenced by the diet of animal. Grass or forage-fed cattle and sheep tend to produce meat with a more intense flavour than grain-fed animals. Grass-feeding increases certain polyunsaturated fatty acid concentrations in the muscle and improves flavour.

Changing cooking methods can also affect the flavor of the meat. Dry heat cooking will change the flavor on the outer portions of thee product, while wet cooking will change more of the inner tissue taste. Reheated products also have a distinctive flavor that is unappealing. This distasteful flavor is caused by changes to the meat components during refrigeration. Smoked and cured products will also have a distinctive flavor.

CONCLUSIONS

Organoleptic evaluation of meat consists in describing the attribute of food, in this special case that can be perceived by the sense organs. The attributes to be evaluated are: appearance with colour, juiciness, tenderness, aroma and flavour.

The evidence shows that postmortem factors have a major impact on the sensorial qualities of meat. The processor must use valuable cooler space to many variables, so must expect for obtained a high quality of meat.

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The influence of pH and temperature against *E. coli* and coliforms growth in frozen and freezing poultry carcasses

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Researches were initiated in a slaughtering unit from Iassy. 216 samples from poultry carcasses were gathered and analyzed microbiologically. Carcass washing was used in order to obtain test samples.

Escherichia coli and coliforms presence represents the markers for flash salubrity.

Samples were gathered during Strategic and HACCP programs.

In order to appreciate the salubrity of the products and unit were used two markers: E coli and coliforms presence.

To reduce the contamination, experimentally, we treated the washing weather with lactic acid. In this way we stopped the evolution of the microorganisms sensible to environmental ph changes.

This ph change reduced the microbiological load, especially, E coli and coliforms from the surface of the carcass.

Key words: poultry, decontamination, lactic acid, skin, coliforms, E. coli

The modern technology of fowl slaughtering raises new hygiene problems. In this context flash contamination with various germs is facilitated by unsuited transport, microorganisms from the processing rooms, deficient hygiene of the slaughter devices, disobeying rules of the technological flux and flows in the cooling process.

Generally the antimicrobial treatment is used in all slaughter units, during technological process and it can be physical, chemical or microbiological. In this way the microbiologic load of the product is diminished, especially from the point of view of the microorganisms considered pathogen.

Carcasses antimicrobial treatment can gather five different techniques: using organic acids, radiation, alkaline solutions, chlorinated water, steam and hot water.

New concepts gain more ground in industry and represents the proceedings meant to remove unwanted germs from carcasses (for example washing techniques with organic acids).

These decontamination procedures can lead to a safety rise in food industry, beyond the actual limits, established using only prevention means.

The following study intends to evaluate the ability of lactic acid to reduce the pathogens concentration in carcasses refrigerated or frozen. This study will use biologic markers represented by microorganisms as hygiene indicators.

Material and method

Researches were initiated in a slaughtering unit from Iassy. 216 samples from poultry carcasses were gathered and analyzed microbiologically.

The parameters investigated were represented by microorganisms used as salubrity indicators, especially E coli and coliforms . The samples were obtained using Carcass washing after cooling and after freezing.

The carcasses evaluation was done considering ANSV programs: 78323/1998 and 74032/1999. Parts of the HACCP program in the slaughter unit are tests for E coli and coliforms.

Tests for E coli and coliforms from poultry carcasses are based on E coli (biotype 1) colony counting, there is used 1 ml washing solution. Complete and not deteriorated carcasses were chosen randomly, in order to obtain the samples.

Sterile bags and 400 ml of sterile physiologic solution with peptone 1% were used, for each carcass surface wash, for 1 minute. The wash liquid was collected in sterile recipients, 30 ml per sample. Using a Vortex mixer all samples were agitated for 30 seconds. Further more dilution with sterile physiologic solution was done up to 10^{-6}.

In order to isolate the specific biologic markers we used selective growth mediums.

Escherichia coli identification followed the protocol indicated by STAS ISO 4832. Determining the probable count of bacteria’s used the protocol indicated by STAS SR ISO 7251.

Considering these standards, three test tubs with Durham tubes and selective growth mediums are used for inoculation with all dilutions of the washing solution. The selective medium used contains tryptose, sodium lauryl sulfate, lactose. The lauryl sulfate largely inhibits the growth of undesirable microbial flora. The presence of E. coli is indicated by fluorescence under a long wavelength UV lamp (figure 1). A positive indole reaction and gas formation due to fermentation of lactose confirm the results.

Figure 1. Positive result (Durham tubes filled with gas, UV light -366 nm- Light blue fluorescence indicates the presence of E. coli.)

This medium is clear and yellowish-brown. Filled in test tubes fitted with DURHAM tubes after sterilizing with autoclaving (15 min at 121°C) is inoculated and incubated at 37^0 C for 24-48 hours.

After this time interval from each positive sample a highly selective medium is inoculated. The second growth medium used is EC broth. Incubation: 24-48 hours at 35°C and/or 44.5°C.

Gas formation at 44.5 °C (and 35 °C)	Escherichia coli, possibly also other coliform bacteria
Gas formation only at 35x°C	Coliform bacteria without E. coli

From the positive samples plates with Levine medium are inoculated and incubated at 37^0 C for 24 hours. After incubation are searched E coli specific colonies: Large, blue-black, green metallic sheen.

Biochemical aspects of E coli species were identified using the API-20E test kit for enteric bacteria. The mini-Api device is used to read the test strip.

An extremely important element is the pH of the environment, which allows larger limits for microorganism's growth, from pH 3.8-11.

Organic acids are more efficient against pathogens implicated in diseases with food origins. In the study initiated we pursued lactic acid 1% action over microbial strains.

Lactic acid is an organic product, obtained on industrial scale using lactic fermentation of vegetal materials reach in sugar. It is free of toxicity for human and animals.

pH measuring was done using a pH meter. Its efficacy was evaluated according 29^{th} (1) article from E.C.178/2002 regulation.

Initially pH carcass was 5,5 but the value dropped to 3,5 after Lactic acid treatment.

Results and discussion

Coliforms and E coli are considered worldwide the main biological markers for feces contamination of the flash. Theirs presence reveals an eventual contamination of the food with a digestive origin pathogen.

Researches regarding aerobe microorganisms from carcasses surface proved that the microbial load is low. The significance of this particularly low load is that technological flux is respected, during all fazes.

In many countries are used various methods to diminish the pathogens load from carcasses surface. There are used physical methods as water flush, immersion of the carcasses in hot water and acid solution. None of these measures satisfies the epidemiological exigencies or justifies the financial efforts.

In order to introduce this salubrity measures, several pathogens were isolated and used for laboratory trials. Lactic acid 1% reduces the microbiological load without deteriorating the organolepticaly proprieties of the flash. (Dincer 2002)

Van der Marel and col. in 1988 studies the effect of the carcasses immersion in lactic acid and he observes a microbiological load diminish after a 15 seconds immersion. The effect is more pregnant with 2% solutions than 1%, and immediate after the treatment the microorganisms load is diminished.

Bautista and col. in 1995 determinates that lactic acid is efficient in reducing the coliforms load from the carcasses surface. It reduces the microbiological load with 90-99% and pathogens with 30-90%.

After samples gather (in order to establish the microbiological load) carcasses were refrigerated at 4^0C and freeze at -18^0C for 24 hours. Between these time intervals more samples were gathered using carcasses wash. The results obtained are presented in table 1.

Coliforms and E coli values from gathered samples ***Table 1***

Species	Samples number	4^0C				-18^0C			
		Positive samples		Negative samples		Positive samples		Negative samples	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
E coli	108	11	10,1	97	89,8	6	5,5	102	94,4
Coliforms	108	28	25,9	80	74	9	8,3	99	91,6

A significant contamination occurs after evisceration. It can appear without a visible contamination with feces.

Immediate after slaughter carcasses are immersed in water tanks at 5-15^0 C. In these tanks is realized the carcasses wash, followed by cooling to 4^0C in special rooms.

Some of the carcasses after evisceration have a raised microbial load and represents a contamination source. In order to reduce the microorganisms load water used for carcasses wash is treated with lactic acid. Reducing the pH microorganisms' growth is stopped and in this way is limited coliforms and E coli contamination. (Table 2)

Coliforms and E coli values after immersion in Lactic acid solution 1%

Table .2.

Species	samples	Without lactic acid 1%				With lactic acid 1%			
		Positive samples		Negative samples		Positive samples		Negative samples	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
E coli	108	18	16,7	90	83,3	5	4,6	103	95,3
Coliformi	108	36	33,3	72	66,6	6	5,5	102	94,4

After primary treatment with lactic acid 1%, carcasses were refrigerated for 24h. In order to observe the concomitant bacteriostaic effect of low temperature and low pH environment samples were gathered after refrigeration.

Combined bacteriostaic effect, of both pH and low temperature, proved to be very effective against monitored pathogen, E coli.

Coliforms and E coli values after treatment with Lactic acid solution 1% and refrigeration

Table 3

Species	samples	4^0C, Without lactic acid 1%				4^0C, With lactic acid 1%			
		Positive samples		Negative samples		Positive samples		Negative samples	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
E coli	108	11	10,1	97	89,8	-	-	108	100
Coliformi	108	28	25,9	80	74	2	1,85	106	98,1

The study continued with freezing of the carcasses treated with lactic acid 1% at the -18^0C. Freezing carcasses stopped bacteria's growth; the effect was a bactericide one.

Coliforms and E coli values after treatment with Lactic acid solution 1%, and freezing

Table 4

Species	Samples	-18^0C, Without lactic acid 1%				-18^0C, With lactic acid 1%			
		Positive samples		Negative samples		Positive samples		Negative samples	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
E coli	108	6	5,5	102	94,4	-	-	108	100
Coliformi	108	9	8,3	99	91,6	-	-	108	100

From the gathered information we confirmed that combined effect of the reduced temperature and low pH reduces the microorganism's growth from carcasses surface.

Conclusions

1. The washing tank represents an important source of contamination;
2. Carcasses wash must be realized using water flush, in order to reduce the microbial load;
3. Organic acids have an important antimicrobial role;
4. E coli and coliforms can be found in a reduced quantity on carcasses surface;
5. 216 samples from poultry carcasses were gathered and analyzed microbiologically. Escherichia coli and coliforms presence represents the markers for flash salubrity. Samples were gathered during Strategic and HACCP programs;
6. The percent of the contaminated carcasses with coliforms was 24.8% and with E coli 18, 57%;
7. Changing water PH with a lactic acid solution 1% reduced coliforms and E coli growth;
8. Lactic acid is used frequently in food industry because of its powerful antimicrobial action.

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Observation concerning of raw milk used for manufacture of white-brined cheese – telemea

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A study concerning the microbiological quality of milk used as raw material in Telemea cheese was conducted. 275 samples of raw milk (241cow's raw milk and 54 ewe's raw mil) were investigated.

Total viable count, coliforms, enterobacteriaceae, B. cereus, Staphylococci, Salmonella spp.,and Listeria spp. were determined. Salmonella enterica ssp. enterica serovar. Saint Paul was identified in a ewe's raw milk sample. Listeria spp. were identified in 7.3% of cow's milk samples and in 36% of ewe's milk samples but no strain of Listeria monocytogenes was isolated. A strain of S. aureus and one of E. sakazakii were isolated from cow's raw milk samples.

Although, the prevalence of these pathogens were low the presence of them in raw milk represent a potential risk to consumers.

Key words: raw milk quality, white-brined cheese, Salmonella spp., Enterobacteriaceae

Introduction

Telemea cheese is a traditional variety of white-brined cheese common in many Balkan countries which despite of the long history it has not been studied in detail till now. In Romania this variety of cheese is manufactured preponderantly from cow's and ewe's milk or a mixture of cow's and ewe's milk (13).

The quality of telemea cheese is influenced by many factors, raw milk quality being one of the most important. White brined cheeses are matured for long period of time in brine and thus the microflora of raw milk make a significant contribution to the maturation process and to some degree regulate the quality and safety of the final product.

The objective of this study was to determine the microbiological quality of raw milk used for manufacture of telemea cheese.

Materials and Methods

Investigations were carried out between October 2006 and April 2007 on 275 samples of raw milk collected as follows: 275 samples (221 samples of cow's milk from a dairy farm from Iasi county and 54 samples of ewe's milk from a sheepfold from Sibiu county).

After purchase, the samples were placed directly into cool boxes and transported to the laboratory. The samples were processed within 24 to 48 h.

The milk samples were analyzed in laboratory in order to establish the microbiological status.

Total viable counts of aerobic bacteria, Coliforms, B. cereus, Staphylococcus spp., Enterobacteriaceae, Salmonella spp. and Listeria spp. were determined on each sample that passes the organoleptic examination.

The bacteriological parameters were determined according to criteria specified by following methods:

- SR ISO 6610/1997
- SR ISO 5541-2/1994
- SR ISO 7932/2005
- SR ISO 7251/1997
- SR ISO 6888/2002
- SR ISO 6579/2003
- SR ISO 11290/2005

Microbiological investigation of sample was done in accordance with criteria specified SR ISO 6887-1/1999 – Food and feed microbiology, SR ISO 7218/1996 - Food and feed microbiology and SR ISO 8261/1996 – Milk and milk products.

The results of microbial examination were evaluated in accordance with Romanian Ministry of Health Order no. 975/1998, 1106/2003 order of Romanian Sanitary Veterinary and Food Safety National Authority and EC Regulation 853/2004.

The bacteria isolated were further biochemical characterized by API test in order to find out the milk contaminant species. The API ID 32 E, API ID32 Staph, and API Listeria were used for characterization of *Enterobacteriaceae*, *Staphylococci* and *Listeria spp.*.

Results and Discussion

In table 1 are presented the results of organoleptic examination of the milk samples. The aspect, consistency, color and smell of milk samples were evaluated according to seasonal normal variation of these characteristics.

From the 275 milk samples undergo the organoleptic examination, 34 (30 cow's milk samples and 4 ewe's milk samples) were improper for further examination according to specifications. The defects of milk samples rate were higher in warm season when the animal feeding and the temperature of milk could not be well oversight. The milk defects affect especially the aspect and color of milk being caused mainly by contamination of milk with spoilage microorganisms capable to produce pigments that affect more or less the color of raw milk. There are diffusible pigments secreting microorganisms capable to change the color of milk with intensification of pigmentation in case of acidification of milk (i.e. *Pseudomonas fluorescens* which induce a slightly yellow nuance of milk) and there are microorganisms that produce undiffusible pigments identified as spots on the surface of milk (i.e. some *Saccharomyces* yeasts which in certain condition impart yellowish, orange or red color to milk) (13).

Table 1 The organoleptical characteristics of raw milk samples intended for use in manufacture of Telemea cheese

Season	Species	Samples	Samples with defects									
			Aspect		Color		Consistency		Smell		Total	
			No	%	No	%	No	%	No	%	No	%
Warm	Cow	146	21	14.4	21	14.4	18	12.3	20	13.7	21	14.4
	Ewe	54	4	7.4	2	3.7	-	-	2	3.7	4	7.4
	Total	200	25	12.5	23	11.5	18	9.0	22	11.0	25	12.5
Cold	Cow	75	9	12.0	6	8.0	6	8.0	6	8.0	9	12.0
Total		275	34	12.4	29	10.5	24	8.7	28	10.2	34	12.4

Table 2 - The microbiological quality of raw milk samples intended for use in manufacture of Telemea cheese

Milk samples		Microbiological parameter													
		TVC/ml		Coliforms/ml		E.coli./ml		Staph.spp/ml		B.cereus/ml		Salmonella /25ml		Listeria spp /25ml	
		C*	U**	C	U	C	U	C	U	C	U	C	U	C	U
Total 241	No	**134**	**77**	**169**	**72**	**179**	**62**	**227**	**14**	**137**	**4**	**240**	**1**	**209**	**32**
	%	**68.0**	**31.9**	**70.1**	**29.9**	**74.3**	**25.7**	**94.2**	**5.8**	**98.3**	**1.6**	**99.6**	**0.4**	**86.7**	**13.3**
Cow 191	No	**129**	**62**	**132**	**59**	**140**	**51**	**179**	**12**	**188**	**3**	**191**	**-**	**177**	**14**
	%	**67.5**	**32.5**	**69.1**	**30.9**	**73.3**	**26.7**	**93.7**	**6.3**	**98.4**	**1.6**	**100**	**-**	**92.7**	**7.3**
Ewe 50	**No**	**35**	**15**	**37**	**13**	**39**	**11**	**48**	**2**	**49**	**1**	**49**	**1**	**32**	**18**
	%	**70**	**30**	**74**	**26**	**78**	**22**	**96**	**4**	**98**	**2**	**98**	**2**	**64**	**36**

C* - Conform with specifications
U** - Unconform to specifications

The improper storage and manipulation of the milk support the growth of bacteria, being a major cause of defects of milk.

The microbiological examination of the milk samples yielded different results depend on the parameter investigated and the origin of milk. In accordance with legal specifications the total viable count was overtaken in 77 samples of milk and coliforms go over the limit of legal specifications in 72 samples. The potential pathogens: *E. coli, Staphylococcus spp.*, *B. cereus*, *Salmonella spp.* and *Listeria spp.* were isolated, exceeding the legal specifications in 25.7, 5.8%, 1.6%, 0.4% and 13.3% respectively *(Table 2)*.

Based on our results contamination of milk with *Salmonella spp*. was low (only one sample) compared with dates published by other researcher (1,2,6,10). *Listeria spp.* was isolated from 13.3% of investigated samples in accordance with data published by other authors (3,7,12).

The prevalence of *Listeria spp.* was bigger in ewe's samples than in cow's milk samples. In a large case-control study the main factors associated with raw milk contamination by *L. monocytogenes* were: poor quality silage, poor animal cleanliness, insufficient lighting of milking barns and parlors, and incorrect disinfection of towels between milkings (4,11).

The serotype of Salmonella isolated from milk samples was *S. Saint –Paul*, a serotype involved in outbreaks of human salmonelosis.

Listeria monocytogenes, the only species of the group proved to be pathogenic for humans till now, was not isolated from milk samples investigated in this study. The species of *Listeria* most frequent isolated from milk samples was *L. ivanovii (Table 3)*.

Tabelul nr:3 - The identification of Listeria spp. strains isolated from milk with miniApi system

Species	No of samples		Strains/ cow's milk		Strains/ ewe's milk	
	cow	ewe	No	%	No	%
Listeria innocua	191	50	4	2,1	6	12
Listeria grayi			5	2.6	7	14.0
Listeria seeligeri			3	1.6	1	2.0
Listeria welshemeri			1	0.5	2	4.0
Listeria ivanovii			1	0.5	2	4.0
Total strains			14	7.3	18	36.0

Staphylococci group of microorganisms including pathogenic species were isolated from 5.8% and the most frequent isolated were Staphylococcus xilosus in cow's milk samples and Staphylococcus chromogenes from ewe's milk samples. S. aureus was isolated from a single sample of cow milk (Table 4). The principal sources of staphylococci in milk are the infection of mammary gland, staphylococcal mastitis being not uncommon among dairy herds.

The Enterobacteriaceae bacteria from milk samples belong to nine species. Among these species the Enterobacter sakazakii, Salmonella enterica ssp. enterica serovar. Saint Paul and Shigella sonnei are recognized as potentialy human pathogens.

Table 4 - The identification of Staphylococci isolated from milk with miniApi system

Species	No of strains		%	
	Cow	ewe	cow	ewe
Staphylococcus sciuri	2	-	1.0	-
Staphylococcs aureus	1	-	0.5	-
Staphylococcus xylosus	3	1	1.5	2.0
Staphylococcus epidermidis	2	-	1.0	-
Staphylococcus equorum	1	-	0.5	-
Staphylococcus chromogenes	2	3	1.0	6.0
Staphylococcus simulans	1	-	0.5	-
Total strains	12	4	6.3	8.0

E. sakazakii (previously, yellow pigmented *E. cloacae*) is usually a commensal of the human intestine, but it has occasionally been isolated as an extraenteral pathogen (5). The thermal resistance of *E. sakazakii* appears to be higher than that of many other *Enterobacteriaceae* (8). *E. sakazakii,* should be viewed as potential agents of foodborne illness, but more epidemiological information on these organisms is required to allow a more reliable assessment of their potential as foodborne pathogens *(Table 6)*.

Table 5 - The identification of Enterobacteriaceae isolated from milk samples with miniApi system

Species		No of strains		%	
		cow	ewe	cow	ewe
Enterobacter spp.	*Enterobacter sakazakii*	1	-	0.5	-
	Enterobacter intermedius	1	1	0.5	2.0
	Enterobacter aerogenes	1	-	0.5	-
Escherichia spp.	*Escherichia coli*	51	11	26.7	22.0
Klebsiella spp.	*Klebsiella oxytoca*	1	-	0.5	-
Citrobacter spp.	*Citrobacter freudii*	2	1	1.0	2.0
Proteus spp.	*Proteus vulgaris*	1	-	0.5	-
Shigella spp.	*Shigella sonnei*	1	-	0.5	-
Salmonella spp.	*S. Saint Paul*	-	1	-	2.0
Total		59	14	30.9	28

Conclusions

1. The study show that microbial load of cow's milk samples were higher than the microbial load of ewe's samples, fact reflected by TVC and Coliforms percents of unconformed samples;
2. *Enterobacter sakazakii* and *Shigela sonnei,* two pathogen species of enterobacteriaceae, were isolated from cow's milk samples;
3. One sample of ewe's milk was contaminated with *Salmonella enterica ssp.enterica serovar. Saint Paul,* recognized as human pathogen;
4. Thirty two strains of *Listeria* belonging to five species were isolated from investigated milk samples. The most of them (18 samples) were isolated from ewe's samples;
5. The presence of these presumptive pathogen species in raw milk used in cheese industry indicate the necessity of implementation of programs for improving the milk quality;
6. Present microbiological indicators used according to legal specifications in assessment of raw milk quality don't exclude the presence of hazardous microorganisms and thus a new approach of this important issue it is necessary.

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The microbiological quality of white-brined telemea cheese manufactured in farm-house facilities

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Microbiological assessment and organoleptical evaluation of 115 samples of Telemea cheese were carried out. The Enterobacteriaceae, Bacillus cereus, Staphylococci, Salmonella spp. and Listeria spp. were determined

From the 115 examined samples, 14 show organoleptical defects and were excluded from microbiological examination. A number of 78 of 101 examined samples were contaminated with one or more of the assessed microorganisms.

Samples made from ewe's milk demonstrate a higher microbial contamination comparing with samples made from cow's milk or mixture of milk. Escherichia coli, Enterobacter sakazakii, Shigella sonnei, S. arizonae, and Staphylococcus aureus were identified according with biochemical profile established with miniAPI test galeries.

Key words: telemea cheese, white brined cheeses, cheese microbiology, cheese safety

White brined cheeses are ones of the most popular varieties of cheese manufactured in the Balkans. They are manufactured from bovine, ovine, caprine or from a mixture of these. Telemea cheese is a variety of Romanian white brined cheese manufactured traditionally from cow's milk, ewe's milk or from a mixture of these, similar in some aspects with Teleme cheese manufactured in Greece (4,12).

For many years traditionally manufactured cheeses were considered safe products. The relative recently problems experienced by dairy industry, generated by the presence of pathogens in cheese, indicate the need of implementation of quality and safety management systems in this sector (5).

The aim of this study is to evaluate the microbiological quality of Telemea cheese artisanaly manufactured in farm house facilities and to identify the potential hazardous microorganisms associated with this kind of cheese variety.

Materials and Methods

The study was performed between October 2006 and April 2007 on 115 Telemea cheese samples (65 samples cheese made from cow's milk, 22 samples cheese made from ewe,s milk and 22 samples made from a mixture of cow's milk and ewe's milk). The Telemea cheese examined in this study didn't suffer maturation prior brining.

After purchase, the samples were placed directly into cool boxes and transported to the laboratory and were processed within 24 to 48 h.

The *Enterobacteriaceae, Staphylococci, Bacillus cereus, Salmonella spp. and Listeria spp.* were determined.

The bacteriological parameters were determined according to criteria specified by following methods:

- SR ISO 5541-2/1994

- SR ISO 7932/2005
- SR ISO 7251/1997
- SR ISO 6888/2002
- SR ISO 6579/2003
- SR ISO 7402/1996
- SR ISO 11290/2005

Microbiological investigation of sample was done in accordance with criteria specified SR ISO 6887-1/1999 – Food and feed microbiology, SR ISO 7218/1996 - Food and feed microbiology and SR ISO 8261/1996 – Milk and milk products.

The bacteria isolated were further biochemical characterized by API test in order to find out the milk contaminant species. The *API ID32 E*, *API 50CHB, API ID32 Staph*, and *API Listeria* were used for characterization of *Enterobacteriaceae*, *Bacillus spp., Staphylococci* and *Listeria spp.*.

Results and discussions

In table 1 are presented the results of the examination of Telemea cheese samples according to investigated parameters and the particularities of cheese samples.

From the 115 samples of cheese examined in this study, 14 (12.17%) were organoleptical improper being excluded from microbiological examination. The main defects group identified in examined cheese samples was consistency defects and was originated in misleading of brining process.

The microbiological examinations were done on 101 samples and reveal a diverse microflora. The enterobacteriaceae were isolated from 17 cheese samples, with greater prevalence in samples of cheese made from ewe's milk (Table 1).

As it resulted from biochemical characterization of isolated enterobacteriaceae, 9 different species were identified.

One strain of *Salmonella arizonae*, two strains of *Shigella sonnei*, three strains of *E. coli* and two species of *E. sakazakii* have been isolated from the investigated Telemea cheese samples and could be considered hazardous for consumers.

There are recognized at the moment five groups of virulent *E. coli*, some being foodborne pathogens. *E. coli O157 H7* was cited as a bacteria capable to survive in brine and in brined cheese (1,3,8).

Table 1 - The microbiological quality of white-brined Telemea cheese manufactured in farm-house facilities

Cheese samples		Organoleptic evaluation		Microbiological parameter									
				Enterobacteriaceae		Staph.spp/ml		B.cereus/ml		Salmonella spp. /25ml		Listeria spp /25ml	
		C	U	P	N	P	N	P	N	P	N	P	N
Total (115)	No	101	14	84	17	77	24	83	18	100	1	83	18
	%	87.83	12.17	83.17	16.83	76.24	23.76	82.18	17.82	99.01	0.99	82.18	17.82
CT* 65	No	60	5	50	10	48	12	46	14	60	-	54	6
	%	92.31	7.69	83.33	16.67	80.00	20.00	76.67	23.33	100	-	90.00	10.00
ET** (22)	No	19	3	15	4	13	6	17	2	18	1	12	7
	%	86.36	13.64	78.95	21.05	68.42	31.58	89.47	10.53	94.74	5.26	63.16	36.84
MT*** (28)	No	22	6	19	3	16	6	20	2	22	-	17	5
	%	78.57	21.43	86.36	13.64	72.73	27.27	90.91	9.09	100	-	77.27	22.73

C – conform P - positive
U – unconform N - negative
** Telemea cheese made from cow's milk*
*** Telemea cheese made from ewe's milk*
**** Telemea cheese made from mixture milk*

There are recognized at the moment five groups of virulent *E. coli*, some being foodborne pathogens. *E. coli O157 H7* was cited as a bacteria capable to survive in brine and in brined cheese (1, 3, 8).

E sakazakii, regarded nowadays as a potential pathogen, especially for children, was isolate from two samples of cheese made from cow's milk. The bacterium was isolated till now relatively frequent from dried infant foods, milk powders, cheese products and various dry food ingredients (7,9,10).

Table 2 - Species of bacteria isolated from Telemea cheese samples after biochemical characterization by specific miniAPI test galeries

Species		Telemea cheese variety		
		CT*	ET**	MT***
Enterobacteriaceae (API ID32 E)				
1.	*Enterobacter sakazakii*	2	-	-
2.	*Enterobacter intermedius*	1	-	1
3.	*Enterobacter aerogenes*	1	-	-
4.	*Escherichia coli*	3	1	-
5.	*Klebsiella oxytoca*	-	-	1
6.	*Citrobacter freudii*	1	1	1
7.	*Proteus vulgaris*	-	1	-
8.	*Shigella sonnei*	2	-	-
9.	*Salmonella arizonae*	-	1	-
Staphylococcus spp. (API ID32 Staph)				
1.	*Staphylococcus sciuri*	4	1	-
2.	*Staphylococcus aureus*	1	3	1
3.	*Staphylococcus epidermidis*	4	1	4
4.	*Staphylococcus chromogenes*	3	1	1

Bacillus spp. (API 50 CHB)				
1.	*Bacillus mycoides*	8	2	2
2.	*Bacillus subtilis*	1	-	-
3.	*Bacillus circulans*	2	-	-
4.	*Bacillus coagulans*	1	-	-
5.	*Bacillus lichemiformis*	-	-	-
6.	*Bacillus thuringiensis*	2	-	-
Listeria spp. (API Listeria)				
1.	*Listeria grayi*	-	1	1
2.	*Listeria welshemeri*	-	1	1
3.	*Listeria innocua*	4	2	3
4.	*Listeria seeligeri*	2	1	-
5.	*Listeria ivanovii*	-	2	-

**** Telemea cheese made from cow's milk***
***** Telemea cheese made from ewe's milk***
****** Telemea cheese made from mixture milk***

The *staphylococci* were isolated from 24 of 101 samples, being more frequently isolated from ewe's milk cheese samples. Based on the biochemical profile, five strains of *S. aures* have been identified. *Staphylococcus aures* seems to resist in brined cheese but the salt together with the low pH acting as an inhibitor against the pathogen. The maturation process also contributes to inhibition of bacteria due to lactobacilli competition. The increasing of salt concentration in brine will enhance the survival of *S. aureus* due to inhibition of lactobacilli (2,5,6).

B. cereus were isolated from 18 of 101 samples but after biochemical characterization by API 50CHB tests none of the isolates were confirmed. Based on actual knowledge none of the six isolated species of *Bacillus* could be considered pathogen.

As Listeria monocytogenes are quite halotolerant, there is an increase concern about the survival of and growth of such contaminant in the brined cheese since it has been isolated by other researcher (5,11). In our study *Listeria spp.* were isolated from 18 of the 101 samples. After biochemical characterization five species of *Listeria* were identified. *Listeria monocytogenes* the only species of the genus undoubtedly pathogenic were not identified.

Except *Staphylococci*, *Listeria spp.* and spores of *Bacillus spp.,* microorganisms that were capable to survive at high salt concentration, presence of the other isolated microorganisms are mainly the consequence misleading of manufacture process and of contamination of cheese during the manipulation.

Conclusion

1. There were a large diversity among the contaminant microorganisms isolated from investigated Telemea white brined cheese samples;
2. From 115 samples of Telemea cheese examined, 92 (80%) were found to be unconfirmed with organoleptical and microbiological specifications;
3. The samples of Telemea cheese made from ewe's milk show higher degree of contamination compared with the other two types of cheese.
4. From the 24 species of isolated bacteria, as resulted after biochemical characterization, 5 could be regard as potentially hazardous for the consumers. Presence in Telemea brined cheese of these microorganisms rise concerns about the safety of these traditional dairy products and indicate the necessity of developing specific requirements for quality of raw milk, sanitation and handling of the equipment and ustensils, water supply and plant process control.
5. Even if the cheese making is a very complex process, the application of good manufacturing practice and implementation the HACCP requirements will ensure the control of the manufacture process and consequently the safety of the end product

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Comparative study regarding the applicability of the IDEXX -quanti-tray/2000 methodology in estimating contamination the raw milk with coliform bacteria and Escherichia coli

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The investigations concerning the bacteriological parameter in 85 samples of raw milk, from the same small farm of Iasi County. The investigation of the presumptive coliforms was examined with Quanty Tray/2000 of IDEXX Technology and compared to the results obtained with the classical standard methods.

Key words: diluted or not raw milk samples, coliforms parameter, standard methods, IDEXX Technology

MATERIAL AND METHOD

The study was conducted June 2006 though March 2007 on a count of 85 crude milk samples, drawn in the milk sampling centers of Iasi County.

The samples were analyzed in order to test the applicability of the Quanty Tray rapid method, upon estimating the *coliform bacteria* load and the *Escherichia coli* count for the milk samples, comparing the results with those obtained, simultaneously, by standard methods.(2,3,4)

The IDEXX Technology, the Quanty-Tray/2000 method were conceived to determine the coliforms in the water samples by the use of Colilert 18 rapid kits.

The Colilert18 kit detects simultaneously the total coliforms and the *Escherichia coli*. The total coliforms metabolize the nutritional indicator – ONPG by means of the β-galactosidase enzyme, phenomenon indicated by the apparition of the yellow color and the *Escherichia coli* metabolizes the MUG nutritional indicator by means of the β-glucuronidase, phenomenon indicated by the fluorescence detection with UV lamps.(8).(Figure 1)

Fig.1.The researches of coliform's and E.coli strains from raw milk with Colilert 18 fast kit

The method's forming stream was represented by the multiple tubes method, the same based on a new DST technology - Double Sublayer Technology, the reactive agent being obtained by the combination of two nutrients. The method affords the quantification without dilution of a count of 2000 UFC/ml of water.

For the quantification of the *coliform bacteria* and of the *Escheichia coli* count in the milk, the milk as such or the dilutions of 10^{-1}, 10^{-2} respectively were used, in a quantity of 100 ml, necessary to the filling up the pour plate.(5,6)

The methods standardized for the estimation of the *coliforms* and of the *E. coli* in the milk which the results obtained by the Colilert-18 method were reported to, in view to their being validated, were:

The standardized techniques used the methods described in:

- SR ISO 5541/1/94, Establishing the colifom bacteria count. Colony count method at 30^0C,
- SR ISO 5541/2/94, Establishing coliform bacteria count. The most likely number method, at 30^0C,
- SR ISO 7251/97, General directives for establishing the *Escherichia coli* presumptive count. The most likely number technique.
- The working protocol of the IDEXX Quanti Tray/2000 Technology, the most likely number technique, quantitative determination – adjusted on milk samples as such or diluted.(1,2,3,4,7,8)

RESULTS AND DISCUSSION

The results achieved are synthetically shown in Tables 1 and 2.

Tabelul nr1

The coliforms contamination of raw milk samples, estimated SO 5541/1-2/1994, SR ISO 7251/1997 and Colilert 18 results

Interval de raportare *Bacterii coliforme* /ml	**Nr. probe**	**Methods of analysis used**						
		SR ISO 5541-1/94		**Nr. probe**	**SR ISO 5541-2/94**		**Nr. probe**	**Colilert 18**
		N	**C_1**		**MPN_1**	**C_2**		**MPN -G**
10- 100	**10**	**58-86**	**26-74**	**17**	**61-93**	**35 - 75**	**19**	**19,2 – 78,2**
100-1000	**28**	**116-986**	**82-964**	**39**	**110-920**	**110 - 750**	**37**	**101,3 – 697**
>1000	**47**	**1140-1854**	**1100-1844**	**29**	**>1100**	**>1100**	**29**	**1184- >2005**

Legendă:

N – coliform count on VRBL

C_1- coliform count confirmed on BBLV

MPN_1 – coliform count expressed by the multiple tubes method

C_2 – Coliform count confirmed on Levine

MPN-G – positive coliform count– yellow color

Tabelul nr.2

The milk samples contamination with Escherichia coli, estimated with, SR ISO 7251/1997 and Colilert 18 results

Interval de raportare *Escherichia coli* /ml	Nr. probe	Methods of analysis used			
		SR ISO 7251/97		Nr. probe	Colilert 18
		MPN_2	C_3		MPN - F
10- 100	17	30 - 92	30-75	19	12,4 -69,7
100-1000	39	110-930	110-740	37	101,3 -782
>1000	29	>1100	>1100	29	1091 -1652

Legendă:
MPN_2 – the presumptive Escherichia coli count
C_3 – confirmed Escherichia coli count confirmed by Kovacs reactive agent
$MPN\text{-}G_1$ – Presumtive Escherichia coli coliform bacteria counts
MPN-F- fluorescent positive Escherichia coli count

The analysis of table 1 reveals the following:

- 1844 colonies on VRBL medium by the method provided for in SR ISO 5541-1/94; more than 1100 colonies by SR ISO 5541-2/94 and a count between 1184 and 2005 by 51-Well Quanti-Tray MPN Table have been confirmed as being *coliform bacteria.*
- the *coliform bacteria* count confirmed by the method provided for in SR ISO 5541-2/94 is closed to the *coliform bacteria* count confirmed by the Colilert 18 kit:
- a higher count of the total positive samples investigated by the IDEXX technology confirm the presence of *coliform bacteria*, in small reporting intervals;
- 51-Well Quanti-Tray MPN Table allows for the estimation of a lower *coliform bacteria* load in the milk samples;
- the differences between the results achieved are higher in the presence of a UFC *coliform bacteria* higher count, the Colilert 18 affording the estimation of a maximum count of 200,5, namely 51 positive counts from the sample as such, undiluted, while the maximum value which can be expressed with the MPN Table by the standard methods is of 110 colonies from the sample as such.

From the analysis of table 2 it results that:

- More than 1100 colonies have been confirmed by SR ISO 7251/97 as being *Escherichia coli* while 51-Well Quanti-Tray MPN Table made possible the confirmation of 1652 *Escherichia coli* colonies, a maximum of 49 plate counts were yellow and fluorescent.
- *The Escherichia coli count* confirmed by the method provided for in SR ISO 5541-2/94 is close to the count confirmed by the Colilert 18 kit.
- 51-Well Quanti-Tray MPN Table affords the estimation of the milk samples' *Escherichia coli* smaller load.

CONCLUSIONS

1. The *coliform bacteria* count confirmed by the method provided for in SR ISO 5541-1/94 is lower than of those confirmed by SR ISO 5541-2/94 and Colilert 18;
2. The most likely *E. coli* count estimated by the method provided for in SR ISO 7251/97 is lower than that confirmed by the use of Colilert 18 kit;
3. The increase sensitivity of the Colilert 18 kit in identifying the total and fecal coliforms, comparatively with the standard methods is due to the possibility of finding the positive ONPG coliforms which do not fully ferment the lactose and finding the negative lactose *E. coli* strains.
4. The falsely negative results when using the standard methods can be caused by a compound incertitude given by: the incertitude of the incubators used, the higher number of the determination times, by the stress exerted upon the microorganisms and upon the media quality, which gives the possibility that the analyzed samples be declared negative when they are actually falsely negative.
5. The Colilert 18 Quanti-Tray method allows for the results' estimation after an incubation of only 18 hours at 35-38^{0}C while the standard methods use a time interval of approximately 3 times higher than in the method provided for in SR ISO 5541-1/94, 5 times higher in the case of SR ISO 5541-2/94 and 8 times higher in the case of SR ISO 7251/97.
6. The Collilert18 Quanti-Tray method represents a specific, rapid and sensitive method which can be applied as well in the detection of coliform bacteria and of the Escherichia coli species in the milk samples.

The use of the Colilert-18 – Quanti-Tray qualitative method would afford the saving of two running times (the filling up of the pour plate and their sealing) so that the estimation of the milk unconformity, in parallel with the NCS/ml, NTG/ml rapid determinations or of certain physicochemical parameters, would become even faster and would give the possibility of the its processing as such, in accordance with the legal provisions.

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"In vitro" assessment of antibacterial effect of silver nanoparticles

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The antibacterial efficiency of nanometer sized silver particles synthesized in water and in polyurethane urea polymer against P. aeruginosa was tested. The antibacterial investigations were performed both in liquid media and on solid media. The silver nanoparticles were found to exhibit a limited antibacterial effect at tested concentration. The antibacterial effect was visible in case of aqueous solution at 90 ppm in the first 24 hours of testing. Doped polyurethane demonstrate a weak antimicrobial activity against tested P. aeruginosa strain

Key words: silver nanoparticles, antimicrobial effect, Pseudomonas aeruginosa

Introduction

Silver has been known for its antibacterial activity since the ancient times (3). Silver ions are highly toxic to bacteria but have a low toxicity to animal cells (1,2,8). While other antibacterial medicines have failed due to emergence of antibiotic resistance, till now the research data didn't reveal unequivocally the resistance to silver (6). Products such as silver nitrate and silver sulfadiazine, have been used to prevent bacteria growth.

Metal silver was added to polymers used in fabrication of medical devices such as catheters for antibacterial functionality (2).

Due to its high cost the application of metal silver as antimicrobial was reduced. Recently obtained nanopowders of silver seem to solve this problem. However, the antimicrobial effects of silver nanoparticles (Ag-NPs) was not fully investigated till now but it can be expected that due to high specific area and high fraction of surface atoms of silver nanoparticles will demonstrate a higher antimicrobial activity compared with bulk silver metal (2,5).

The purpose of this study was to evaluate the antibacterial activity of silver nanoparticles against *Pseudomonas aeruginosa*.

Materials and Methods

The antibacterian properties of silver nanoparticles was tested on *P. aeruginosa*, strain ATCC 27583 both on solid and in liquid media.

The silver nanoparticles used in this study was synthesized both in aqueous solution in presence of sodium citrate as reducing agent and surfactant additive substances to prevent the agglomeration of particles and in polyurethane- urea matrix. The size of silver particles in both situations was preponderantly lower than 10 nm as it can see from the analysis of UV spectra (Fig. 1 and 2).

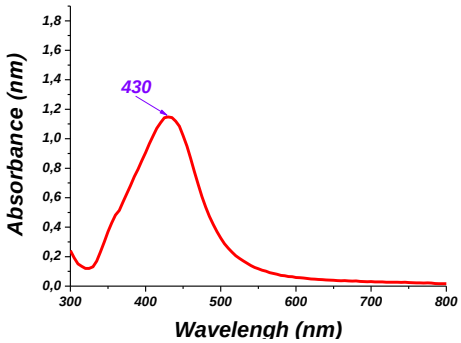

Fig. 1. - UV-VIS spectra of silver nanoparticle synthesized in water. Diameter of particles between 10 – 60 nm

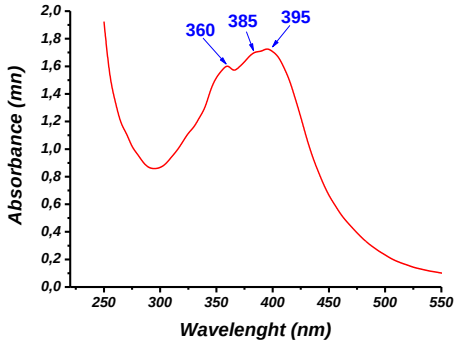

Fig. 2. - UV VIS spectra of silver nanoparticles synthesized in polyurethane matrix. The 360, 385 and 395 nm absorbent peaks indicate the size of silver particles, all of them under 10 nm diameter.

The antibacterial activity was tested on solid media and in liquid media.

a) On solid media a disk diffusion method was used to assay the bactericidal activity against *P. aeruginosa*. Disks from absorbent paper (5 mm diameter) were impregnated just before testing with 10 µl of freshly prepared solution. Three decimal dilutions of the solution were tested (90 ppm, 9 ppm and 0.9 ppm). Disks with the same diameter were prepared from doped polyurethane membrane.

The bacterium inocula were prepared from a culture of *P. aeruginosa* ATCC 27583 incubated overnight at 37°C in nutrient broth by diluting with 0.9%NaCl to a 0.5 Mc Farland standard. The inocula were applied by inundation to Petri plates with Mueller Hinton agar and the prepared disks were placed on the surface of the inoculated media.

After the incubation of the plates at 37°C for 24 hours the zone of bacterial inhibition were measured. The assays were performed in triplicate and the average was calculated.

b) For testing of antibacterial effect of silver nanoparticles in liquid media 8 tubes with 9 ml of Mueler Hinton broth were inoculated with 1 ml of an overnight culture of *P. aeruginosa* prepared as above. Two of the inoculated tubes were used as baseline for measuring the growth of bacteria.

In each of the other 6 tubes (2 for each tested concentration) 1 ml of silver nanoparticles solution was added. All the tubes were incubated at 37°C for 72 hours and the bacterial growth was monitored macroscopically and by measuring of optical density at 24 hours, 48 hours and 72 hours from the beginning of the incubation.

For testing the polymer membranes doped with silver nanoparticles, three conic flasks of 150 ml, each with 45 ml Mueler Hinton broth, were inoculated with 5 ml of an overnight culture of *P. aeruginosa* prepared as above. One of the flasks was used as baseline and in each of the other two, two fragments (2 cm^2) of polyurethane membrane doped with silver nanoparticles were immersed. The flasks were incubated at 37°C for 72 hours. The microbial growth was monitored as above.

After 48 hours and 72 hours of incubation the fragments were extracted, washed in distilled water, sterilized in UV and examined with atomic force microscope (AFM) (scaning frequency 20 Hz, lateral resolution 10 nm, vertical resolution 0,1 nm) in order to identify the bacterial film on the surface of the membrane.

Results and discussions

On the solid media the Ag-NPs aqueous solution demonstrate a variable antimicrobial effect, the intensity of the effect was proportionally with the concentration of solution. The average diameter of the bacterial growth inhibition zone was 86.6 mm for 90 ppm solution and 64.9 mm for 9 ppm solution. In case of 0.9 ppm solution the inhibition zone was macroscopically imperceptible denoting the lack of antibacterial effect at this concentration.

The absence of antibacterial activity was observed in case of polyurethane membrane doped with Ag NPs too *(Fig. 3 and 4)*.

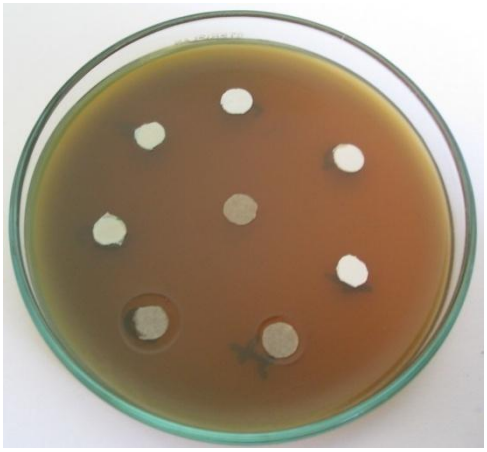

Fig. 3a - The zone of growth inhibition around the disks impregnated with Ag-NPs (1 - solution 90; ppm, 2 –solution 9 ppm; 3 – solution 0.9 ppm; 4 – doped polyurethane)

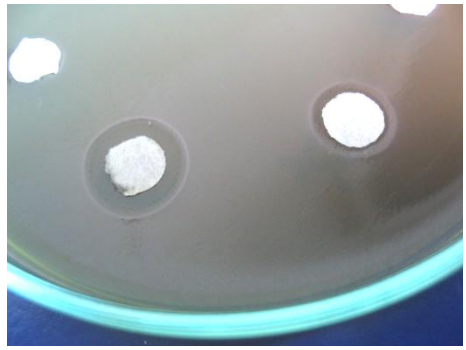

Fig. 3b - The zone of growth inhibition (detail)

In liquid media the 90 ppm aqueous solution of Ag-NPs inhibited the growth of bacteria in the first 24 hours but at 48 and respectively 72 hours the macroscopic signs of bacterial growth were obvious. At 9 and 0.9 ppm concentrations the antimicrobial activity was negligible, signs of bacterial growth being visible even after 24 hour of incubation.

The polyurethane doped membrane didn't exhibit any antimicrobial activity despite of the high quantity of the Ag-NPs incorporated.

The optical density measurements of the bacterial growth confirm the results obtained by macroscopically examination. OD values obtained at 72 hours were grossly influenced by the modification of the media induced by bacterial growth exceeding the limits of an acceptable interpretation in case of the control tubes and of low concentrations of Ag NPs tubes.

Concentration of 90 ppm inhibits the bacterial growth, the average value of OD determined at 24 hours being near similar with the initial average value. Between 24 and 48 hours the OD of the media grew up indicate the falling down of antimicrobial activity. The other two tested concentration showed no obvious antibacterial effect (*Fig. 5*).

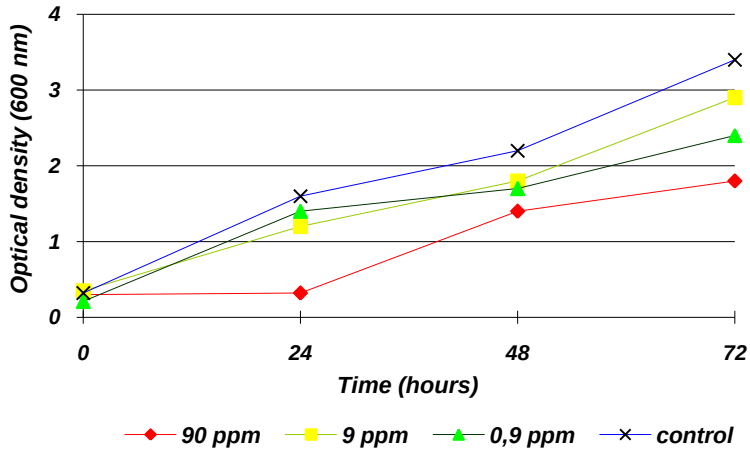

Fig. 4 Growth of P. aeruginosa ATCC 27583 on liquid media in presence of silver nanoparticles

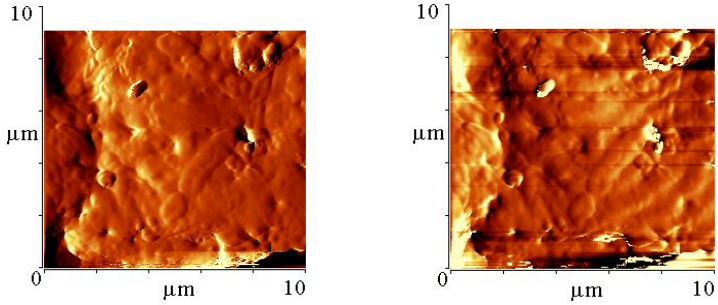

Fig. 5a and 5b – Aspects of atomic force microscopy of doped polymer surface after microbial exposure

Atomic force microscopy examination of doped polyurethane membrane exposed to *P. aeruginosa* culture revealed at the surface of the polymer a thin layer of bacteria proving the weak antimicrobial activity of Ag-NPs captured in polymer (*Fig. 5 a and 5b*).

The weak antibacterial effect of Ag-NPs founded in condition of our study could be explained in case of doped polyurethane by the very slow rate of diffusion of Ag-NPs from the polyurethane matrix, phenomenon observed by other authors too (4). Soaking of the polymer prior to testing antimicrobial activity could reduce the time necessary for reaching the appropriate concentration for antimicrobial activity. A high permeable coating material on the surface of Ag-NPs particles would accelerate the release of Ag-NPs and consequently will improve the antibacterial activity.

Another cause of the results obtained could be the low concentration o Ag-NPs used in this study, other authors claiming that for intense and rapid antimicrobial activity higher concentration of Ag-NPs are needed (4, 7).

The initial antibacterial activity observed in case of aqueous solution with 90 ppm Ag-NPs could be generated by parasite Ag ions located at the surface of Ag-NPs particles appeared as a consequence of synthesizing process. Also the composition of the microbiological media used in this study could interfere with Ag-NPs and inactivate them after a short period of time.

The detailed mechanism of action of Ag-NPs is complex and still incompletely known, further investigation made in different condition (media, microorganisms, concentrations and coating materials) are necessary to fully understand the antimicrobial activity of Ag-NPs.

Acknowledgements

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Encouraging factors in starting infections with portaje embryos of nursery pond fish

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The increasing mortality from a fish farm from Iaşi imposed the carring out of laboratory exams to establish a diagnosis and find out the causes of their death. The microbiological and morphopathological exams of fish and the microbiological and chemical exams of water has been carried out. Based on the results obtained it has been diagnosed aeromonosis having as the main cause thermic stress.

Key words: aeromonas, fish disease, environment factors

Although viral pathology is the main limitative factor of intensive productions, the bacterian disease of fish are more frequent. High density of fish in intensive systems of exploitation determines the increase of the concentration on the volume unity of the embryos eliminated in the aquatic medium and implicit, of the chance to produce a chain infection with pathogene and opportunist micro-organisms. The starting factors of infections with portaje embrios, conditioned by pathogens, are thermic stress, manipulating stress, over crowded places, water eutro-physation, etc.

In the case in which we suspect an infectious disease, the certain diagnosys is based on showing, isolation, and identification of etiologic agent, as the clinical and morpho-pathological expression are often non-characteristic.

The present paper is the presentation of a single case, in which stems of *Aeromonas hydrophila* and *Aeromonas caviae* were involved..

Material and method

There have been made anamnetic investigations and morpho-pathological and bacteriological examinations on 5 samples of phytophagous and 5 samples of ordinary carp of 2 summers, with the aim to identify the determining and favourable factors that cause fish disease and the looses by mortality.

The dead phytophagous was taken from a fish breeding nursery of Iasi county, (F1) from the cages that were hold on the shore for fishing and daily delivery.

The carps were taken from a exploitation in „policulture system" (carp, crucian carp, *Polyodon spatulla*) –(unity F2), being recently bought with the aim to populate a fishpond with a surface of 4,5 ha.

Morpho-pathological and bacteriological examinations made on fish were according to the protocol of work specific to each type of investigation.

At the same time, samples of water taken from the two unities were investigated from a microbiological and physico-chemical point of view.

Results and debates

Anamnetic investigations showed the following aspects:

- the increase of morbidity and mortality at fish in may of the year 2006;
- the water temperature had values between 12 ^{o}C in F1 nursery and 15^{o} C in F2 nursery; - inferior values to the optimal temperature of breeding the two affected species (22 – 25^{o} C);
- the carps were subjected to the manipulation stress, transport and adapting to the new conditions, and phytophagous was subjected to the capture stress and the one due to the over crowded population in cages;
- the loose by mortality were reduced step by step, at the same time with the warming of water.

Clinical examination

In both nurseries, the main simptoms of the sick fish were the hipodinamy state and cutaneous injures.

Anathomo-pathological examination

The exterior examination of the three samples of phytophagous showed cutaneous ecchymosis, lepidortose areas and the lack of scales. (figure 1), hyper-secretion of mucous gills and, at one single sample, the fragmentation of the caudal swimmer.

The internal examination revealed muscular haemorrhages of different dimenssions, catharal - haemorrhage inflamation of viscera and aero-cystitis (figure 2 and 3).

Figure 1: Phytophagous with haemorrhages at the basis of pectoral swimmers and nude cutaneous areas

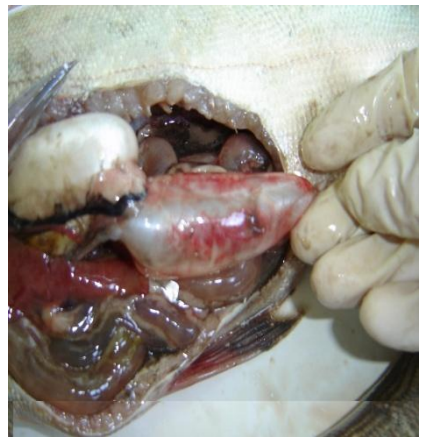


Figure 2 and 3. Phytophagous: internal injures by haemorrhage and inflammatory type

Carps investigated from a clinical and morpho-pathological point of view presented: unilateral exophthalmia (figure 4) ecchymosis in the stomach area, at the basis of the swimmers and peri-rectal, lepi-dortose and the loose of the scales on large areas, light and deep cutanoeus ulcers (figure 5).

Internal examination revealed the presence of aescitic liquid, sometimes sanguine (figure 6 A and B), the adherence of the viscera to the walls of the abdominal cavity, catharal enterite, hypertrophy liver, friable and with small congestive and haemorrhage hotbeds

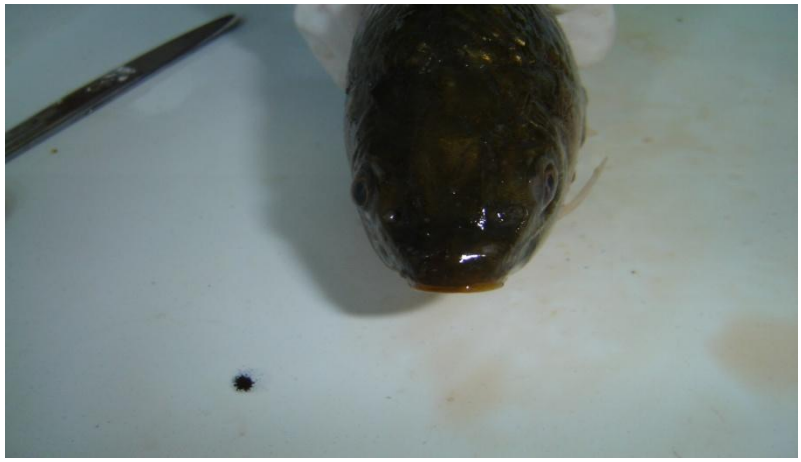
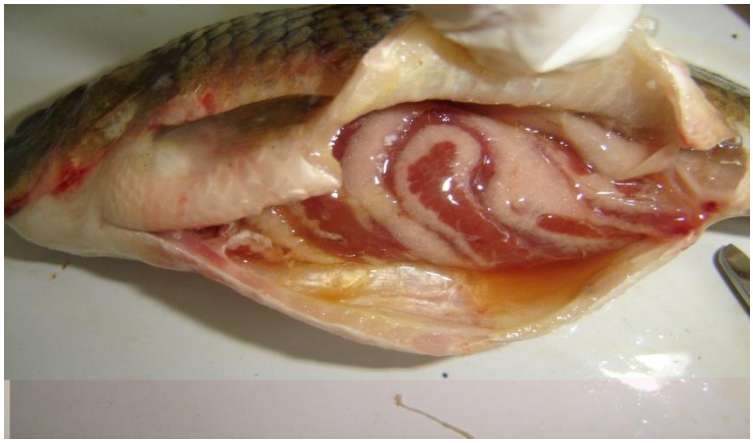
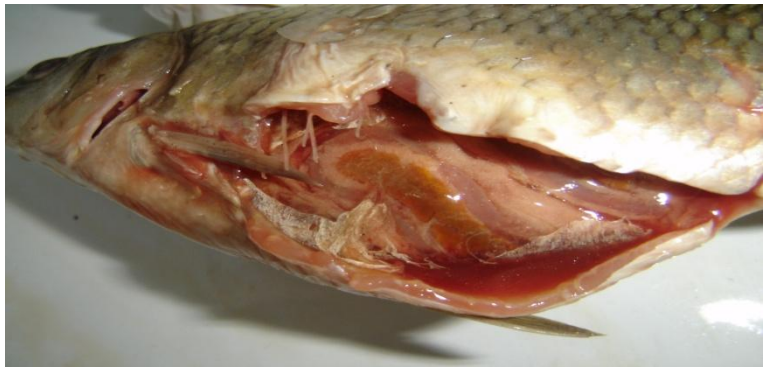


Fig.4 . Carp with exophthalmia

Figure. 5. Carp with cutaneous ulcer and nude areas



A



B

Figure 6. Carps with aescite : A – aescitic serous liquid; B - aescitic sanguine liquid

Bacteriological examination

Direct bacterioscopic examination of cutaneous ulcers and of the signs from liver and kidneys in Gram coloured froties revealed a poli-morphe negative micro-flora Gram with bacilus and coco-bacilus bacterias in a large number (figure 7).

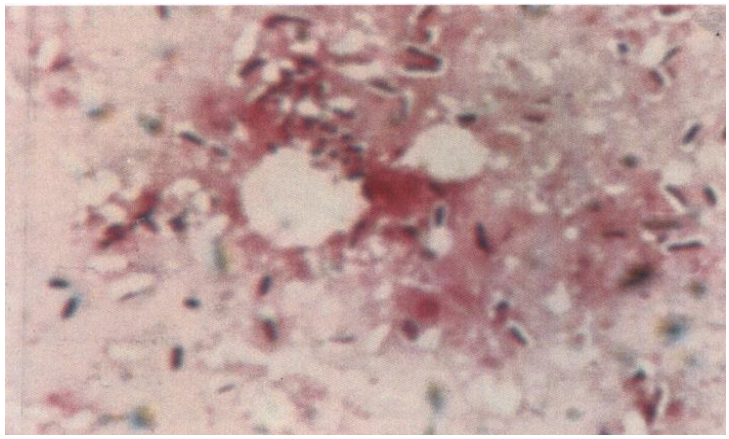


Figure. 7 Froties from cutaneous ulcer at carp

From cutanoeus ulcers mixt cultures of bacterious stems were obtained and they were framed into *Aeromonas, Lisobacter, Cytophaga, Flavobacterium types* – types that form ordinary resident microbiota of fish.

The inseminations made from heart and kidney, on tomato juice and sangiune-gealose had as a result, for all the examined fish, isolation in pure cultures of some hemolitic stems of *Aeromonas hydrophila* (β-hemolitic) and *Aeromonas caviae (α.- hemolitic).*

Identification of isolated bacterious stems was made based on cultural, morphological and biochemical characteristics.

Testing sensibility of stems of *A. Hydrophila* interacting with antibiotics and chemio-terapeutics drugs by anti-biograma – difusimetrical method indicated a sensibility of 100% for spectinomicin, furasolidon and gentamicine. Interacting with other tested anti-bacterian drugs (amoxyciline, polimixine, cloramfenicol, tetracicline, so on), the behaviour was changed from one stem to another, showing moderate sensibility or resistance.

Micro-biological and physio-chemical parameters of samples of water situated within the limits admitted by the present STAS norms.

Conclusions

1. Appearance of disease in post-hibernal period, at water temperatures inferior to the thermic confort of fish, the stopping of loose at the same time with the increase of the environmental temperature and the injured analysed situation lead to the diagnosis of spring viremia.
2. Bacteriological diagnosis is of septicemia with *A. Hydrophila* and *Aeromonas caviae (*aeromonose, the disease of mobile aeromonades*).*
3. It is possible that the two diseases to evolve together, aeromonose evolving seldom as a complication of spring viremia.
4. Factors that favourised the starting and evolution of diagnosticated infections were the low temperature of water which reduces until disappearance the activity of immune system, in association with manipulation stress and the one of transport.

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Mysterious resistance of fungal biofilms

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Pathogenic fungi of the genus Candida can cause both superficial and systemic diseases, and are now recognized as major agents of hospital - acquired infections. Candida infections involve the formation of biofilms on catheters or prosthetic heart valves. Biofilms of Candida albicans consist of matrix enclosed microcolonies of yeasts and hyphae, arranged in a superposed structure. The biofilms are resistant to a range of antifungal agents currently in clinical use, including amphotericin B and fluconazole, and it appears to be a multiple resistance mechanism. The lack of reactivity of these organized communities to main antibiotics, antifungals or other existing decontaminants is already proved. The mechanisms by which Candida biofilms resist to the action of antifungal agents are not known. Possible resistance mechanisms include drug exclusion by the biofilm matrix and phenotypic changes resulting from nutrient limitation or a low growth rate. In a previous investigation, a perfused biofilm fermenter was used to generate C. albicans biofilms at different growth rates and the susceptibility of the biofilm cells to amphotericin B was compared with that of planktonic cells at the same rates in a chemostat. The results showed that biofilms were resistant to the drug at all growth rates tested whereas planktonic cells were resistant only at low growth rates. A subsequent study using a different model system demonstrated that glucose-limited and iron-limited biofilms grown at the same low rate were equally resistant to amphotericin B.

This paper describes the most important issues about the mechanisms of fungal biofilms resistance to antifungals and other antimicrobial substances.

Key Words: Candida, biofilm, antifungal resistance

Introduction

Biofilms are of considerable interest in the context of food and medicine hygiene.

In most natural environments, micro-organisms exist predominantly as biofilms than as planktonic or free-floating cells. Biofilms are structured microbial communities that are attached to a surface. Individual micro-organisms in biofilms are embedded within matrix of-often slimy-extracellular polymers, and characteristically display from that of planktonic cells. The aggregation of microbial cells in biofilms and the complex multicellular interactions that ensue dependend on the production of an extracellular matrix. Bacteria and fungi that build biofilms secrete extracellular polymeric substances (EPS) that form a highly hydrated slime in which cells are embedded and held in dense agglomeration.

Microbes often construct and live within surface-associated multicellular communities known as biofilms. The precise structure, chemistry and physiology of the biofilm, all vary with the nature of its resident microbes and local environment.

However, an important characteristic among biofilms is their structural integrity critically depends upon an extracellular matrix produced by their constituent cells. Extracellular matrices might be as diverse as biofilms, and they contribute significantly to the organization of the

community (2). Biofilms offer their member cells several benefits - protection from environmental injuries and assaults is the most important. Given their ubiquity and importance in the microbial world, it is hardly surprising that biofilms have attracted the attention of the scientific community.

The first example of a biofilm to be recognized in medical systems was dental plaque on tooth surfaces, but recent estimates suggest that a substantial proportion of human infections involve biofilms (3).

Micro-organisms enclosed in biofilm matrix can also be detected in tissues taken from non device-related chronic infections such as cystic fibrosis (4). Bacterial biofilms and their role in disease have been studied intensively in recent papers and there is now a considerable amount of information available on their structure and properties, particularly those of Gram-negative bacteria (5, 6). Much less is known about fungal biofilms.

Organisms commonly known as fungi have been recognized for their utility in developing and testing many kind of hypotheses, as pure beauty and delight.

The fungi are heterotrophic micro-organisms and exhibit absorptive nutrition. In the filamentous fungi, growth is restricted to the apical region of the filament such that cells produced in previous generations which are now situated in a sub apical position do not contribute to growth. In some species, it is possible to observe some structures called "appresoria", a flat swelling that forms on the end of a vegetative fungal hypha and which adhere to the surface before penetrating it. It was found a key gene that allows fungi to stick to plastic surfaces and form thin coatings called biofilms. The gene, called FLO 11, is required for fungal biofilm formation.

Candida albicans is a human commensally and opportunistic pathogen. In addition to colonizing cutaneous and mucosal surfaces, the micro-organism can colonize and form biofilms on medical devices. Catheters (intravascular and urinary), dentures, gastrostomy tubes, voice prostheses, and prosthetic valves are among the device that may be colonized (7,8). A relatively small number of *Candida* species are pathogenic for immunocompetent humans. These organisms are capable of causing a variety of superficial and deep-seated mycoses that are distributed worldwide. All are opportunistic pathogens, liable to attack immunocompromised or debilitated hosts. The principal pathogen of the genus is the fungus responsible for thrush, *C.albicans*, which can grow lither as oval budding cells, as continuous septated hyphae or as pseudohyphae; all of these morphological forms are usually seen in infected tissue (9). *Candida* species are now recognized as major agents of hospital-acquired infections. Their emergence as important nosocomial pathogens is related to specific risk factors associated with modern medical procedures, notably the use of immunosuppressive and cytotoxic drugs, powerful antibiotics that suppress the normal bacterial flora, and implanted devices of various kinds.

Candida biofilms have been obtained in vitro in a number of model systems that include various abiotic supports such as catheter disks, acrylic strip, microtitre plate, cylindrical cellulose filter, perfused biofilm fermenter, glass, and with both static and continuous medium replenishment. The surface supporting biofilm formation and strain used for that might influence the extend of biofilm formation, the morphological features and the abundance of extracellular matrix material.

Various factors may affect the biofilm formation *in vitro*:

- *Candida* species and strain
- Nature of colonized surface
- Presence of conditioning film
- Liquid flow
- Bacteria

There was some correlation between the ability to form biofilms and pathogenicity when different *Candida* species were tested in the catheter disk system. Isolates of *Candida parapsilosis, Candida kefyr* and *Candida glabrata* all gave significantly less biofilm growth than the more pathogenic *Candida albicans* (10).

The fact that *Candida albicans* isolates consistently produce more biofilm *in vitro* than non-*Candida albicans* isolates has been confirmed recently (11). On the other hand, *Candida tropicalis* and *Candida parapsilosis* form biofilms quite readily when grown in medium containing 8% glucose (12).

From a yeast cells inoculum, *Candida albicans* will form a multilayered biofilm on catheter surfaces that consists of yeast cells, germ tubes, hyphae, and pseudohyphae and extracellular matrix material (13).

To colonize any surface, fungal cells must first adhere to biomaterial surfaces. The initial attachment of *Candida* cells to biomaterials is mediated by both non-specific factors (cell surface hydrophobicity and electrostatic forces) and by specific adhesions on the fungal surface recognizing ligands in the conditioning films, such as serum proteins (fibribogen and fibronectin).

Recent studies suggest that specific adherence events may also be mediated by cell surface proteins such as those encoded by members of the ALS family of adhesin- producing genes and EAP 1 (14, 15). *Candida* cells can also coaggregate and / or bind to bacteria (16, 17).

The initial focal attachment of individual cells to a substratum is closely followed by cell division, proliferation and biofilm development.

Mature form of *Candida* biofilms exhibit a three-dimensional structure and display extensive spatial heterogeneity (18, 19). This structural complexity is through to represent the optimal spatial arrangement to facilitate the influx of nutrients, the disposal of waste products, and establishment of most biological and artificial surfaces.

Fundamental to microbial biofilm formation is the cell-cell signaling. This strategy of cell - cell communication benefits well - being by controlling competition for nutrients and preventing unnecessary overpopulation. It has important implication in the infectious process, particularly for dissemination and for establishment of distal sites of infections.

The antifungal resistance of biofilms

Microbial biofilms are notoriously resistant to a variety of antimicrobial agents, including antibiotics, antiseptics and industrial biocides. Corresponding resistance of candida biofilms to antifungal agents was first demonstrated in 1995. In 2000, the mechanisms by which *Candida* biofilms to antifungal agents was not known. Possible resistance mechanisms include drug exclusion by the biofilm matrix and phenotypic changes resulting from nutrient limitation or a low growth rate (20, 21).

Various mechanisms have been proposed to explain the recalcitrance of biofilms to antimicrobial agents. Proeminent among these is the suggestion that the matrix of extracellular polymeric material, sometimes known as the glycocalyx, may exclude or limit the access of a drug to organisms deep in the biofilm. Possible drug exclusion by the matrix of bacterial biofilms seems to depend on a number of factors, including the nature of the antibiotic and the binding capacity of the matrix towards it (22).

Since the drug resistance of *Candida* biofilms cannot be attributed to a matrix barrier effect or to a low growth rate, it seems that mechanism by which drug resistance is acquired could be the synthesis of new proteins which occurs after attachment of the yeast to certain surfaces .

Biofilm resistance is a complex multifactorial phenomenon which still remains to be fully elucidated and understood. Different mechanisms may be responsible for the intrinsic resistance of *Candida* biofilms:

- the hight density of cells within the biofilm

- the effects of the biofilm matrix
- decreased growth rate and nutrient limitation
- the expression of resistance genes, particularly those encoding efflux pumps
- the presence of “persister” cells .

Several studies have examined the effects of growth rate and nutrient limitation in relation to drug resistance in *C.albicans* biofilms. Baillie and Douglas demonstrated that mature biofilms were resistant to amphotericin B at all growth rates tested and also at different levels of nutrient limitation. In addition, Chandra and colleagues reported that a progression of drug resistance was associated with an increase in the metabolic activity of the developing biofilm and not with a lower growth rate, which clearly indicates that drug resistance develops over time, coincident with biofilm maturation.

Subsequent studies have demonstrated drug resistance for *Candida* biofilms grown on cellulose, polystyrene, silicone elastomer, polyurethane and denture acrylic. It has been reported that some of the newer antifungal agents are active against *Candida* biofilms. The biofilms of *C.albicans* and *C. parapsilosis* were clearly resistant o new triazoles (voriconazole and ravuconazole), there appeared to be some antibiofilm activity with lipid formulations of amphotericin B and two echinocandins (caspofungin and micafungin). The efficacy of caspofungin against *C.albicans* biofilms in vitro has now been confirmed by other workers. Caspofungin is the first antifungal agent to be licensed that inhibits the synthesis of β 1,3-glucan, the major structural component of *Candida* cell walls; glucan synthesis might prove to be a particularly effective target for biofilms if, as seems possible from analytical data, the biofilm matrix also contains this polysaccharide. Under planktonic conditions, one of the main mechanisms through which azole resistance develops in *C. albicans* is the active efflux of these drugs mediated by ABC transporter and major facilitator proteins (23). The expression of genes encoding both types of efflux pumps was found to be up-regulated during the different phases of biofilm development, both in vitro and in vivo (24, 25). Interestingly, however, biofilms formed by mutant strains deleted for genes encoding several of efflux pumps retained their drug- resistant phenotype, although they were more susceptible during the early adherence phase of biofilm formation.

The sterol analyses have revealed that ergosterol levels are significantly decreased in the intermediate and mature phases of biofilm growth compared to those in the early phases of development. Since sterol metabolism is the primary cellular process affected by the most widely employed antifungal drugs, the diminished levels of ergosterol present in sessile *C.albicans* may reflect a physiological state more conducive to resistance in these cells.

All of these observations reinforce the notion that biofilm resistance is a multifactorial phenomenon. These intriguing recent findings could lead to important developments in the treatment of fungal infections of implants.

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Benzalkonium chloride – as preservative and antifungal activity enhancer in topical formulations used in cutaneous mycoses

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The aim of this paper is to perform an evaluation of preservative activity of benzalkonium chloride (BZK) in a topical formulation designed for treatment of dermatomycoses and to appreciate its improving effects on antifungal activity of the drug. We prepared four experimental batches of cream: three with benzalkonium chloride (in different concentrations e. g. 2‰, 1‰ and 0.5‰), and a free BZK variant as witness. After preparation, an evaluation of antimicrobial preservative effectiveness according to European Pharmacopoeia 5.0 was performed for each lot. Also, we tested in vivo these variants of cream in dogs affected of different ringworms in order to establish the healing period. The free BZK cream didn't correspond to Ph Eur 5.0 rules. From the three BZK variants, the formulation with 0.5‰ BZK could be used successfully in dermatomycoses therapy due to its improved properties.

Key Words: benzalkonium chloride, antifungal activity enhancer, cream, cutaneous mycoses

Introduction

Dermatomycoses like ringworms are commonly diseases both in humans and animals. The therapeutic armamentarium comprise pharmaceutical products which contain azoles, terbinafine, undecilenic acid (1, 2). Among azoles, clotrimazole, econazole, ketoconazole, and miconazole are the most prescribed in therapy. It is well known that all azoles are only fungistatic through the ergosterol synthesis impairement. In this study we proposed to perform an evaluation of preservative activity of benzalkonium chloride (BZK) in a topical formulation designed for treatment of dermatomycoses and to apreciate its improving effects on antifungal activity of the drug. BZK belongs to a group of chemicals known as quaternary ammonium compounds and it acts by disrupting the cell wall of diseases causing micro-organisms (3). It is capable in various concentrations to inactivate viruses and killing bacteria, fungi, algae and mildews (4). BZK consists of a mixture of alkyldimethylammonium chlorides, the alkyl chains having lengths of C_8-C_{18}. The *Ph. Eur* 5.0 requires that benzalkonium chloride should contain not less than 95% and not more than 104% of alkyldimethylammonium chlorides calculated as $C_{22}H_{40}NCl$. Regarding its toxicity, BZK is not dangerous and has a long history of safe use in human medicinal products.

Experimental methods

We prepared three experimental batches of cream using the following ingredients: clotrimazole, Lanette 16, Lanette 0, glycerin, vaseline, tween 80, benzalkonium chloride (in different concentrations e. g. 2‰, 1‰ and 0.5‰), purified water and a vacuumatic homogenizing device. In the same time, we prepared a free BZK variant as witness. All active substances and ingredients were of pharmaceutical use and high putity, each of them having a complete *Drug Master File (DMF)* according to *European Agency for the Evaluation of Medicinal Products (EMEA)*

recommandations. After preparation we perform the reccomended tests according *Ph Eur* 5.0: aspect, colour, odour, homogeneity, pH, particles diameter, active substances concentration, total aerobic count, and specific micro-organisms presence (5). Also, a test comprising the evaluation of antimicrobial prezervative effectiveness was performed for each lot using specific microbial strains from *American Type Culture Collection* (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*) – approximately 0.5 x 10^6 cfu/g of cream (5). In order to evaluate the improving therapeutic effect of clotrimazole, we tested *in vivo* these variants of cream in dogs affected of different ringworms and we have determined the healing period. The healing period was interpretated as the range of time (in days) passed between the begining of the treatment and clinical signs disappearance.

Results-discussions

For the BZK containing variants, the microbial counts performed on days 2, 7 (for bacteria) and on day 14 (for fungi) have demonstrated a decrease of viable micro-organisms with at least 2log, 3log and 2log respectively; no increase of total viable micro-organisms was noted in day 28 for all these variants of cream (Table 1).

Table 1

Logaritmic reduction of micro-organisms depending of BZK concentration

Variant	Bacteria			Fungi	
	Day 2	Day 7	Day 28	Day 14	Day 28
2‰ BZK	3log	4log	No increase	3log	No increase
1‰ BZK	3log	4log	No increase	3log	No increase
0.5‰ BZK	2log	3log	No increase	2log	No increase

The free BZK variant didn't exhibit a decrease of viable micro-organisms according to Ph Eur 5.0 (Table 2), because lack of preservatives. Although, the low reduction in fungi level after 28 days may be done to the clotrimazole which suppress the development of these micro-organisms.

Table 2

Logaritmic reduction of micro-organisms in the free BZK variant

Variant	Bacteria			Fungi	
	Day 2	Day 7	Day 28	Day 14	Day 28
BZK free cream	No reduction	Increase	Increase	No reduction	1log

We didn't observe significant differences of the mean of healing period for the three BZK variants of cream ($p >> 0.05$), but we could note a significant difference between these and the free BZK variant ($p < 0.05$) (Table 3). The reduced healing period is dued to the interraction between clotrimazole and the BZK against dermathopyte cells, probably through the membrane impairment.

Table 3

Results of ringworm treatment -The range of healing period

Variant	Range of healing period (days)	Mean (days)
2‰ BZK	6-10	8.3
1‰ BZK	6-12	9.1
0.5‰ BZK	7-13	9.7
BZK free cream	9-15	12.5

Conclusions

The *in vivo* synergistic effect between clotrimazole and BZK has been demonstrated in therapy of ringworms.

The concentration in BZK of 0.5‰ is an appropriate level which assure a good preservation of the cream.

This formula may be used in pharmaceutical industry as an improved variant of topical product designed for dermatomycoses control both in humans and animals.

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Researches concerning the residues of chlorinated pesticides to the raw milk croped from diverse routes in Constanta county

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Milk, as a food product, contains to the up most level all the necessary trophines the human body needs.

Although we are dealing with a food product containing, in balanced proportions, all the necessary trophines the human body needs, some physical, chemical and biotic risks for the consumer might occure.

The hygiene of food products has become a point of interest to the specialized, national and international institutions, as integrating part of the bio-protection of the environment, of the ecosystems and especially of the humans. Newly arisen problems refer only to the level of the chemical noxes leading to acute diseases, but also to the small and repeated doses a person consumes in periodical or sporadic manner with the intake of the base food products.

The consumption of these food products asks for cautious thinking because of the major risks to the health, risks that occur due to the presence of pesticides in both vegetal and animal food products. Although organochlorinated pesticides present a moderated toxicity have serious implications to the health and some very important characteristics can be traced (chemical stability, remanence, lipo-solubility and cumulative effect).

The use of pesticides leads to the appearance of their residues in food products. The ways leading to the contamination with pesticides of the food precuts are numerous. The atmosphere, the soil and the hydrosphere play the main role in the different ways food products are contaminated (7, 8,10, 11, 13).

The penetration of the human body by pesticides leads to some of the most serious diseases of the nervous system, the cardio vascular system, the hemo-poetic organs, the liver, the kidneys, and to the decline of the immunity. Considering the impact of pesticides on the human body, we must specify that some pesticides have a mutagenic, embrio-toxic, teratogenic, gonado-toxic influence. The carcinogenic effect of the organochlorurates continues to remain a controversial subject. Suspicions exist that some of these, as for example Lindane, might have an oncogenic effect (7,8,9,10).

The present paper contains investigations regarding the organochlorine pesticide residues in raw milk.

Key Words: organochlorine residues, milk

MATERIAL AND METHOD

The researches have been done four years in a row on a number of 160 milk probes coming from milk collected in large quantities. The milk came from four sources placed in the Dobrogea province and was collected and processed by S.C.Multicom Grup S.A.Pantelimon, a company producing milk products.

The research work regarding the organochlorurates pesticide residues was done with the means of a VARIAN chromatographic gas device, using the chromato-graphic method (mg/l).

RESULTS AND DISCUSSIONS

The final results with regards to he tracing of organochlorurate residues in raw milk are presented in table no. 1 and diagram no. 1. The analyzing of the obtained data show that the majority of the raw milk probes present the following values: 0,002-0,004 for αHCH; 0,011-0,025 for βHCH; 0,001-0,0057 for γHCH; 0,015-0,045 for the DDT total.

In 2002, 2003, the values of αHCH were situated between 0,002-0,004 with an average of 0,003 in 80 probes (50%); in 2004,2005 the values were contained between 0,002-0,004, with an average of 0,002 in 80 probes (50%).

For βHCH, the values were contained between 0,011-0,025, with an average of 0,01 for all the above mentioned years in all 160 probes (100%).

For γHCH, the values were situated between 0,0017-0,0057 in 2002; 0,002-0,004 in 2003; 0,001-0,005 in 2004, with an average of 0,002 in 10 probes (75%), and 0,0015-0,0028, with an average of 0,001 in 2005 in 40 probes (25%).

For the DDT total, the obtained values were contained between 0,017-0,035, with an average of 0,02 in 80 probes (50%) in 2002, 2004; 0, 015-0,030 in 2003, with an average of 0,01 in 40 probes (25%) and 0,019-0,045, with an average of 0,03 in 2005 in 40 probes (25%).

Table 1

Results regarding the oganochlorurated pesticide residues

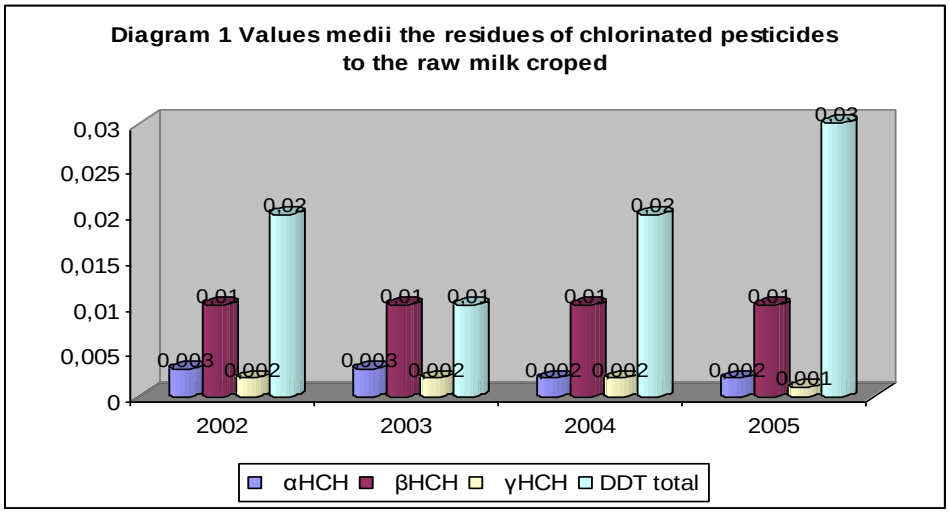
Year	No. of probes	Detection limits (mg/l milk) – organochlorurates			
		αHCH 0,005*	βHCH 0,01*	γHCH 0,002*	DDT total -0,04*
2002	40	0,002-0,004 0,003	0,011-0,020 0,01	0,0017-0,0057 0,002	0,017-0,029 0,02
2003	40	Undet.-0,003 0,003	0,011-0,025 0,01	0,002-0,004 0,002	0,015-0,030 0,01
2004	40	0,002-0,003 0,002	0,018-0,025 0,01	0,001-0,005 0,002	0,017-0,035 0,02
2005	40	0,002-0,004 0,002	0,011-0,019 0,01	0,0015-0,0028 0,001	0,019-0,045 0,03
Total	160	0,002-0,004 0,002	0,011-0,025 0,01	0,001-0,0057 0,001	0,015-0,045 0,02

****Detection limits αHCH 0,005, βHCH 0,01, γHCH 0,002, DDT total-0,04***

Reference values-LMA:- αHCH- 0,004 ppm, αHCH-0,003ppm, γHCH-0,008 ppm, DDT total- 0,04 ppm.

Following the conducted research during the entire period of time the obtained results were contained between 0,002-0,004, with an average of 0,002 in 80 probes (50%), respectively 0,003 for the remaining 80 probes (50%) for αHCH; 0,011-0,025, with an average of 0,01 in all 160 probe (100%) for βHCH; 0,001-0,0057, with an average of 0,002 in 120 probes (75%) and 0,015-0,028 with an average of 0,001 in 40 probes (25%) for γHCH; for the DDT total, the obtained values are contained between the following limits: 0,017-0035, with an average of 0,02 in 80 probes (50%),

0,015-0,030, with an average of 0,01 in 40 probe (25%) and 0,019-0,045, with an average of 0,03 in 40 probes (25%).



The analyzed data brings to the evidence that the majority of the processed raw milk probes presented values between: 0,002-0,004 for αHCH; 0,011-0,025 for βHCH; 0,001-0,0057 for γHCH; 0,015-0,045 for the DDT total.

CONCLUSIONS

The investigations concerning the organochlorine pesticide residues in processed raw milk used to produce acid milk products produced within Dobrogea zone to SC Multicom Grup SA Pantelimon emphasized the following:

1.The results obtained reported in mg/l milk, had the values contained between 0,002-0,004 for αHCH; 0,011-0,025 for βHCH; 0,001-0,0057 for γHCH; 0,015-0,045 for DDT totals for the organochlorine residues.

2.Comparing the values obtained with the admitted standard limits. The residues stayed below the admitted standard limits in the milk used for our research.

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13. **xxx, 2001** - *Ordinul MAAP nr.356*

Researches concerning the residues of arsen(as) and heavy metals of collected raw milk and processed in acid milk products to "SC MULTICOM GRUP SA" in Constanta county

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Milk can be directly contaminated with some toxic metals during its machine processing, during preservation, transportation, from some of the packing materials and through the trophic chain.

Some metals reaching the animal and human body over specific limits can lead to toxic state of health. In milk and fooder, heavy metals and As can originate from different sources. The pollution through industrial emissions constitutes the source weighing the most. A relatively small part of these metals, like for example Zn and Cu contained in salts are used for their therapeutical qualities. Copper, as metal, and arsenic can be used as fooder additives for their stimulative role in obtaining some weight gains (7,8).

Measure toxicity depend of kind metals,of his solubility and of their compound,of cumulative effect of certain metals in some tissue, of measure lack of poise electrolytic product in organism, of measure of metal and of duration of action.

In our county have established as limited maxim:0,01 mg/kg milk-cadmiu, 0,10 mg/kg lapte-plumb, arsen(7,8)

Key Words: heavy metals, raw milk

MATERIAL AND METHOD

The investigations have been done four years in a row on a number of 120 milk probes coming from milk collected in large quantities. The milk came from four source

placed in the Dobrogea province and was collected and processed by S.C.Multicom Grup S.A.Pantelimon, a company producing milk products.

The research work regarding the residues of Arsen(As) and heavy metals of milk

raw, was done with the means of a GBG AVANTA through spectrophotometer of absorption atomic

RESULTS AND DISCUSSIONS

The final results with regards to he tracing of residues heavy metals in raw milk are presented in table no. 1 and diagram no. 1. The obtained values for Zn were contained between 2,1-3,9 with an average of 2,6 in 30 probes (25%) în 2002; between 2,1-3,9 with an average of 3,0 in 60 probes (50%) for years 2003,2005 and between 2,2-3,8 with an average of 3,2 in 30 probes (25%) in 2004.

The research work regarding the residues for Cu were the values situated between 0,2-0,4 with an average 0,33 (50%) in 2002,2004; between 0,1-0,4 with an average 0,20 in 30 probes (25%) in 2003 and between 0,2-0,4 with an average 0,26 in 30 probes (25%) in 2005.

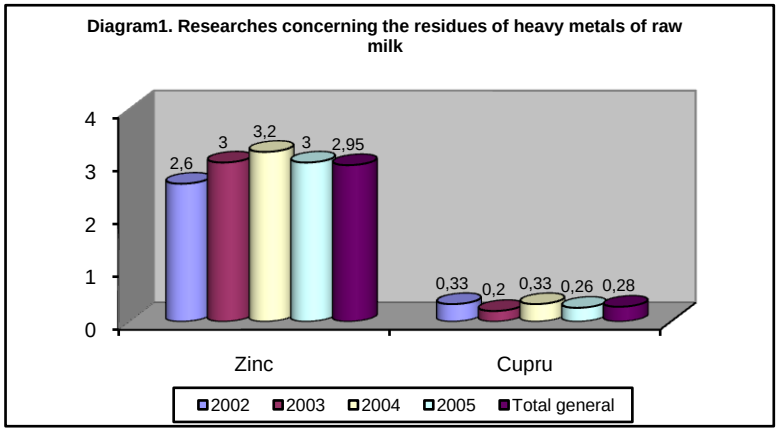
The researches concerning the residues of Cd, Pb and As on whole period to the raw milk were untraceable, with the Pb exception which had the value 0,1 in 30 probes (25%) in 2002.

Table 1

Researches concerning the residues of Arsen (As) and heavy metals of raw milk

Year	Zone	No. probes	Heavy metals and arsen (spectrophotometer of absorption atomic)				
			Zinc	Cupru	Cadmiu	Plumb	Arsen
2002	I	10	2,3-2,8	0,2-0,4	nedetectabil	0,1	nedetectabil
	II	5	2,2-2,4				
	III	10	2,1-2,8				
	IV	5	2,1-3,9				
	Media		2,6	0,33			
2003	I	5	2,2-2,7	0,1-0,4	nedetectabil	nedetectabil	nedetectabil
	II	10	2,8-3,9				
	III	5	2,1-3,7				
	IV	10	2,1-2,8				
	Media		3,0	0,20			
2004	I	10	2,7-3,8	0,2-0,4	nedetectabil	nedetectabil	nedetectabil
	II	5	2,2-2,7				
	III	10	3,6-3,8				
	IV	5	2,8-3,7				
	Media		3,2	0,33			
2005	I	10	2,8-3,9	0,2-0,4	nedetectabil	nedetectabil	nedetectabil
	II	5	2,2-2,7				
	III	10	2,1-2,5				
	IV	5	2,8-3,7				
	Media		3,0	0,26			
Total medie		120	2,95	0,28	nedetectabil	nedetectabil	nedetectabil

Reference values-LMA: - Zn= 5ppm,Cu= 0,5ppm,Cd=0,01ppm,Pb=0,1ppm,As=0,1ppm,Hg=0,01ppm



CONCLUSIONS

The researches concerning the residues of Cd, Pb and As on whole period to the raw milk were untraceable, with the Pb exception which had the value 0,1.

To As and heavy metals the values were 0 to Cd and As, Pb detected merely to the samples from 2002, with and average value of 0,1mg/l. For Zn the values were contained between 2,1-3,9mg/l with the media of 0,1mg/l, and Cu had the limits contained between 0,1-0,4 with and average of 0,28. The values obtained framed in the standard norms, as forethought Ord.MAAP no.356/2001.

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Researches concerning the genetic history of a Leghorn hen line

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Investigation of the genetic history of a Leghorn hen line was carried out by the Writht's pedigree sampling method. Data consisted in pedigrees of a White Leghorn line, across eight generations.

For males, the total inbreeding was 3.12% from which 2.5% was generated in the first fine generation (non-current inbreeding), and 0.62% in the two last generations (current inbreeding). For females, the total inbreeding was 1.25%, all of it being generated in the first fine generation. Average inbreeding per generation was 0.44% for males and 0.17% for females.

Inter-se relationship per generation was higher for males (4.65%) than for females (4.12%), thus, the expected inbreeding followed the same pattern (2.48% for males, respectively 2.10% for females).

The genealogic and genetic analyzes are as a matter of fact of the practical breeding. The data collecting in the genealogic books, these registers of the domestic animal populations permits the passing to more detail analyzes of the breeding work.

The aim of the present researches consists of studying some aspects of the genetic dynamics in a Leghorn hen line, selected during many generations. There were established some indices which emphasize its genetic history, as following:

- *achieved inbreeding (total and per generation);*
- *current and non-current inbreeding;*
- *possible inbreeding;*
- *inter-se population relationship;*
- *line and important reproducers relationship.*

Key Words: genetic history, Leghorn hen

Matherials and methods

The researches were carried out on a Leghorn hen line, which represented the base for the founding schemes of Albo 67 hybrid.

The data were obtained from 80 roosters and 100 hens in 2005 generation. There were made up four line randomized ascendants pedigrees, starting from grandparents, a large used procedure by A. Robertson (4) and J. Lush (2).

There was identified the appearances number of individuals, no matter the generation they took part, and that permitting the use of some simplified formulas (1.2.3.4) to calculate: total achieved inbreeding, per generation inbreeding, current and non-current inbreeding.

Total achieved inbreeding = number of double realized lines/ number of possible double lines. The doubled lines are represented by common ancestors in the both sides of the pedigree (2).

Per generation achieved inbreeding = the total achieved inbreeding/ generation number.

Current inbreeding = double lines number achieved during the last two generations/ possible double lines.

Non-current inbreeding = total achieved inbreeding – current inbreeding.

Inbreeding calculation, generally is made counting the appearance of the same ancestors at individuals among the assessment of inbreeding is searched.

For the particular case of inter-se population inbreeding these common ancestors appearances are counted in each pedigree analyzes, comparing each male with the other females, excepting the one it was mated for giving a head pedigree individual. There will be n (n-1) comparisons if there are studied n pedigrees.

Inter-se relationship = number of achieved appearances/ number of possible appearances. The number of the possible appearances = 4 n (n-1), where n is the number of studied individuals. Knowing the average relationship in a population it could be estimated the possible or expected inbreeding, by the randomized mating of individuals.

The possible inbreeding = inter-se relationship/2- inter-se inbreeding.

Results and discussions

The achieved inbreeding

In males, by the studied on the 80 pedigrees of the 4 randomized lines, there were found 10 double lines from which 2 in the last two generations that leading to a total achieved inbreeding of 3.12% and 0.44% per generation (table 1).

The current inbreeding in males was of 0.62%, and the non-current one of 2.5%. In females, upon the made up study on the 100 four lines randomized pedigrees there were found 5 double lines, all of them with further ancestors than the two last generations, this fact leading to a total achieved inbreeding of 1,25%, and per generation of 0.17%. The total achieved inbreeding in females was by the whole a non-current one.

Table 1

Total and per generation inbreeding

Specification	Males	Females
Number of generation at expected inbreeding	7	7
Total inbreeding (%)	3.12	1.25
Inbreeding per generation (%)	0.44	0.17
Current inbreeding (%)	0.62	0
Non-current inbreeding (%)	2.50	1.25

Inter-se relationship and the possible inbreeding

In table 2 it could notice that the highest inter-se relationship (4.65%) is recorded in males, this fact leading to a possible inbreeding of 2.38%. The inter-se relationship in females was 4.12% that makes possible a 2.10% inbreeding value.

Table 2

Inter-se relationship and expected inbreeding

Specification	Inter-se relationship (%)	Expected inbreeding (%)
Males	4.65	2.38
Females	4.12	2.10

Line relationship with important reproducers

The identifying of the important reproducers in a population represents an informational source linked to the applied breeding method and its consequences. For specifying the genetic similarity of the researched hen line with some reproducers (roosters) which founded it or activated in it, there was calculated the relationship coefficient of the analyzed population with a series of important reproducers (r_{G-R}). There was reported the number of achieved appearances for these reproducers at the total possible number of appearances (the number of pedigrees multiplied by 4 randomized lines). After the calculations, it was considered that 9 reproducers were important, all of them having the value of the inbreeding coefficient over 1% (table 3).

Table 3

Line relationship with main breeding individuals

Main breeding individuals	r_{G-R} (%) Total	r_{G-R} (%) Males	r_{G-R} (%) Females
700811 (1997)	4.72	5.62	4.00
280311 (1997)	4.17	3.44	4.75
520113 (1997)	2.92	3.75	2.25
100213 (1997)	2.92	3.44	2.50
650211 (1997)	2.36	3.12	1.75
410311 (1997)	2.50	3.44	1.75
460711 (1998)	2.36	4.06	1.00
230611 (1998)	4.86	4.69	5.00
190612 (2002)	5.14	5.62	4.75
TOTAL	35.14	42.87	30.50

The maximum relationship was 5.14% for rooster 190612 in 2002 generation. The genetic similarity coefficient of the 80 studied roosters with the 9 roosters was 42.87%, and the one of the 100 hens with the same 9 roosters was 30.5%. From these 9 roosters, 6 owed to 1997 generation, the genetic similarity coefficient of the line with these 6 roosters is 19.59%.

Conclusions

The achieved inbreeding per generation of 0.44 in the males of the studied line keep this population within the limits of a non-inbred line.

The value of the inbreeding coefficient achieved per generation was lower in females (0.17%), this being a consequence of the special measures made during the selection program, certifying the fact that the line is under the total control of selection.

From the total achieved inbreeding in males of 3.12%, the current inbreeding represented 0.62%, and in females, the total achieved inbreeding was by the whole non-current, 1.25%.

Inter-se inbreeding was higher in males (4.65%), than in females (4.12%), that leading to a possible inbreeding of 2.48% in males and 2.10% in females.

The genetic similarity coefficient of the line with the 9 roosters which exceeded the relationship coefficient of 1%, recorded a value of 35.14% of the total up to date population genetic fund.

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The diagnosis of infection of *Brucella ovis*

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The diagnosis of infection with Brucella ovis in Vaslui county was effected through the complement fixation test (CFT) and bacteriological examination.

During 2000-2005, a number of 64 226 blood samples were processed about CFT. As a result of this test, a number of 434 samples blood were serological positives (0, 68 %). After sacrificed a number of 82 rams serological positive, the infection with Brucella ovis was confirmed at 57 samples (69,51%).

The samples with anticomplementary results - 45 (0,07%) have been processed over 21 days from the first examination. 10 of the samples were serologically positive.

Key words: Brucella ovis, diagnosis, complement fixation test

MATERIALE ŞI METODE

Supravegherea infecţiei cu Brucella ovis la berbecii de reproducţie se realizează prin examen serologic - reacţia de fixare a complementului. Berbecii cu reacţii serologice pozitive se sacrifică, confirmarea diagnosticului realizându-se prin examen bacteriologic (6).

Cultivarea brucelelor s-a realizat pe medii deshidratate speciale. La agarul nutritiv s-a adăugat şi ser bovin în proporţie de 2-5%. De asemenea, s-au folosit antibiotice pentru inhibarea contaminanţilor (3).

După 48-72 ore de creştere la 36-38^{o}C, la un pH de aproximativ 7 şi în atmosferă cu CO_2, s-au evidenţiat colonii rugoase, de tip R, cu aspect uscat, granular, de culoare galben-auriu (1).

Metodele de colorare (3) folosite pentru evidenţierea brucelelor au fost : coloraţia Gram şi coloraţia Ziehl - Neelsen modificată (figura 3, 4).

Identificarea brucelelor (2) s-a realizat pe baza caracteristicilor de creştere, antigenice (reacţia de aglutinare) şi biochimice (testul catalazei, testul oxidazei şi testul de reducere a nitraţilor în nitriţi).

REZULTATE ŞI DISCUŢII

EXAMENUL SEROLOGIC

REACŢIA DE FIXARE A COMPLEMENTULUI

În perioada 2000-2005, în Laboratorul Sanitar Veterinar Judetean Vaslui s-au examinat 64 226 probe în vederea depistării infecţiei cu Brucella ovis prin reacţia de fixarea a complementului - RFC (4). S-au depistat un număr de 434 de berbeci serologic pozitivi, iar la 45 probe s-au înregistrat rezultate de anticomplementaritate.

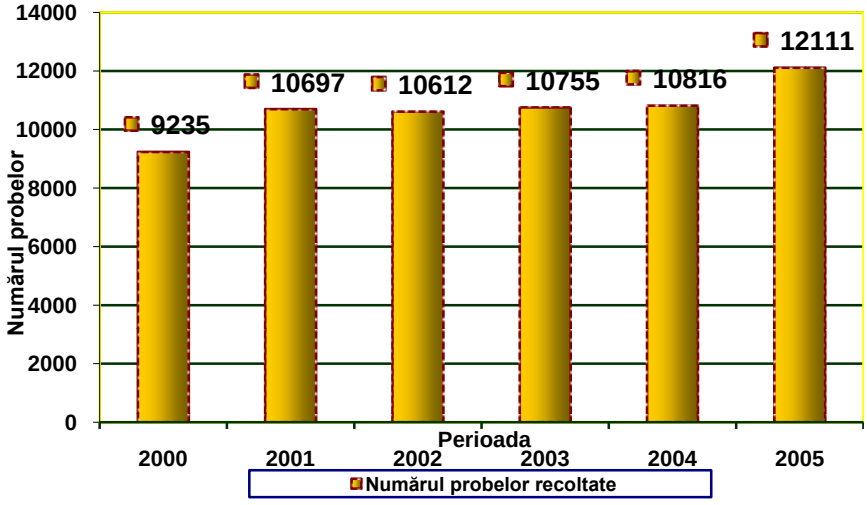
Probele anticomplementare s-au reexaminat printr-un nou control la 21 de zile, iar la 10 dintre ele s-au obţinut (4) rezultate pozitive (tabelul nr.1).

Situaţia probelor analizate în laborator prin R.F.C. precum si situaţia probelor pozitive prin examen serologic în perioada 2000-2005, este evidentiata in graficele 1, 2.

Tabelul nr.1

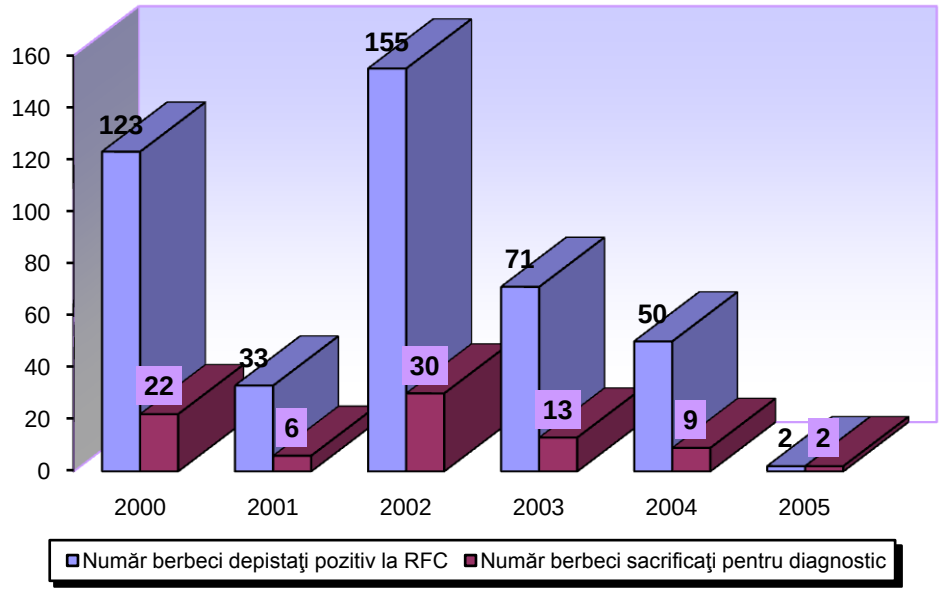
Rezultate examene supraveghere bruceloză la berbeci în judeţul Vaslui în perioada 2000-2005

Nr. crt.	Specificare		U/M	2000	2001	2002	2003	2004	2005	Total
		Efectiv ovine	cap.	222 475	225 086	212 226	216 226	225 533	238 238	1 340 209
1	Examen supraveghere	Efectiv existent berbeci	cap.	4 836	5 567	5 448	5 616	5 765	6 158	33 390
2		Efectiv controlat	cap.	9 235	10 697	10 612	10 755	10 816	12 111	64 226
3		Rata controalelor	2	1,91	1,92	1,95	1,92	1,88	1,97	1,92
4		Număr berbeci depistati pozitiv la RFC	cap.	123	33	155	71	50	2	434
			%	1,33	0,31	1,46	0,66	0,46	0,02	0,68
5		Număr berbeci sacrificati pentru diagnostic	cap.	22	6	30	13	9	2	82
6			%	17,89	18,18	19,36	18,31	18,00	100	18,89
7	Total pozitive confirmate după sacrificare		cap.	17	2	23	10	4	1	57
8	Rezultat examen serologic	P	cap.	123	30	148	71	50	2	424
		D	cap.	2	1	-	-	-	-	3
		Ac	cap.	-	3	27	-	15	-	45
		N	cap.	9110	10663	10437	10684	10751	12109	63754
9	Retestare serologica la 21 zile	P	cap.	-	3	7	-	-	-	10
		D	cap.	-	-	-	-	-	-	-
		Ac	cap.	-	-	-	-	-	-	-
		N	cap.	-	-	20	-	15	-	35
10	Rezultat final	P	cap.	123	33	155	71	50	2	434
		D	cap.	2	1	-	-	-	-	3
		Ac	cap.	-	-	-	-	15	-	15
		N	cap.	9110	10663	10457	10684	10751	12109	63774



Graficul nr.1

SITUAŢIA PROBELOR ANALIZATE ÎN LABORATOR PRIN R.F.C. ÎN PERIOADA 2000-2005



Graficul nr.2
SITUAŢIA PROBELOR POZITIVE PRIN EXAMEN SEROLOGIC ÎN PERIOADA 2000-2005

EXAMENUL BACTERIOLOGIC

De la animalele sacrificate (82) pentru stabilirea diagnosticului de bruceloză la berbeci (18,89 %), s-au trimis probe la laboratorul naţional de referinţă (testicule şi epididim – figura nr. 1, 2). În urma examenului bacteriologic (figura nr. 3, 4), s-au confirmat 57 de cazuri (tabelul nr. 1).

În urma examenului bacteriologic s-au izolat 7 tulpini bacteriene cu următoarele caractere culturale şi morfologice, biochimice şi antigenice (tabelul nr.2):

Tabelul nr.2

Identificarea culturilor de Brucella

Nr. crt.	Tip cultural	Examen macroscopic al culturii	Aglutinare cu antiser Brucella R	Catalază	Oxidază	Reducerea nitraţilor
1.	R	Colonii cu margini neregulate, gălbui, ϕ1-2 mm	+	+	-	-
2.	R	Colonii granulare, uscate, gălbui -aurii, ϕ 2-3 mm	+	+	-	-
3.	R	Colonii cu margini neregulate, gălbui, ϕ1-2 mm	+	+	-	-
4.	R	Colonii granulare, uscate, gălbui -aurii, ϕ 2-3 mm	+	+	-	-
5.	R	Colonii cu margini neregulate, gălbui, ϕ1-2 mm	+	+	-	-
6.	R	Colonii granulare, uscate, gălbui -aurii, ϕ 2-3 mm	+	+	-	-
7.	R	Colonii cu margini neregulate, gălbui, ϕ1-2 mm	+	+	-	-

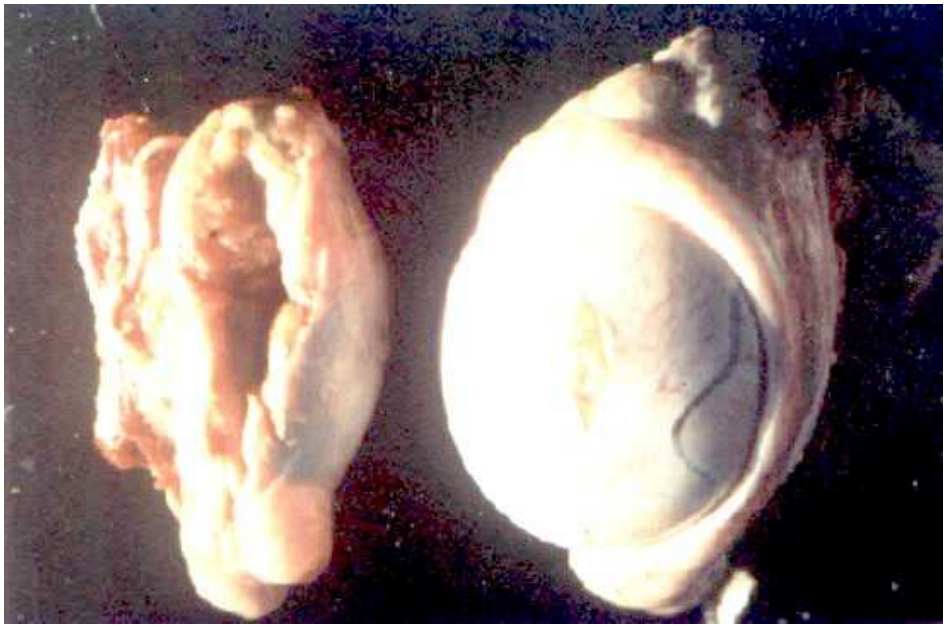


Figura nr.1
Orhiepididimită brucelică. Testicule şi epididim recoltate de la berbecii sacrificați în urma depistării prin examen serologic.

Figura nr.2
Testicul şi epididim de berbec cu inflamație brucelică.

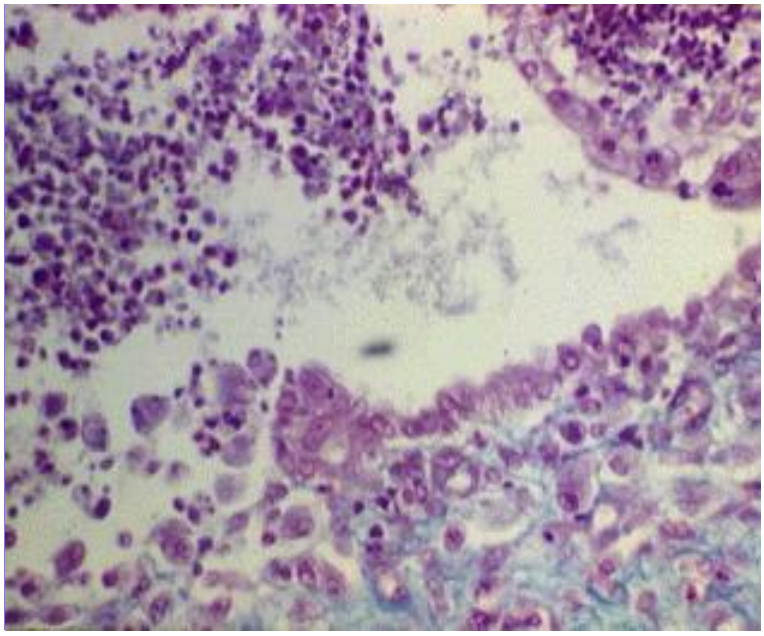


Figura nr.3
Frotiu din epididim berbec.Evidențierea Brucellei ovis prin colorația Ziehl-Neelsen modificată.

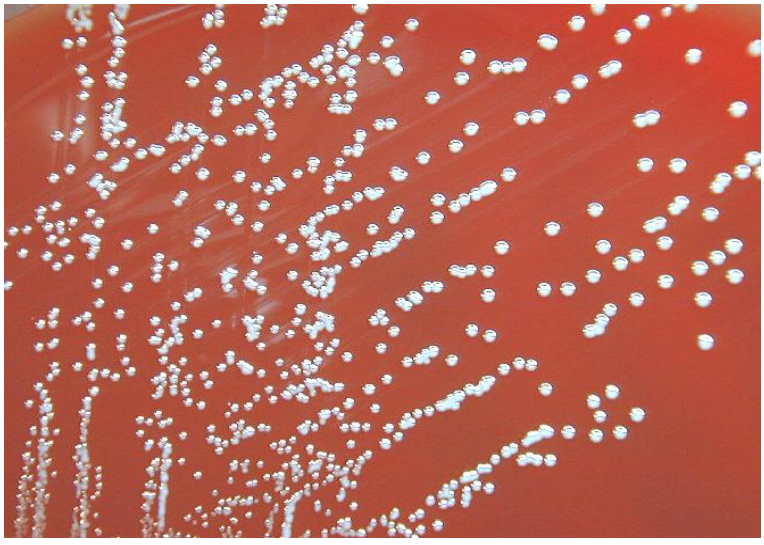


Figura nr.4
Culturi de Brucella ovis pe mediu cu sânge.

Concluzii :

1. În perioada 2000-2005, s-au primit în laborator 64 226 probe provenite de la berbecii de reproducție, care au fost analizate pentru supravegherea infecției cu Brucella ovis prin RFC.
2. Prin examenul serologic s-au pus în evidență un număr de 434 probe pozitive şi 45 de probe anticomplementare.
3. Din totalul animalelor sacrificate pentru stabilirea diagnosticului de bruceloză, boala s-a confirmat în 57 de cazuri (69,51%).
4. Au fost analizate prin examen cultural, antigenic şi biochimic un număr de 7 probe, rezultatele examenelor au demonstrat infecții probabile cu Brucella ovis.

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Occurence of Paramphistomum microbothrium (Fischoeder 1901) in deer (Cervus elafus)

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P. microbothrium was well known like parasites of domestic large and small ruminant, especially cattle. In Serbia they occured in small ruminants and cattle. Our examination we performed in period 1996-1997. at hunting ground located at Northwest part of Vojvodina near by Danube flown. From six hunting deer (Cervus elafus) examined by necropsy, paramphisomidae parasites were found at 4 animals. Numerous adult parasites were occurred in rumen (more than 250 per animals), but they also occur in reticulum and omasus in smaller number. Find parasites of genus Paramphistomidae were determinate using key given by Nesmark (1937), Vishnyakov (1980) and Samnaliev (1981) which use type of matter of acetabulum from determination of genus and metter of atrium genital and acetabulum to determination of paramphistomidae species. After microscopic examination we concluded that occurred paramphistomided belonging to the species Paramphistomum microbothrium (Fischoeder 1901). Our results present a first occurence of P. microbothrium at deer (Cervus elafus) in Serbia.

Key words: Paramphistomum microbothrium, cervus elafus

Introduction

Deer are widely distributed, and hunted, with indigenous representatives in all continents except Antarctica and Australia. The majority of large deer species inhabit temperate mixed deciduous forest, mountain mixed coniferous forest, tropical seasonal/dry forest, and savanna habitats around the world. Red Deer (*Cervus elaphus*) is one of the largest species of deer in the world. The deer of Central and Western Europe vary greatly in size with some of the largest deer found in the Carpathian Mountains in Central Europe. In Serbia deer lived at Đerdap national park and at forest at Northwest part of Vojvodina near by Danube flown.

Parasitoses caused by helminthes produce significant health problems in deer, especially with Planthelminthes which was the frequently infection transmitted through intermediate host in those population. Paramphistomiasis was one of important planthelminth infection occurred at southern and Easter Europe, and one species, *P.microbothrium,* has been almost exclusively incriminated. In our paper we give survey of those infection occurred at deer in one hunting ground in Serbia.

Material and methods

Our examination we performed in period 1996-1997. at hunting ground located at Northwest part of Vojvodina near by Danube flown. Six hunting deer (Cervus elafus), were examined by necropsy. We have examined trachea, lung, heart, complete gastrointestinal tract, liver, kidney and urinary bladder. The intestine and the other organs were slit opened and visible helminths removed. The mucous is scraped and many young parasites were found in debris. The contents and washing were scanned over a gauze sieve, under jet water and the retained material examined, small quantities at a time, in a large whistle enamel tray.

Parasites found were collected and fixed in acetic formalin (after Railliet). At laboratory of Scientific Veterinary Institute of Serbia, parasites of genus *Paramphistomidae* were fixed 10% formalin, embedded in paraffin, mediosagital sectioned at 5-6 micrometers and stained by HEA. Determination were performed by key given by Nesmark (1937), Vishnyakov (1980) and Samnaliev (1981) which use type of matter of acetabulum from determination of genus and metter of atrium genital and acetabulum to determination of paramphistomidae species.

Results and discussion

From six hunting deer (Cervus elafus) were examined, paramphisomidae parasites were found at 4 animals. Numerous adult parasites were occurred in rumen (more than 250 per animals), but they also occur in reticulum and omasus in smaller number. Immature parasites were found attached to the wall of the abomasus, where the wall and folds were be so thickened as almost to occlude the lumen of the organ. Young parasites were found behind the pylorus attached to the mucus, and in brownish-pink clusters in the contents. Erosion and small hemorrhages were present and even the contents themselves may show discoloration from hemorrhage.

After microscopic examination we concluded that occurred paramphistomided belonging to the species *Paramphistomum microbothrium* (Fischoeder 1901)(picture 1).

Picture 1. Mediosagital cut of *P. microbothrium*.

P. microbothrium was well known like parasites of domestic large and small ruminant, especially cattle (Horak, 1971). Disease was characterized by sporadic epizootics of acute parasitic gastro-enteritis with high morbidity and mortality rates, particulary in young stock. Development of parasites circulated through intermediat host snail from genus *Bulinus*. After ingestion of metacercaria by finaly host development was acomplished during passage throught the rumen, abomasus and small intestine. In Serbia they occured in small ruminants and cattle (Babić, 1966; Cvetković, 1968; Vujić and Petrović, 1971).

Conclusion

Our results present a first occurence of *P. microbothrium* at deer (*Cervus elafus*) in Serbia.

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Serological screening for anti-felin coronavirus antibodies detection using indirect immunofluorescence technics

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Serology can be useful as an aid in diagnosis of feline infectious peritonitis, but when is used alone is practically meaningless. There were analized 23 blood samples from cats with ages between 6 months and 1,5 years , with or without clinical signs of the disease. It was determined the antibody titer using indirect immunoflourescence on serum samples, the results indicating whether or not the cat has been exposed to FCoV, but without distinguishing between enteric coronavirus and FIPV. From the total number of 23 analized blood samples, 3 (13,04%) of them resulted positive in RT-PCR were tested over the antibodies presence and were find positive as well. The rest of the samples (86,96%) were find negative in RT-PCR and indirect immunoflourescence. Two of the positive cats were females (66,6%) and 1 of them was male (33,4%), all of them without clinical signs. One tested animal resulted positive a year before was negative this time.

Key words: antibodies, FIP, indirect immunofluorescence

Feline Infectious Peritonitis (FIP) is caused by a coronavirus that can infect any cat, though young cats and very old cats (14 years and up) appear most susceptible. The FIP virus (FIPV) is very similar to the coronavirus that causes a transient, usually mild, self-limiting diarrhea (Feline Enteric Corona Virus, FECV). In fact, there is some evidence that FECV can mutate to FIPV in some individuals.

MATERIALS AND METHODS

For the indirect immunofluorescence reaction there were used plastic 96 microwells or Lab-Tek chamber slide system with 8 wells, an infected cell culture (PK), a positive control with known titre (16000), diluted serum samples (1/25,1/125, 1/625, 1/3125, 1/16000), dilutions being realized in PBS 1ml-BSA 1mg (bovine serum albumine), a conjugate anti-IgG cat-FITC , UV microscope.

RESULTS AND DISCUTIONS

From the total of 23 analysed serum samples using RT-PCR, only 3 were positive, revealing the fact that the animals were exposed to the feline coronavirus.

After the serological exam using indirect immunofluorescence, all the 3 samples were positive in a titre of 1/625 in case of polyclonal antibodies (fig. 1, 3, 4).

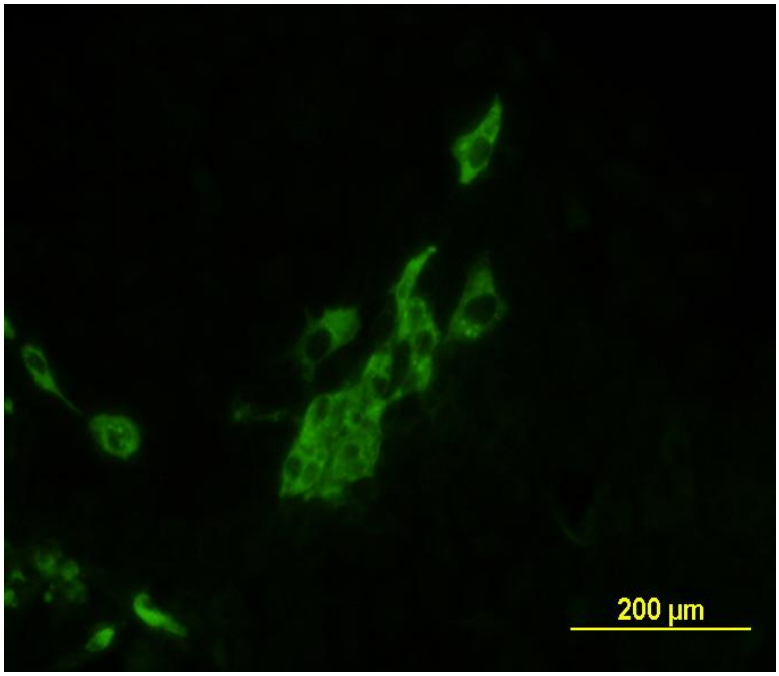

Fig.1 – Cytoplasmatic fluorescence, x20

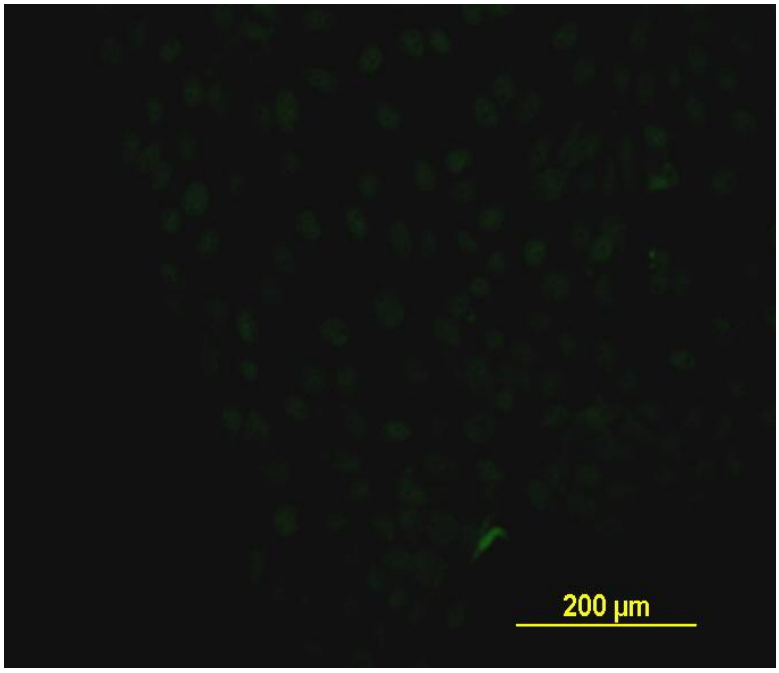

Fig.2 – Negativ control, x20

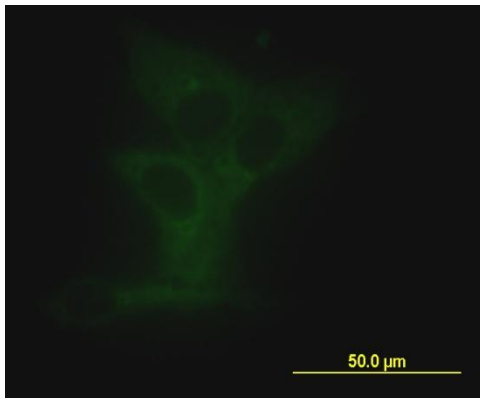

Fig.3 –Cytoplasmatic fluorescence, x60

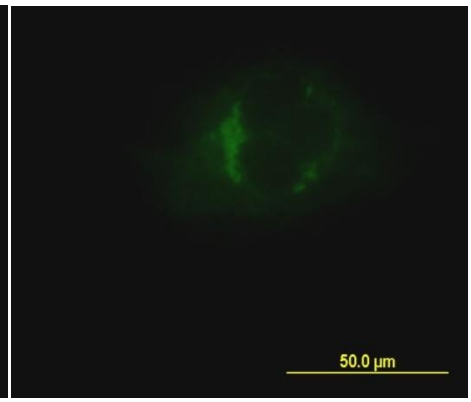

Fig.4 – Cytoplasmatic fluorescence, x60

CONCLUSIONS

1. From the total number of 23 analized blood samples, 3 (13,04%) of them resulted positive in RT-PCR were tested over the antibodies presence and were find positive as well.
2. The rest of the samples (86,96%) were find negative in RT-PCR and indirect immunoflourescence.
3. Two of the positive cats were females (66,6%) and 1 of them was male (33,4%), all of them without clinical signs.
4. One tested animal resulted positive a year before was negative this time.

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Reasearches regarding some allergic diseases diagnosis in dogs and cats

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Allergic diseases are quite frecquent in animals, especially cats and dogs with a good shape, too well nurtured or with parasitic infestesion.

To establish the diagnosis of allergic diseases 30 animals (6 cats and 24 dogs) were tested using some in vivo and in vitro tests. After the clinical examination only the subjects which presented signs of a possible allergy were selected.

Results interpretation was made in function of the cutaneous reaction, the number of eosinophilis and IgE levels. Usually the three parameters were related.

In the 30 suspected animals were diagnosticated: atopic dermatitis in 15 dogs (62,5%) and 2 cats (33,33%), food allergy in 6 dogs (25%) and 1 cat (16,67%), contact dermatitis in 3 dogs (12,5%) and 3 cats (50 %).

Key words: allergy, cat, dog, cutaneous test, IgE level

There are a lot of favourizing factors which produse some cutaneous affections in cats and dogs, but most common are the allergies. Pets (dogs and cats) can develop allergies at any age, but most of all in animals after 2 years old. (1,2,4,5,6)

In allergies, the organism reacts to some molecules called *allergens*. Those allergens can have a vegetal nature (trees and plants pollen, grass etc.), textures (wool, nylon), rubber or plastic material, aliments and alimentary additives, various types of meat, cereals, dairy products, dust and fleas bite. (1,2,5,6)

The main dermatopathies with immune substrate are: atopic dermatitis, hipersensitivity dermatitis in fleas bite, food, drugs and contact allergy. Another frequent immunopaties are: rheumatoid polyarthritis, immune nephritis, bronchic astma, etc (1,2,5,6).

MATERIAL AND METHODE

A number of 30 animals were examined in the Interne Clinics of Veterinary Medicine Faculty and in various privat clinics. The animals presented clinical signs which indicated a type of allergy. The exact allergy diagnosis and the identification of the allergens were accomplished using one of the following methods:

- Allergic test (intradermic innoculation or blood tests);
- Removal of each possible allergen from the animal environement untill the cause is identified;
- Therapeutical answer, although is not very concludent, usually produces de the faster improvement in animal state and is the most advantageous method from the economical point of view.

The tests used for the allergy diagnosis establishment can be realised in vivo (cutaneous tests) or in vitro (number of eosinophilis and IgE levels)

I. Cutaneous tests (scratch, prick or patch) are considered the most revealing for the diagnosis (3,6).

A small amount of the incriminated allergen is administrated on the skin or intradermical in order to follow if there is an allergic reaction. There are 3 types of cutaneous tests: Prick test, intradermic test and patch test (diagnosis of contact allergic dermatitis).

Prick test is the most used and contains standard allergens (Halcis – Pricktest kit) for allergies induced by the environement allergens. The negative control is the diluant of the solution and the positive control is the histamine solution.

HALCIS-PRICKTEST contains an allergenic extract with allergen AU/ml, or %, phenolum 5mg, Di-Natriumhydrogenphosphat-dihydrat 1,6mg/ml, Natriumhydrogenphosphat – monohydrat 0,6mg/ml, glicerine 630 mg/ml, water until 1 ml.

The allergens are: graminaceous, cereals, trees pollen, *Artemisia vulgaris*, moulds mixture, *Aspergillus fumigatus, Penicillium notatum, Mucor mucedo, Cladosporium cladosporoides, Candida albicans, Saccharomyces mellis*, dog hair, cat hair, *Dermatophagoides pteronissimus, Dermatophagoides farinae,* negative control and positive control.

Results interpretation

The cutaneous reaction was evaluated after 20-30 minutes from application and the interpretation was made in function of the papula size: no papula and erythem < 1 mm (negative reaction), no papula and erythem > 3 mm (+ positive reaction), papula < 3 mm and visible erythem (++positive reaction), papula 3-5 mm and visible erythem (+++positive reaction), papula > 5 mm (++++ positive reaction)

II. In vitro tests followed to determine the number of eosinophilis and seric IgE titres.

1. The number of eosinophilis was established in blood taken from extern saphene vein.

2. The values of seric IgE were established using ELISA technique. The test is less sensitive compared to cutaneous tests being used a human kit.

The test is posistive if the specific Ig E titre is 4 times bigger than usual.

RESULTS AND DISCUTIONS

Allergies diagnosis is based on allergologic anamnesis, clinical examination, allergic tests in vivo and in vitro .

The allergologic anamnesis followed some objectives with diagnosis value:

- The allergic syindrom
- Demonstration of the allergic nature (determine factors)
- Allergen identification

There were evaluated the aspect related to the time (pollenic season) and space (dust allergy), the reactions intensity at the contact with the allergen, the symptoms repeat, the elimination test.

The diagnosis methodes were used to test type I atopic hipersensitivity in 30 animals (24 dogs and 6 cats) with different ages and from various species.

Tested animals react normally, observing more intensity degrees of the reaction (from + to ++++) in case of positive reaction. There weren't registrated secondary effects or accidents, proving the applicability of those tests in animals and humans as well..

There were tested various breeds, but the biggest number of cases was registrated in the common breed (10 dogs and 3 cats). In pure breeds were diagnosticated 6 cases in the German Shepard, 2 cases each in Dalmatian, Cocker Spaniel and Golden Retrevier and 1 case each in Boxer and Pekinez. In cats were diagnosticated 2 cases each in Persian and 1 case in Birman.

The obtained results in cutaneous tests are synthetised in table no.1

4 dogs (13,33%) of the tested animals reacted at negative control, being removed. That is the reason why animals should be tested previously with the negative and the positive control for a proper interpretation.

In tested animals were followed the reactions (reactions intensity, secondary reactions, cutaneous reactions evolution) and the allergen types which determined the hipersitivity state.

From the used allergens posistive reactions were obtained in : graminaceous pollen (7 dogs and 1 cat), cereals pollen (2 dogs), trees pollen (1 dog), *Artemisia vulgaris* (3 dogs and 2 cats), mixture of moulds (5 dogs and 2 cats), *Aspergillus fumigatus* (4 dogs and 1 cat), *Penicillium notatum* (2 dogs), *Mucor mucedo* (1 dog), *Cladosporium cladosporoides* (3 dogs), *Dermatophagoides pteronissimus* (4 dogs), *Dermatophagoides farinae* (4 dogs and 1 cat).

It was observed that from the 24 suspected dogs, in 15 (62,5%) was diagnosticated atopic dermatitis, in 6 (25%) food allergy and in 3 (12,5%) contact dermatitis.

In the 6 examined cats were diagnosticated atopic dermatitis in 2 (33,33%) cases, food allergy in 1(16,67%) case and contact dermatitis In 3 (50 %) cases.

From the used allergens it wasn't obtained a cutaneous reaction in *Saccharomyces mellis*, *Candida albicans,* dog hair and cat hair allergens.

Tabel 1 – Results obtained after the allergens using in cutaneous test

Crt. No.	ALLERGEN	Habitate					
		Outside		Outside and inside		Inside	
		Dogs	Cats	Dogs	Cats	Dogs	Cats
1	Graminaceous pollen	1	-	2	1	4	-
2	Cereals pollen	1	-	1	-	-	-
3	Trees pollen	-	-	1	-	-	-
4	Artemisia vulgaris	-	-	2	1	1	1
5	Mixture of moulds	2	1	2	1	1	-
6	Aspergillus fumigatus	1	-	2	1	1	-
7	Penicillium notatum	1	-	1	-	-	-
8	Mucor mucedo	1	-	-	-	-	-
9	Cladosporium cladosporoides	1	-	2	-	-	-
10	Candida albicans,	-	-	-	-	-	-
11	Saccharomyces mellis	-	-	-	-	-	-
12	Dog hair	-	-	-	-	-	-
13	Cat hair	-	-	-	-	-	-
14	Dermatophagoides pteronissimus	2	-	2	-	-	-
15	Dermatophagoides farinae	2	1	2	-	-	-
16	Negative control	2	-	2	-	-	-
17	Positive control	4	-	12	4	8	2

Rapported to age, the middle age subjects presented the most positive reactions and rapported to sex it wasn't observed the preponderence of the one or the other.

The biggest case incidence was registred in good shape subjects (hyperproteic nutrition), in animals exposed excesively to some cosmetic treatment (shampoo, hair styling) or in the ones with parasitic infections.

Hypereosinophilia was observed in the majority of investigated cases with values between 9 and 12%.

The IgE levels were more or less concludent, in function of the each subject immunitary system. In cases where allergy overlapped with some other affections, the results weren't too relevant.

For a better interpretation the levels of IgE was established in healthy dogs, obtaind values being between 0,0011 – 0.0017 mg/ml.

In the suspected animals variable values (0,0043mg/ml - 0,0019mg/ml) were obtained.

CONCLUSIONS

1. To diagnose some allergic diseases 30 animals (24 dogs and 6 cats) with different ages, breeds and habitates were examined.
2. The tests were realised in vivo (cutaneous tests: Prick test, intradermic test) and in vitro (number of eosinophilic and IgE level).
3. in the 30 suspected animals were diagnosticated: atopic dermatitis in 15 dogs (62,5%) and 2 cats (33,33%), food allergy in 6 dogs (25%) and 1 cat (16,67%) and contact dermatitis in 3 dogs (12,5%) and 3 cats (50 %).
4. 4 (13,33%) of the tested animals reacted at negative control, being removed.
5. From the used allergens posistive reactions were obtained in : graminaceous pollen (7 dogs and 1 cat), cereals pollen (2 dogs), trees pollen (1 dog), *Artemisia vulgaris* (3 dogs and 2 cats), mixture of moulds (5 dogs and 2 cats), *Aspergillus fumigatus* (4 dogs and 1 cat), *Penicillium notatum* (2 dogs), *Mucor mucedo* (1 dog), *Cladosporium cladosporoides* (3 dogs), *Dermatophagoides pteronissimus* (4 dogs), *Dermatophagoides farinae* (4 dogs and 1 cat).
6. From the used allergens it wasn't obtained a cutaneous reaction in *Saccharomyces mellis*, *Candida albicans,* dog hair and cat hair allergens.
7. In the suspected animals variable values (0,0043mg/ml - 0,0019mg/ml) were obtained.
8. Hypereosinophilia was observed in the majority of investigated cases with values between 9 and 12%.
9. The biggest case incidence was registred in good shape subjects (hyperproteic nutrition), in animals exposed excesively to some cosmetic treatment (shampoo, hair styling) or in the ones with parasitic infections.

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Identification of microbiene flora isolated from various affections in dogs and cats

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U.S.A.M.V. Faculty of Veterinary Medicine - Iaşi

The researches includ the synthesis of the bacteriological examinations results, carried out between 2006-2007 in the Faculty of Veterinary Medicine from Iasi.

In 55,35% cases a mixt flora was isolated and in 44,64% cases bacteria or yeasts pure culture were obtained.

The Gram positive microflora was isolated in 57,14% cases and the Gram negative microflora in 42,85% cases.

The results of the antibiograms made on total flora plead for the necessity of treatment as a consequence of the responsiveness to antibiotics of the involved tested microorganisms.

Key Words: microbiene flora, various affections, antibiograms

In speciality literature are more frequent the works in which are presented various diseases at the natural barriers level (skin and greenhorn). The majority of authors (1,2,4,5) are agreed that in the frequent appearance of all this affections, a central part has the environement factors depreciation (ozone layer reduction, the direct action of the sun radiations, the chemical pollution, etc) which produce the local and general organism immunity decresing in animals. In this context some epiphite, comensale, opportunisticand pathogen microrganisms produce a serie of infections at this level.

The aim of this work was the inventorizing of microorganisms from infections with various localizations in dogs and cats and the sensibility testing in antibiotics and antimycotics of germs isolated in order to establish the corect therapeutical scheme.

MATERIALS AND METHODS

The microbiological investigations were made on 45 dogs and 10 cats which presented on clinical examination: otits, conjunctivitis, pyodermitis, vaginitis, cystitis and enteritis (table.1)

The pathological material inoculation was realised on usual mediums specific to aerobic and anaerobic bacteria, on mediums with serum and as well on mediums for mycetes (Sabouraud, Czapeck).

For the microbiological flora isolation, mediums were incubated in thermostat at 37^0 C, during 24-28 h for bacteria and at 22-25^0C, during 3-5 days for mycetes.

The next step after the culture development was the purufication and the identification of the bacteria and mycetes, according to morphocultural and biochemical characteristics.

To establish the etiological responsibility of the isolated germs, there were accomplished pathogenity tests „in vitro” (haemolysis test, coagulation test of rabbit citrated plasma).

The antibiogrames were made on total flora through the diffusimetric methode using the OXOID dispenser microcomprimates with antibiotics OXOID and PHIZER.

Tabel 1

Clinical diagnosis of the studied cases

Crt. No.	Specie	Cases no.	Otitis		Conjuncti-vitis		Pyodermitis		Vaginitis		Pharingo-laryngitis		Cystitis		Enteritis	
1	Câine	45	14	25	3	5,35	12	21,4	1	1,7	2	3,5	4	7,1	1	1,7
2	Pisică	19	2	3,5	1	1,7	4	7,1	1	1,7	2	3,5	8	7,1	1	1,7

RESULTS AND DISCUTIONS

In the 31 (55,35%) of the 56 cases a mixt flora with bacteriene and mycotic strains from Staphylococcus, Streptococcus, Arcanobacterium, Escherichia, Clostridium, Proteus, Pseudomonas, Candida, Malassezia genuses was isolated.

In the other 25 cases (44,64%) pure cultures of germs from the same taxons were obtains

In the cases with polymicrobiene ethiology, the most frequente were the associations between 2 and 3 microbiene species: 68,65% in otitis, 50% in conjunctivitis, 31,25% in pyodermitis, 100% in vaginitis, 50% from pharyngo-laryngitis, 58,3% in cystitis, 100% in enteritis.

The associations between bacteria and yeasts had a reduces incidence and those were presented in otitis (6,2%) and cystitis (8,3%) cases.

In infections produced by a single pathogen agent, 25 cases (44,64%) had bacteriene cause: 31,25% otitis, 50% conjunctivitis, 56,25% pyodermitis, 50% pharyngo-laryngitis, 41,66% cystitis.

The isolated germs frequence in function of the infection location is presented in tabel 2.

From the tabel 2 analysis was observed that in dog otitis ethiology more frequent incriminated was Pseudomonas aeruginosa (80%), Escherichia coli (26,3%%), Arcanobacterium pyogenes (23%) and Stapylococcus aureus (22,2%). In a reduced percentage Streptococcus spp., Clostridium spp. and Malassezia spp. Were isolated. From the cats otitis cases 15,7% Escherichia coli and 3,7% Staphylococcus aureus were isolated.

From urinary tract infections were colected urine samples in which were isolated more frequent : in dogs Escherichia coli (26,3%) and Clostridium perfringens (42,8%) in associations with Staphylococcus aureus, Streptococcus spp., Pseudomonas aeruginosa; in cats Arcanobacterium spp. (15,3%) in associations with Staphylococcus spp., Streptococcus spp and Candida spp.

Staphylococcus spp. was the main cause of pyodermitis in dogs as well as in cats (40,7% respectivlly 14,8%, followed by Arcanobacter spp. and Streptococcus spp. associated in small percentage with Pseudomonas aeruginosa, Proteus vulgaris and Clostridium perfringens stains.

In dogs and cats conjunctivitis Staphylococcus aureus and Arcanobacterium pyogenes was isolated in equal proportions.

In a more reduced percent (1%) in the ethiology of dogs and cats pharyngolaryngitis cases species from Staphylococcus, Arcanobacterium, Escherichia genuses were incriminated.

Vaginitis cases were produced by Escherichia coli (5,26%) in bitches and cats and by Arcanobacterium pyogenes (7,16%) only in bitches.

From the 2 coprocultures made in enteritis cases Escherichia coli strains were isolated.

A dates analysis from table 2 show the fact that 57,14% from the isolated strains are Gram positive and 42,85% are Gram negative bacteria, without significant differences regarding the implications of pathogen specie in cats and dogs infectious ethiology.

The isolated strains behaviour was different from case to case in function ofthe isolated flora structure. Antibiotics towards wich the isolated bacteriene flora sensitivity was tested, were: Norfloxacine, Euroxyl, Lincospectin, Gentamicine, Rifampicine, Synulox, Clamoxyl, Spectinomicin, Penicilline, Ciftiofur, Erythromicine, Doxycicline, Lincomycina, Ampiclox, Nistatin, Dioflucan.

The most effective antibiotics, in which the majority of flora was sensitive were: Ciftiofur, Euroxyl, Synulox, Norfloxacine, Lincospectin which proved their effiency „in vivo", too.

The mycotic strains isolated from the cystitis cases in cats and otitis cases in dogs, were sensitive towards Nistatin and Dioflucan.

CONCLUZII

1. Microbiological exams were made on 45 dogs and 19 cats with different infections: otits, conjunctivitis, pyodermitis, vaginitis, cystitis and enteritis
2. In 55,35% cases a mixte flora was isolated and in 44,65 cases pure bacteria or yeasts cultures were obtained.
3. The isolated microflora was represented by a big number of bacterien and mycotic strains (57,14 % Gram positive germs and 42,85% Gram negative germs).
4. The The results of the antibiograms made on total flora plead for the necessity of treatment as a consequence of the responsiveness to antibiotics of the involved tested microorganisms

Tabel 2

Frequence of germs isolated in function of the infection location.

Crt. No	Pathogen agent	Otitis				Conjunctivitis				Pahryngo-laryngitis				Pyodermitis				Vaginitis				Cystitis				Enteritis			
		Dogs		Cats		Dogs		Cats		Dogs		Cats		Dogs		Cats		Dogs		Cats		Dogs		Cats		Dogs		Cats	
		nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%	nr	%
1	*Staphylococcus spp.*	6	22,7	1	3,7	2	7,4	1	3,7	1	3,7			11	40,7	4	14,8					1	3,7	1	3,7				
2	*Streptococcus spp.*	1	14,2									1	14,2	2	28,5	1	14,2					1	14,2	1	14,2				
3	*Arcanobacterium spp.*	3	23			1	7,6	1	7,6	1	7,6	1	7,6	3	23			1	7,1	1	5,2			2	15,3				
4	*Escherichia spp.*	5	26,3	3	15,7							1	5,2	1	5,2			1	5,2			5	26,3			1	5,2	1	5,2
5	*Pseudomonas spp*	8	80											1	10							1	10						
6	*Proteus spp*													1	100														
7	*Clostridium spp.*	1	14,2											1	14,2							3	42,8						
8	*Candida spp.*																							1	10				
9	*Malassezia spp.*	1	100																										

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The level of residual nitrates in some ranges of cheese

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The level of residual nitrates of various ranges cheese was investigated for 262 samples trough spectrocolorimetric method.

The average of values in mg NO^-_3/kg was:

1,23 for raw cow cheese;

1,04 for green cheese of cow milk;

0,51 for green cheese of ewe's milk;

1,51 for Balkan cheese;

1,08 for „Caşcaval" cheese.

Key words: level, nitrates, cheese

Brânzeturile sunt produse alimentare de mare valoare nutritivă prin conţinutul lor ridicat în proteine, grăsimi, săruri minerale şi chiar unele vitamine (1, 2, 10, 14). Ele sunt produse care au o bună digestibilitate şi unele categorii din ele sunt recomandate în diverse diete alimentare în anumite boli gastro-intestinale, osteodistrofice, etc.

Pentru a corespunde rolului lor alimentar deosebit se impun din start unele măsuri:

- laptele materie primă folosit la procesarea lor, trebuie să provină de la animale sănătoase, să fie condiţionat corespunzător şi să nu conţină noxe fizice, chimice şi biologice peste LMA admisa;
- pausterizarea laptelui trebuie făcută corespunzător, iar prcesarea lui în brânzeturi se va executa în condiţii de igienă şi cu respectarea întocmai a tehnologiei;
- asigurarea unor condiţii optime de igienă şi tehnologice în perioada maturării, îngrijirii, conservării, depozitării, etc (10, 13, 14).

Brânzeturile sunt considerate ca surse relativ sărace în nitraţi pentru om chiar şi atunci când în laptele folosit la producerea lor s-a adăugat nitraţi în scop antibacterian (11, 13, 17).

Nitraţii conţinuţi iniţial în lapte cât şi cei adăugaţi în scop antibacterian (admişi în unele ţări) au următoarea evoluţie (10, 15, 17):

- o parte din ei se vor elimina prin zer în momentul sinerezei şi scurgerii brânzeturilor;
- metabolizarea altei părţi de nitraţi de către microorganismele din maielele folosite la fabricarea brânzeturilor.

Cu toate aceste aspecte o parte din nitraţii rămaşi vor fi convertiţi în nitriţi, care vor constitui sursele principale de nitrozare a aminelor şi a acizilor aminaţi rezultaţi în procesul de maturare a brânzeturilor şi formare de nitrozamine (7, 8, 9, 11, 13).

În brânzeturile maturate se găsesc atât amine primare, dar în deosebi amine secundare, care se nitrozează relativ uşor în prezenţa nitriţilor şi nitraţilor formându-se NDMA, NDEA, etc (4, 10, 11, 13).

În lucrare ne-am propus să stabilim conţinutul de nitraţi din trei sortimente de brânzeturi nematurate şi două sortimente de brânzeturi maturate.

MATERIAL ŞI METODĂ

Cercetările s-au efectuat pe 40 probe de brânză proaspătă de vacă, 20 probe de caş din lapte de vacă şi 20 probe de caş din lapte de oaie, ca brânzeturi nematurate.

Pentru brânzeturile maturate s-au făcut investgații pe 90 probe de brânză telemea şi 92 probe de caşcaval.

Pentru determinarea nitraților s-a folosit metoda spectrocolorimetrică cu reducere pe coloana de cadmiu. Rezultatele s-au exprimat în mg NO^-_3/kg.

REZULTATE ŞI DISCUŢII

Rezultatele investigațiilor atât pentru brânzeturile nematurate cât şi pentru cele maturate sunt redate în tabelul 1 şi diagrama nr. 1 şi nr. 2.

Tabelul 1

Nivelul nitraților reziduali în unele sortimente de brânzeturi

Categoria de brânzeturi	Nr. probe	Sortimentul	mg NO^-_3/l		Depăşiri ale LMA
			Limite	Media	
Nematurată	40	Brânză proaspătă de vacă	0,40-2,30	1,232	0
	20	Caş din lapte de vacă	0,60-1,80	1,040	0
	20	Caş din lapte de oaie	0,10-1,00	0,510	0
Total/Media	80	-	0,10-2,30	1,00	0
Maturată	90	Brânză telemea	0,40-5,00	1,51	0
	92	Caşcaval	0-6,80	1,11	0
Total/Media	182	-	0-6,80	1,31	0
Total/Media	262	-	0-6,80	1,20	0

Analiza datelor a evidențiat următoarele valori medii pentru brânzeturile proaspete: 1,23 mg NO^-_3/kg la brânza proaspătă de vaci; 1,40 mg NO^-_3/kg la caşul din lapte de vacă; 0,510 mg NO^-_3/kg la caşul din lapte de oaie (diagrama nr. 1).

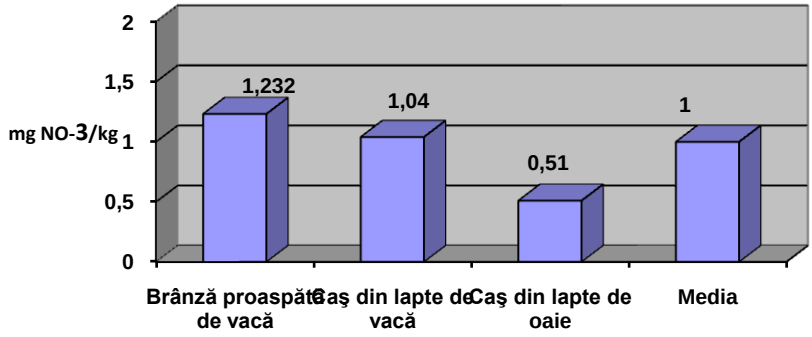

Diagrama nr. 1: Nivelul nitraților reziduali în unele brânzeturi nematurate (mg NO^-_3/kg).

Pentru cele două sortimente de brânzeturi maturate, telemea şi caşcaval din lapte de vacă nivelul nitraților reziduali a avut valori medii de 1,51 mg NO^-_3/kg la telemea şi 1,11 mg NO^-_3/kg la caşcaval (tabelul 1 şi diagrama nr. 2).

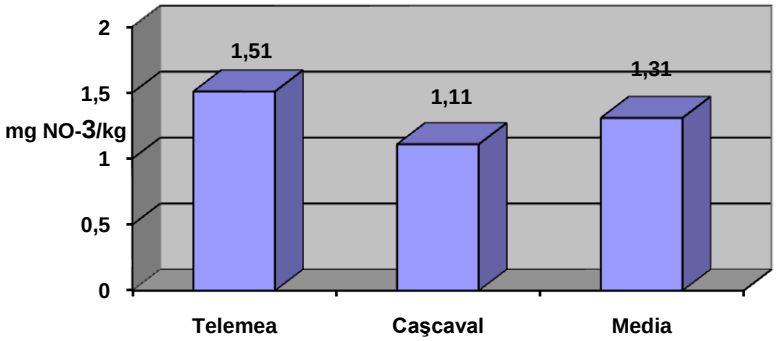

Diagrama nr. 2: Nivelul nitraţilor reziduali din unele brânzeturi maturate (mg NO^-_3/kg).

Faţă de nivelul nitraţilor din laptele de vacă (2,7 mg NO^-_3/l) nivelul lor a reprezentat 45,55% în brânza proaspătă de vaci; 38,52% în caşul din lapte de vacă şi 23,28% la caşul din lapte de oaie (2,19 mg NO^-_3/l lapte de oaie).

Pentru brânza telemea care se conservă prin sărare şi în zer, nitraţii reziduali au reprezentat circa 56,00% din conţinutul celor din lapte, iar în caşcaval 41,12%.

CONCLUZII

Cercetările nitraţilor reziduali din câteva sortimente de brânzeturi ne permit a formula următoarele concluzii:

1. În brânzeturile proaspete nematurate, nitraţii reziduali, la toate probele cercetate, au avut valori fără semnificaţie sanitară, situându-se valoric mult sub limita de toleranţă;
2. La brânzeturile maturate, telemea şi caşcaval, nitraţii reziduali s-au situat la nivele fără riscuri pentru consumatori şi inferioare celor stabilite pentru lapte.

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Reseaches concerning the nitrate level in raw milk pasteurized milk, powder milk and raw ewe's milk

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We are examined 210 samples of raw, pasteurized and powder milk trough spectrocolorimetric method.

The results regarding the residual level of nitrates on an average the next values in mg NO^-_3/l : 2,70 for raw cow milk; 2,61 for pasteurized milk; 2,13 for reconstituted powder milk; 2,19 for raw ewe's milk.

Key words: nitrates, level, milk

Principalele surse de nitraţi pentru om sunt reprezentate în unele vegetale care îi acumulează în rădăcini, frunze sau tulpini, apa cu conţinut ridicat de nitraţi, unele produse animale cum sunt preparatele din carne la care se folosesc nitraţi în amestecul de sărare (1, 2, 3, 7, 11, 15, 16).

Laptele şi produsele lactate pot fi considerate ca surse fără importanţă toxicologică pentru consumatori, în condiţiile când laptele provine de la animale în echilibru fiziologic şi nu i s-a adăugat nitraţi fraudulos (corecţia densităţii) sau în scopul acţiunii anticlostridene a nitriţilor rezultaţi din reducerea nitraţilor (9, 10, 14, 17, 19).În privinţa originii nitraţilor din lapte s-au conturat două ipoteze:

- ipoteza poluării laptelui cu nitraţi numai din sursa externă directă ca apa folosită la spălarea vaselor şi utilajelor de muls, care conţinea nivele ridicate de nitraţi, îndepărtarea „pietrei de lapte” cu acid azotic de pe plăcile pasteurizatoarelor fără clătirea lor corespunzătoare, adaosul de nitraţi laptelui fraudulos în scop de conservare şi adaosul intenţionat în laptele folosit la prepararea unor sortimente de brânzeturi pentru a preveni „balonarea târzie” (2, 5, 8, 16, 17, 19).
- ipoteza prezenţei nitraţilor în lapte prin secreţia lactată de către animalele care au ingerat furaje cu conţinut ridicat de nitraţi şi/sau adăpat din surse de apă cu nivel ridicat de nitraţi. Se consideră că există o corelaţie strânsă între nivelul azotaţilor din sol, care se transferă plantelor şi ingesta acestora animalelor prin furajare. Lanţul trofic – sol – plantă – animal – produse animale – nu poate fi neglijat.

În laptele de oaie şi vacă nivelul nitraţilor reprezintă 48-78% cu o medie de 60% din cel sanguin şi constitue un suport care indică laptele ca o cale de eliminare a nitraţilor (2, 6, 9, 11, 13, 16, 17, 18, 20, 21, 22). În lapte deci, nitraţii pot proveni direct din surse externe, dar şi intern din ingesta cu nivel ridicat de nitraţi. Nivelul nitraţilor din lapte poligastricelor este totuşi redus, având în vedere că acestea pot folosi azotatul ca sursă de azot asimilabil la intervenţia simbionţilor rumenali (15, 16).

Dacă cantitatea nitraţilor depăşeşte posibilitatea activităţii de sinteză în azot asimilabil de către simbionţi, mai ales în stări de disfuncţii rumenale, nivelul nitraţilor sangvini şi din lapte poate creşte şi deveni periculos. Pentru laptele de vacă şi oaie, nivelul nitraţilor din lapte reprezintă cam 60% din cel sangvin (20, 21, 22).

În lucrare ne-am propus evaluarea nivelului de nitraţi din laptele crud de vacă şi oaie, laptele pasteurizat şi laptele praf reconstituit.

MATERIAL ŞI METODĂ

Investigațiile s-au făcut pe 95 probe de lapte crud de vacă, 40 probe de lapte pasteurizat, 45 probe lapte praf reconstituit şi 30 probe de lapte de oaie.

Pentru determinarea nitraților s-a folosit metoda cu reducere pe coloană de cadmiu (spectrocolorimetrie), iar măsurarea absorbanței s-a făcut la lungimea de undă 538 nm. Exprimarea nitraților s-a făcut mg ioni de NO^-_3/l.

REZULTATE ŞI DISCUŢII

Investigațiile făcute pe cele 210 probe de diverse sortimente de lapte sunt redate în tabelul nr. 1 şi diagrama nr. 1. Exprimarea azotaților s-a făcut în mg NO^-_3/l conform metodei folosite.

Datele obținute, arată că la cele 95 probe de lapte colectură, nitrații au avut limite de variație cuprinse între 0 şi 7,5 mg NO^-_3/l şi o medie de 2,7 NO^-_3/l. Cuantificând nivelurile de nitrați s-a putut observa:

- nivel 0 mg NO^-_3/l la 2 probe (2,1%);
- nivel între 0,1-2 mg NO^-_3/l la 20 probe (21,05%);
- nivel între 2,1-4 mg NO^-_3/l la 61 probe (64,21%);
- nivel între 4,1-6 mg NO^-_3/l la 9 probe (9,47%);
- nivel între 6,1-7,5 mg NO^-_3/l la 3 probe (3,16%).

Tabelul 1

Nivelul nitraților în diverse sortimente de lapte

Sortimentul de lapte	Nr. probe	Mg NO^-_3/l		Depăşiri ale LMA
		Limite	Media	
Lapte proaspăt	95	0-7,50	2,70	0
Lapte pasteurizat	40	0,-4,10	2,61	0
Lapte praf reconstituit	45	0-2,90	2,13	0
Lapte de oaie	30	0,4-3,90	2,19	0
Total/Media	210	0-7,50	2,50	0

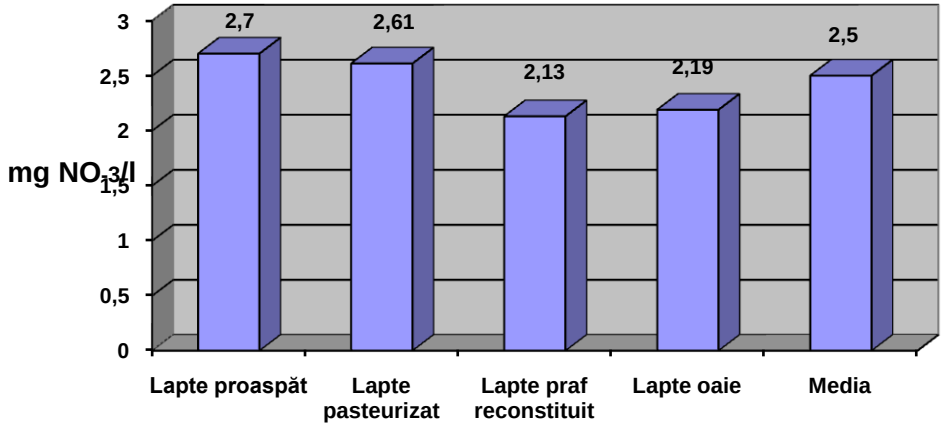

Diagrama nr. 1: Valori medii ale nivelurilor de nitrați în diverse sortimente de lapte (mg NO^-_3/l).

Această cuantificare a nivelurilor de nitrați din lapte a evidențiat faptul că la majoritatea probelor (64,21%) nitrații reziduali s-au situat între 2,1-4 mg NO^-_3/l de lapte proaspăt.

La laptele pasteurizat valoarea medie a nitraților a fost de 2,61 mg NO^-_3/l, fiind foarte apropiată de ceea a laptelui nepasteurizat. Limitele de variație la această categorie de lapte au fost cuprinse între 0 şi 4,10 mg NO^-_3/l.

Conținutul de ioni de NO^-_3/l la cele 40 probe de lapte pasteurizat cercetate s-a clasat astfel:

- nivel 0 de mg NO^-_3/l la 2 probe (5,00%);
- nivel până la 2 mg NO^-_3/l la 5 probe (12,50%);
- nivel între 2,1-3,00 mg NO^-_3/l la 22 probe (55,00%);
- nivel între 3,1-4,1 mg NO^-_3/l la 11 probe (27,50%).

Pentru laptele praf reconstituit cu apă bidistilată, conținutul mediu de nitrați a avut o valoare medie de 2,13 mg NO^-_3/l, iar limitele au fost cuprinse între 0 şi 2,90 mg NO^-_3/l.

Procesarea laptelui în lapte praf evidențiază o conservare în proporție de circa 80% a nivelului inițial de nitrați din lapte, dacă se ține cont de datele stabilite la laptele crud (2,7 mg NO^-_3/l) şi cele ale laptelui praf (2,13 mg NO^-_3/l).

Datele obținute pentru laptele de oaie au stabilit o medie de 2,19 mg NO^-_3/l şi limitele cuprinse între 0,4-3,90 mg NO^-_3/l.

Nivelul nitraților la cele 30 de probe de lapte de oaie, s-au situat valoric astfel:

- nivel între 0,4 şi 0,8 mg NO^-_3/l la 5 probe (16,66%);
- nivel între 1,3 şi 1,9 mg NO^-_3/l la 7 probe (23,33%);
- nivel între 2,1 şi 2,9 mg NO^-_3/l la 11 probe (36,66%);
- nivel între 3,1 şi 3,9 mg NO^-_3/l la 7 probe (23,33%);

CONCLUZII

Investigațiile ne permit să enunțăm câteva concluzii:

1. Nivelurile nitraților în laptele proaspăt de vacă, în laptele pasteurizat şi în laptele praf reconstituit au avut valori în mg NO^-_3/l, care nu constitue riscuri pentru sănătatea publică. Aceste valori au fost cuprinse între:
   - 0 şi 7,5 mg NO^-_3/l la laptele de colectură;
   - 0 şi 4,10 mg NO^-_3/l la laptele pasteurizat;
   - 0 şi 2,90 mg NO^-_3/l la laptele praf reconstituit;
2. Procesarea laptelui proaspăt prin pasteurizare şi uscare în lapte praf pare a nu influența deosebit nivelul de nitrați inițial din lapte.
3. Nivelurile de nitrați stabilite la laptele de oaie au avut valori fără semnificație toxicologică variind între 0,4 şi 3,9 mg NO^-_3/l.

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Confirming PRRS virus presence using laboratory tests

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In order to eradicate PRRS some countries use vaccination, in those farms diagnostic approach is based on virus isolation. Serological exams are used only to appreciate the effective immunity. Serologic exams are highly efficient in atipical disease forms.

In the following paper are presented laboratory protocols used to confirm disease presence.

Key Words: P.R.R.S., serology, confirmation

Printre entităţile morbide ale porcului recent diagnosticate şi cu implicaţii economice deosebite se înscrie şi sindromul tulburărilor de reproducţie şi respiratorii la porcine (porcine reproductive and respiratory syndrome – PRRS). În evoluţia focarelor de boală, pe lângă persistenţa virusului în efectiv, un rol deosebit se atribuie prezenţei anticorpilor postinfecţioşi.

Utilizarea unor examene care asigură un diagnostic rapid şi precis are un rol deosebit în combaterea şi eradicarea PRRS. Diagnosticul în PRRS reprezintă un cumul de date, ce conţin aspecte epidemiologice şi clinice, examene anatomopatologice şi histologice confirmate prin diagnosticul de laborator efectuat prin teste ce evidenţiază anticorpii umorali anti-PRRS post-infecţioşi sau post-vaccinali.

MATERIAL SI METODE

După studierea tabloului clinic şi efectuarea examenului necropsic s-au întreprins examene de laborator.

În scopul precizării diagnosticului şi stabilirii tratamentului optim au fost prelevate probe de la toate categoriile de animale. S-au efectuat 567 examene bacteriologice, virusologice, serologice şi de profil metabolic.

Testările serologice au avut ca scop decelarea anticorpilor antivirus P.R.R.S. şi s-au efectuat pe două categorii de probe de ser, la fel ca şi supravegherea nutriţional-metabolică:

- **o primă categorie de probe** a fost prelevată anterior apariţiei primelor semne clinice atribuite infecţiei P.R.R.S., recoltate în alte scopuri (decelare anticorpi GET şi pestă porcină) şi păstrate în laborator;
- **o a doua categorie de probe** a fost recoltată după apariţia primelor semne clinice de boală.

Au fost efectuate examene serologice şi în direcţia altor infecţii virale şi bacteriene.

În prima etapă, s-au analizat 118 probe de sânge, de la vieri de reproducţie, scroafe gestante şi tineret înţărcat.

S-au testat 27 de parametri, care au inclus indici proteici, glucidici, lipidici, enzimatici, vitaminici şi minerali.

În etapa a doua s-au examinat 125 probe de sânge recoltate de la scroafe gestante, purcei sugari, tineret şi porci graşi. S-au analizat principalii parametri hematologici şi biochimici serici.

REZULTATE ŞI DISCUŢII

În cadrul examenului de laborator au fost prelucrate în vederea precizării diagnosticului bacteriologic un număr de 567 probe. Rezultatele investigaţiilor efectuate sunt redate în tabel nr. 1.

Tabel nr. 1

Investigații bacteriologice în perioada evoluției P.R.R.S.

Categoria de animale	Probe examinate	Rezultatele examenului bacteriologic				Infecții bacteriene secundare
	Nr.	Negative		Pozitive		
		Nr.	%	Nr.	%	
Sugari	217	108	49,7	109	50,2	*E.coli*, *Streptococcus spp*., C*l. perfringens*, *S.choleraesuis*, *Camplylobacter spp*.
Tineret	322	46	14,3	276	85,7	*S. choleraesuis*, *E coli., Streptococcus spp., Actinobacillus spp., Mycoplasma spp.,* P*asteurella spp*.
Graşi	28	17	60,7	11	39,3	*Pasteurella spp., Actinobacillus pl*. *Pneumococcus pneumoniae*

Din tabel se constată următoarele:

- prezența infecției cu virus P.R.R.S. în unitate a mărit incidența şi a exacerbat gravitatea evoluției unor boli cu etiologie bacteriană existene în efective;
- speciile bacteriene izolate şi identificate din probele prelevate în perioada evoluției P.R.R.S. sunt identice cu cele izolate şi identificate în perioada anterioară acesteia.

Investigațiile efectuate în direcția decelării unor infecții bacteriene au relevat, în special la categoriile purcei sugari şi porci graşi, prezența unui procent ridicat de probe la care examenul bacteriologic a fost negativ, (49,7% şi respectiv 14,3%).

La categoria tineret în creştere, deşi nu s-au înregistrat entități morbide bacteriene diferite față de perioada anterioară evoluției P.R.R.S., s-a constatat o creştere a frecvenței cazurilor de salmoneloză, poliserozită infecțioasă, enterotoxiemie colibacilară şi dizenterie cu *Serpulina spp*., ceea ce a impus aplicarea unor tratamente antiinfecțioase în doze terapeutice administrate parenteral sau/şi sub formă de premixuri medicamentate.

Testarea sensibilității față de o serie de antiinfecțioase a diverselor tulpini de *E.coli* şi *Salmonella spp*. izolate de la purcei sugari şi tineret pe perioada de evoluție a P.R.R.S. a scos în evidență o variabilitate a activității acestor antibiotice.

De asemeni, s-au efectuat şi testări serologice pentru P.R.R.S. prin testul ELISA (tabel nr. 2), folosind trusa de diagnostic Ingenasa, şi P.R.S.S. Antibody Test Kit, în scopul decelării anticorpilor antivirus P.R.R.S. pe cele două categorii de probe de ser.

Tabel nr. 2

Rezultatul testărilor serologice pentru P.R.R.S. pe categorii de animale

Nr. crt.	Categoria de animale	Nr. probe testate	Rezultate obținute	
			Pozitive	Negative
1	Scroafe lactante	10	-	10
2	Scroafe lactante	15	15	-
3	Scroafe lactante	5	5	-
4	Scroafe lactante	19	-	19
5	Purcei sugari	12	-	12
6	Scroafe lactante	7	7	-
7	Scroafe lactante	7	1	6
8	Scroafe lactante	7	6	1
9	Tineret	13	13	-
10	Vieri	13	7	6
11	Vieri în carantină	14	-	14
12	Scroafe lactante	60	56	4
13	Tineret	27	24	-

Prin testări serologice efectuate pe probe recoltate de la scroafe lactante din toate fermele de maternitate s-a constatat că acestea au fost seropozitive 93,3%, ceea ce evidenţiază faptul că grupele de scoafe lactante prezintă o imunitate postinfecţioasă.

Au fost efectuate examene serologice şi în direcţia altor infecţii virale şi bacteriene. Rezultatele sunt redate în **tabel nr. 3**.

Tabel nr. 3

Alte examene serologice efectuate

Afecţiunea suspectă	Testul utilizat	Categoria de animale	Nr. probe examinate	Rezultatele obţinute			
				Pozitive		Negative	
				Nr.	%	Nr.	%
Boala lui Aujeszky	ELISA	Scroafe lactante	52	24	46	28	54
Parvoviroza	ELISA	Scroafe lactante	60	59	98,3	1	1,7
Leptospiroza	RML	Scroafe lactante	158	0	-	158	-

Din tabelul de mai sus reiese că 98,3% din scroafele lactante au prezentat reacţii pozitive pentru parvoviroză.

Titrurile serologice decelate faţă de virusul bolii lui Aujeszky se corelează cu aplicarea vaccinului anti-Aujeszky în unitate, însă nu se exclude şi o posibilă circulaţie a virusului sălbatic în efectiv. Referitor la infecţia leptospirică, în condiţiile aplicării imunoprofilaxiei specifice şi a supravegherii serologice permanente, boala nu reprezintă o problemă sanitară-veterinară.

Supravegherea nutriţional-metabolică prin teste de laborator poate fi prezentată în două etape:

- în prima etapă este vorba de probe prelevate până la apariţia bolii , iar
- a doua include analize efectuate în lunile în care a evoluat P.R.R.S..

În prima etapă, anterioară diagnosticului P.R.R.S., s-au analizat 118 probe de sânge, de la vieri de reproducţie, scroafe gestante şi tineret înţărcat.

S-au testat 27 de parametri, care au inclus indici proteici, glucidici, lipidici, enzimatici, vitaminici şi minerali. Interpretarea rezultatelor obţinute a evidenţiat unele tulburări nutriţionale metabolice, care au constat în principal din: valori mărite ale albuminemiei şi ureei serice, creşterea activităţii unor enzime (TGP, GCT, CPK), scăderea activităţii fosfatazei alcaline serice, valori mai reduse ale unor vitamine (A şi E) şi microelemente (Cu, Zn, Se), anemie nutriţională. Rezultatele indică prezenţa unor afecţiuni hepato-renale primare sau secundare şi a unor carenţe în unele microingrediente esenţiale (vitamine şi microelemente) (**tabel nr. 4)**.

Tabel nr. 4

Rezultatele testelor biochimice

Indice testat	**Rezultat**	**Indice testat**	**Rezultat**
Albuminemie	↑	Fosfataza alcalină	↓
Uree serică	↑	Vit.A, vit. E	↓
Enzime (tgp,gct,cpk)	↑	Microelemente(Cu,Zn,Se)	↓

În etapa a doua s-au examinat 125 probe de sânge de la scroafe gestante, purcei sugari, tineret şi porci graşi. S-au analizat principalii parametrii hematologici şi biochimici serici. S-au constatat în special la purceii sugari modificări hematologice exprimate prin anemie, leucopenie, hipoglobulinemie asociate cu hiperalbuminemie, creşterea activității unor enzime serice (TGP, CPK) şi unele carențe în vitamine (A şi E) şi microelemente (Fe, Se, Cu). Modificările menționate se corelează cu acțiunea imunosupresoare a virusului P.R.R.S., modificări citate şi în literatura de specialitate.

CONCLUZII

Din analiza rezultatelor testărilor serologice efectuate în scopul evidențierii anticorpilor antivirus P.R.R.S., reies următoarele concluzii:

1. Probele prelevate anterior apariției primelor semne de boală au fost seronegative.
2. Odată cu apariția primelor semne clinice de boală toate probele prelevate şi investigate în direcția PRRS au fost seropozitive.
3. La testările serologice efectuate în aceeaşi perioadă în fermele 1 şi 7, unde manifestările clinice erau incipiente, reacțiile seropozitive, au fost decelate numai la o parte din probele examinate.
4. Investigațiile serologice efectuate la categoria tineret crescătorie, au evidențiat faptul că acestea erau în totalitate pozitive.
5. Probele de ser provenite de la vierii aflați în carantină au fost negative.

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Researches concerning the residues of heavy metals (Zn, Cu, Pb, Cd) and As in fresh whole milk from Moldavian area using atomic absorption spectrofotometry method

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The purpose of this study was to identify and evaluate the residues of heavy metals (Zn, Cu, Pb, Cd) and As in fresh whole milk from different centres from Moldavian area, during year 2005 and 2006.

In this period were analysated 160 samples of fresh whole milk. Heavy metals and As were assesed using atomic absorption spectrofotometry method.

The results shown that 100% of analysed samples contained zinc, 62.5% contained cooper, 25% contained lead, 37.5% contained cadmium. Values of zinc concentrations in samples varied between 2.1 ppm and 3.9 ppm, values of cooper concentrations varied between 0.2 ppm and 0.4 ppm, value of cadmium concentration was 0.001 ppm and value of lead concentration was 0.01 ppm. In 160 samples of fresh whole milk, arsenium was undetected. The results of the researches were under the maximal limits accepted, estabilished and foreseed by the Ord. ANSVSA 97/2005.

Key Words: residues of heavy metals, milk

The development of industry, transports, agriculture through using fertilizers in excess, has generated harmful effects in environment. The results of the undertaken researches until now, are showing that a lot of animal products are chemically contamined, having harmful agents of great risk.

Animal products become a potential danger for the human body, being harmful according to their results contamination degree, from different chemical pollutions. .[1, 3]

Heavy metals take a particular place among contaminants, because of their toxicity, long survival time, accumulation in nature and circular course in biosphere. That in why, the heavy metals toxicity is the results of their attach on enzimatic sistems of animals cell or on certain compounds of membranes cells

Some heavy metals arrived humans bodies over limits and determinate toxical states. The degree of toxicology depends on the chemical contaminant, or its solubility, on the accumulative effect in tissues, on the metal dose intake and on the heavy metal action time. [7]

Fresh whole milk could be contaminated with heavy metals directly in time of conservation, transports from packings and also through troffic chain.

Heavy metals have a toxic effect and oncological action, made hepatic, cutaneous and pulmonary cancer and changed hematological parameters. [2, 4, 5, 6]

Material and method

These researches were set up to identify and evaluate the concentration of the residues of heavy metals (Zn, Cu, Pb, Cd) and As in fresh whole milk.

The investigations were made on 75 samples of fresh whole milk during 2005 year and on 85 samples of fresh whole milk during 2006 year. All samples were proceeded from 6 routes fron Moldavian area, gathered and processed with a view to obtain dairy products.

The harvesting and the preparation of fresh whole milk samples for analysis were made according to sanitary- veterinary norms.

The determination of Zn, Cu, Pb ,Cd and As concentrations from the samples was made by using atomic absorption spectrofotometry method in flame, with burner feedet with mixted air-acetylene at maximum 2250°C.

The heavy metals and arsenium were determined after previous mineralization using atomic absorption with flame GBC-AVANTA apparatus.

Results and discussion

The results of heavy metals and arsenium concentrations in fresh whole milk samples are presented in tables 1 and 2.

Table 1

The results of heavy metals and arsenium concentrations in fresh whole milk samples in 2005

Year	Centre	No. Samples	Zn(ppm)	Cu(ppm)	Cd(ppm)	Pb(ppm)	As(ppm)
2005	I	10	2.2-2.7	0.2-0.3	n.d.	0.01	n.d.
	II	15	2.3-2.5				
	III	10	2.1-2.8				
	IV	5	2.4-3.6				
	V	20	2.2-3.4	n.d.		n.d.	
	VI	15	2.8-3.6				

The results researches concentrations efectuated on 75 samples fresh whole milk in 2005 year shown the pressence of heavy metals. That, in all 75 samples analysated the zinc values concentrations were ranged from 2.1 to 3.6 ppm, at 40 samples the cooper values concentrations were ranged from 0.2 to 0.3 ppm, at 40 samples the lead concentrations values were 0.01 ppm. In 2005 year, cadmium and arsenium were undetected to the limit of sensibility of method.

Table 2

The results of heavy metals and arsenium concentrations in fresh whole milk samples in 2006

Year	Centre	No samples	Zn (ppm)	Cu (ppm)	Cd (ppm)	Pb (ppm)	As (ppm)
2006	I	15	2.1-3.9	0.2-0.4	0.001	n.d.	n.d.
	II	5	2.4-3.2				
	III	15	2.5-3.7				
	IV	25	2.2-2.6				
	V	15	2.8-3.8	n.d.	n.d.		
	VI	10	2.1-2.7				

The results researches concentrations efectuated on 85 samples fresh whole milk in 2006 year shown the pressence of heavy metals. That, in all 85 samples analysated the zinc values concentrations were ranged from 2.1 to 3.8 ppm, at 60 samples the cooper values concentrations were ranged from 0.2 to 0.4 ppm, at 60 samples the cadmium concentrations values were 0.001 ppm. In 2006 year, lead and arsenium were undetected to the limit of sensibility of method.

The average concentrations values of zinc and cooper in samples in 2005 and 2006 are shown in figures no 1 and 2.

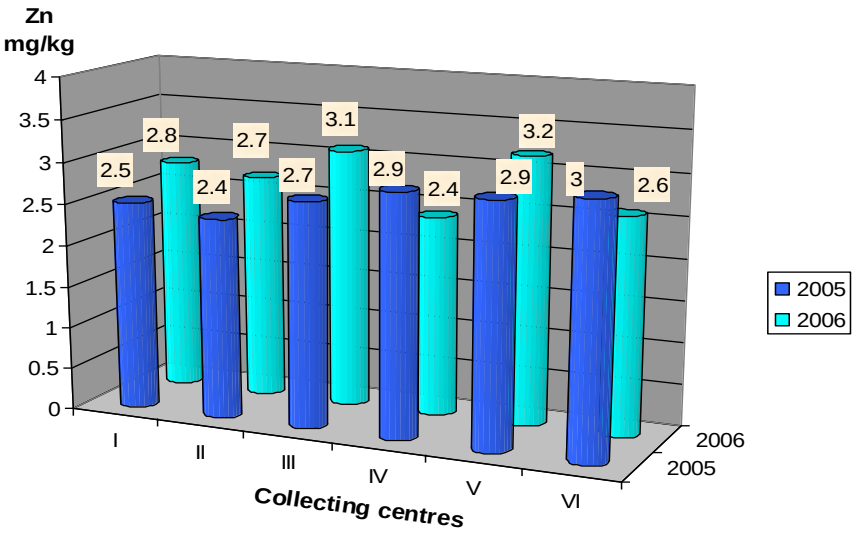

Figure 1. Average concentrations values of zinc in 2005 and 2006

Levels of zinc, cooper, lead, cadmium and arsenium concentrations in 160 samples of fresh whole milk were relative low and the assesed values were under the admited maximal limits estabilished by the ANSVSA Ord. no. 97/2005: 0.01 mg/kg milk – cadmium, 0.10 mg/kg milk – lead, 5 mg/kg milk – zinc, 0.5 mg/kg milk – cooper, 0.1 mg/kg milk – arsenium. [8]

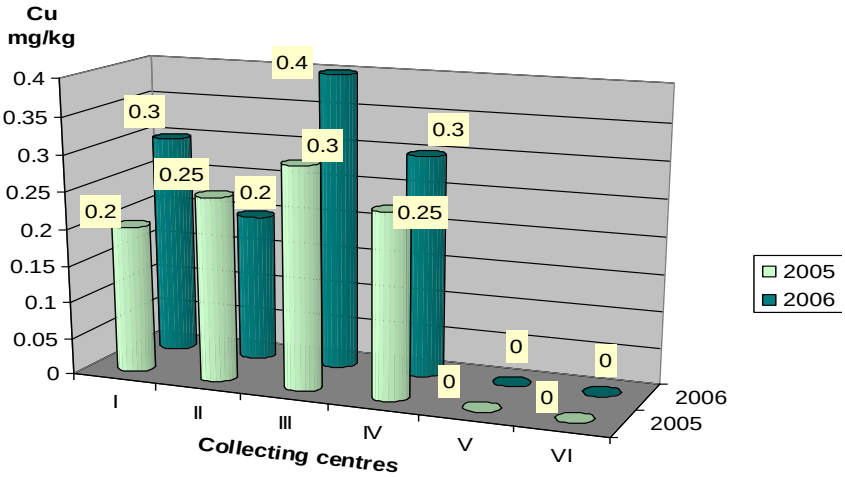

Figure 2. Average concentrations values of cooper in 2005 and 2006

Though the levels concentrations of contaminants were relative low, through long time accumulative effect in tissues; is necessary to continue the investigations, supervisions and monitoring in heavy metals concentration of fresh whole milk.

CONCLUSIONS

After the analitical researches of heavy metals had been expressed the following conclusions:

1. From 160 analised samples of fresh whole milk in 160 (100%) analised samples of fresh whole milk was found values of Zn concentration, in 100 (62.5%) analised samples of fresh whole milk was found values of Cu concentration, in 60 (37.5%) analised samples of fresh whole milk was found values of Cd concentration, in 40 (25%) analised samples of fresh whole milk was found values of Pb concentration.

2. Values of As concentration in analised samples of fresh whole milk were 0 analitical (undetected).

3.The values of the contaminants in the analised samples of fresh whole milk variate betweens samples in same collecting centre also, the values of content in heavy metals were variate betweens samples from different collecting centre; those variations were different because the samples were harvestind from different polluates zones of Moldavia.

4. The levels concentrations of contaminants were relative low under the maximal limits admited and estabilished by the ANSVSA Ord. no. 97/2005.

5. The results obtained by analiysis in this research, determined to continue the investigations, supervisions and monitoring in heavy metals of fresh whole milk on Moldavian area.

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Researches regarding milk salubrity

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Researches were done on 14 milk samples. Milk salubrity was appreciated using total germs count and nine samples were considered unsuited for human consume while the other five samples were microbiological salubrious. Another parameters considered in milk salubrity level were the number of somatic cell and probable number of coliforms. Coliforms level was between 9, 5 – 1100 coliforms / ml and the number of somatic cells was between 90300 NCS/ml- 483000NCS/ml.

Keys words: milk, N.T.G.,coliforms, NCS

Laptele face parte din categoria celor mai des folosite „ingrediente"în meniul zilnic al oamenilor şi în mod deosebit al copiilor, datorită, mai ales, proprietăţilor nutritive pe care le posedă.Principalii indicatori igienico-sanitari ai laptelui de consum sunt încărcătura microbiană şi, nu în ultimul rând, conţinutul lui în celule somatice.

Numărul celulelor somatice reprezintă un important criteriu de evaluare a stării de sănătate a glandei mamare şi, totodată un important criteriu de apreciere a salubrităţii laptelui.

MATERIAL ŞI METODĂ

Laptele s-a recoltat în eprubete sterile (cca. 2o-25 ml) . Probele au fost examinate în laboratorul de microbiologie a Facultăţii de Medicină Veterinară determinându-se:

- proba reductazei, metoda cu resazurină;
- determinarea numărului probabil de bacterii coliforme;
- determinarea numărului de celule somatice.

Proba reductazei

Resazurina, din punct de vedere chimic este o oxazonă care introdusă în laptele crud integral dă o coloraţie albastră. Sub influenţa enzimelor bacteriene din lapte aceasta este redusă în rezorufină de culoare roz şi apoi în dihidrorezorufină incoloră . Pe baza aprecierii nuanţei de culoare la care ajunge amestecul lapte-resazurină după o oră de incubare la 37°C laptele se clasifică după calitatea microbiologică în patru clase.

Într-o eprubetă sterilă de 18xl8o mm se introduc cu pipeta 1 ml soluţie resazurină peste care se adaugă 10 ml lapte crud integral. Eprubetele se închid cu dopuri de cauciuc, se omogenizează proba .

Se introduce într-o baie de apă reglată la temperatura de 37°C (sau termostat adus la temperatura respectivă). După o oră se scoate proba din baia de apă (sau termostat), se agită prin răsturnare, o dată sau de două ori pentru uniformizarea conţinutului şi se examinează proba de culoare . Se evită atât efectuarea analizei cât şi citirea rezultatului în lumină solară directă care provoacă modificări rapide de culoare.

Conform normelor interne pentru laptele crud integral se admit 500000germeni/ml lapte. În schimb normele europene sunt foarte severe în privinţa calităţii igienice a laptelui. Astfel, se cere ca acesta să conţină sub 100000 germeni/ml lapte; depăşirea acestei norme determină interdicţia de livrare a laptelui.

Relația între calitatea microbiană a laptelui şi numărul prezumtiv de germeni este redată în tabelul 1.

Tabel nr.1

Interpretarea testului reductazei şi aprecierea calității microbiologice a laptelui

Nuanța de culoare	Calitatea laptelui	Clasa de calitate microbiologică	Clasa de alitate microbiologică	Număr prezumtiv de germeni/ml lapte
Albăstrui-oțel, albăstrui-palid	Bună	I	I	Sub 500.000
Violet-albăstrui, violet-roşu	Satisfăcătoare	II	II	500.000- 4.000.000
Roz,roz spre alb	Nesatisfăcătoare	III	III	4.000.000- 20.000.000
alb	Total nesatisfăcătoare	IV	IV	Peste 20.000.000

Determinarea bacteriilor coliforme

Principiul se bazează pe proprietatea acestor germeni de a fermenta lactoza cu producere de gaze. Dezvoltarea culturilor se face pe mediul cu bulion-bilă-lactoză-verde briliant repartizate în eprubete cu tubulețe de fermentație(Durham), iar calculul numărului de germeni probabil se face după tabelul Mac-Grady.

Din laptele de cercetat se fac diluții: 1/10; 1/100; 1/1000; 1/10000; 1/1000000. Se însămânțează serii de trei tuburi cu câte 1ml din laptele nediluat şi din fiecare diluție. Tuburile însămânțate se incubează la termostat 48 de ore la 37°C. Prima citire a tuburilor se face la 24 de ore de incubație, iar citirea definitivă la 48 de ore.

Când cel puțin 1/10 din coloana de lichid din tubul Durham a fost dislocată de gaze se consideră test pozitiv prezumtiv pentru coliformi.

Conform standardelor actuale, pentru laptele crud integral se admit maxim 1000 bacterii coliforme/ml, cu excepția laptelui crud materie primă pentru laptele praf, lapte condensat şi lapte praf pentru copii, la care se admit maxim 100 bacterii coliforme/ml. Pentru laptele de consum se admit maxim10 bacterii coliforme/ml.Confirmarea pentru coliformi s-a făcut pe mediul Levine.

Din fiecare eprubetă considerată pozitivă se fac treceri cu ansa pe mediul Levine.Se incubează la 37º C, timp de 24 ore.Pot apare colonii tipice sau atipice.Coloniile tipice au culoare închisă, albastră- verzuie, cu luciu metalic.

Determinarea numărului de celule somatice

Pentru determinarea numărului de celule somatice s-a folosit aparatul SOMACOUNT 150.

Aparatul determină numărul de celule somatice/ml lapte, prin numărarea pulsurilor electrice, determinate de trecerea acestor celule printr-un capilar transparent plasat în fața unei raze laser de culoare verde.

Proba de lapte se recoltează în flacoane de plastic de 50 ml, care se încălzesc la 40º C, după care se analizează. Aparatul este prevăzut cu un agitator care se introduce în fiecare flacon pentru omogenizarea probei de lapte. După agitare, aparatul extrage din proba de analizat 2,5 ml lapte, printr-un impuls, apoi 3 ml de soluție colorantă. Din amestec, aparatul preia 1 ml şi îl trimite prin capilarul transparent la dispozitivul de citire.

Conform normelor europene pentru laptele crud se admit

400000număr celule somatice/ml

REZULTATE ŞI DISCUȚII

Examenul probelor de lapte a relevat un nivel de contaminare microbiologic foarte variat. Numărul total de germeni (NTG) în cele 14 de probe examinate a avut valori cuprinse între

$7,14x10^4$ germeni/ml şi $9,75x10^5$ germeni/ml, corelându-se cu rezultatul testului reductazei.Numărul celulelor somatice a variat între 90300NCS/ml şi 483000NCS/ml (tabel nr.2*)*

Tabel nr. 2

Valori observate la investigaţiile efectuate pe laptele de vacă

Proba	NTG/ml	Testul reductazei	NCS/ml
1	$6,24x10^4$	satisficător	120500
2	$5,62x10^5$	satisficător	72170
3	$5,68x10^5$	satisficător	29160
4	$9,62x10^4$	bun	67170
5	$7,14x10^4$	bun	34833
6	$1,28x10^5$	bun	53000
7	$1,64x10^5$	bun	33170
8	$7,24x10^5$	satisficător	483000
9	$8,16x10^5$	satisficător	438330
10	$8,64x10^5$	satisficător	371429
11	$7,22x10^5$	satisficător	481500
12	$9,75x10^5$	satisficător	201500
13	$6,30x10^5$	satisficător	134830
14	$1,25x10^5$	bun	90300

Analizând datele din tabel rezultă că din cele 14 de probe:

-5(35,71%) au avut o încărcătură microbiologică situată sub limita maximă admisă (500.000 germeni/ml);

-9(64,28%) au depăşit limita maxima admisă fiind improprii pentru consumul uman;

-11(78,57%) s-au încadrat în limita normală admisă în ceea ce priveşte numărul de celule somatice (400000/ml);

-3(21,42%) au depăşit limita maximă admisă.

Rezultatele obţinute la testul reductazei concordă cu NTG-ul, indicând 2 categorii de calitate: bun (5probe), satisfăcător (9 probe).

În ceea ce priveşte numărul de bacterii coliforme, acesta variază între 9,5 coliformi/ ml şi 1100 coliformi/ml; 5 de probe au avut încărcătura sub 1000coliformi/ml şi 9 probe peste 1000coliformi/ml.

CONCLUZII

- S-a urmărit aprecierea salubrității laptelui de vacă pe baza a 3 indicatori igienico-sanitari: încărcătura microbiană(NTG-ul;testul reductazei); numărul probabil de bacterii coliforme; numărul de celule somatice.
- Numărul de germeni/ml a variat între $7,14x10^4$ germeni/ml şi $9,75x10^5$ germeni/ml
- Proba reductazei efectuată pe laptele recoltat a fost bună, satisfăcătoare, corelându-se cu NTG-ul.
- În funcție de rezultatele celor două teste microbiologice (NTG, reductaza), 9(64,24%) din probe au depăşit limitele bacteriologice admise(500.000 germeni/ml).
- Numărul de germeni variază în funcție de sezon, fiind în general mai scăzut în sezonul rece (toamna, iarna) şi mai crescut în sezonul cald (primăvara, vara). Numărul bacteriilor coliforme a variat între 9,5 coliformi/ml şi 1100 coliformi/ml lapte.
- Numărul bacteriilor coliforme în lapte este mai mare mai ales atunci când igiena corporală a animalelor şi a mulsului sunt neglijate şi posibilitatea poluării laptelui cu materii fecale este mai mare.
- Numărul de celule somatice/ml a variat între 90300NCS/ml şi 483000NCS/ml.

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Detection limit – a core step in a method validation protocol in residues analyse

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Establishing the detection limit is the first experimental step in the validation of an analytical method. Method detection limits (MDL) are statistically determined values that define how easily measurements of a substance by a specific analytical protocol can be distinguished from measurements of a blank (background noise).

A common misconception is that the MDL is the smallest concentration that can be measured. Instead, it is the concentration at which we can decide (with a specified degree of confidence) whether an element is present or not. Method detection limits are matrix, instrument and analyst specific and require a well defined analytical method.

Key Words: detection limit, method validation, residues analyse

Matherials and Methods

Method validation is a very important requirement in the practice of chemical analysis. Checks need to be carried out in order to demonstrate that method is scientificaly sound under the conditions in whitch it is to be applied and can produce results according to the specified needs. These checks are gathered together in the “validation protocol”.

According to EURACHEM Guide, “ method validation” is defined as a process of establishing the following informations related to the analitical method(4, 5):

- the performance characteristics and the influences that may change these characteristics (and the extent of these changes);
- the limitations of the analitical process (what analytes it can determin and in which matrices);

The conclusion of a validation process is that the analytical method complies or not with the criteria applicable for the relevant performance characteristics.

The detection limit, pat of the validation process, is an analytical figure of merit that, owing to the complex statistics involved, deserves a separate treatment.

It is a parameter of vital importance in trace analysis. One expects that the ever-increasing concern with respect to food safety will continue to stimulate efforts to characterize sophisticated instruments with a realistic estimate of their detection capability.

At low concentrations, an increasing variety of effects becomes important, including, for example,

- the presence of noise or unstable baseline,
- the contribution of interferences to the (gross) signal,
- the influence of any analytical blank used, and
- losses during extraction, isolation or clean-up.

Because of such effects, as analyte concentrations drop, the relative uncertainty associated with the result tends to increase, first to a substantial fraction of the result and finally to the point

where the uncertainty interval includes zero. This region is typically associated with the practical limit of detection for a given method.

In residues analyses, the term 'limit of detection' only may imply a level at which detection becomes problematic. Nowadays it is widely accepted that the most important use of the “limit of detection” is to show where method performance becomes insufficient for acceptable quantitation, so that improvements can be made. Ideally, therefore, quantitative measurements should not be made in this region.

Yet in trace analyses, many analytes are important at very low levels so it is inevitable that measurements must be made, and results reported, in this region.

Results

One of the many procedures existing for method detection limits (MDL) calculation imply the usage of samples spiked with the analyte of interest (a slightely different procedure can be used for in naturaly contaminated samples). Spiked samples can be described as "best case limits", and the detection limits achievable in such samples may not be analytically achievable in natural contaminated ones. Nonetheless, calculating the MDL in spiked samples is useful for comparing detection limits among many laboratories.

The protocol for MDL calculation has to take into consideration all the variables that may cause a determination to be invalid (such as calibration range, spike level, and blank contamination, etc) and will includes the following sections :

1 Checking the performances of the analytical system

The routine instrument maintenance will ensures that the instruments are sufficiently clean and responding properly.The MDL study should be performed on instruments that have passed all of the necessary quality control checks specified in the method of choice. If there is more than one instrument for a similar test, either the MDL will be calculated for the instrument destined to be used for the low - level screenings (if there is such a separation: an instrument for the low-level and one for high-level screening), or there will be used a different protocol for multiple instruments MDL determination.

2 The calibration of the instrument

Proper calibration is essential for the determination of the MDL. For most trace analyses, it is recommended that the lowest calibration standard should be approximately equal to the limit of quantitation (or estimated limit of quantitation -LOQ) and the remaining standards cover the full range of sample concentrations typically encountered by the laboratory (for the specified analytical procedure). It may not be possible to achieve a low MDL using an instrument calibrated for analysis of highly contaminated samples.

Usually, a manufacturer of an analytical device or of an analyse kit will specify the limit of quantitation either for the instrument or the kit and this will be a reasonable approximation of the lab's LOQ until sufficient data has been generated to make a statistical determination.

A new calibration curve should be generated prior to analyzing MDL samples. If this is not possible, at least the operator will check the working calibration curve at least at the beginning of the analytical shift using the appropriate calibration check standard. At least three standards will be used for this checks in order to presume that the calibration is within the linear range of the curve for the analyte of concern.

The number of calibration points will be determined based upon the

width of the working range and the shape of the calibration curve:non-linear, or quadratic curves require a minimum of five calibration standards to fully

characterize the curve. For most residues analyses, the blank should be included as a point on the calibration curve.

3 Choosing the spiking level

One of the methods of calculation is based upon the standard deviation of 20 blank readings (1, 3). This method is not aplicable with most residues analyses. For example, for an ELISA test, the above method leads to an abnormaly low MDL with no practical suport.

That is why the MDL calculation is based upon the standard deviation of several replicates run at identical concentrations and that is why the initial the initial spike level selected for the MDL samples is important.

Since the MDL is an estimate for the lower level of the calibration curve, the best spiking level is 1 – 1,5 – 2 times the estimated detection level, as specified in the 2002 / 657 / EC procedure (5). The calculated MDL must be greater than one-tenth of the spike level. This is the maximum concentration for an MDL study, and concentrations below this maximum are preferable. At the other extreme, the calculated MDL must not be higher than the spike level. The following inequalities are useful for valuating a calculated MDL:

Calculated MDL < Spike Level < 10 x Calculated MDL (2)

From the definition of the CCβ raise another condition for the spiking level (the β error should comply the specifications of the operator for that spiking level).

If these conditions are met the spike level is appropriate.

4 Preparation of the spiked samples

The procedure requires a minimum of 20 replicates of a sample spiked at the appropriate concentration for the analyte of interest. Because MDL highly depends on the method, the replicate samples must be prepared and processed exactly as prescribed, in the same time and with the same instruments, reagents, etc. MDLs for residues determination should be calculated starting from preparing a single stock solution large enough to divide into at least 20 replicates. These individual samples must be analyzed exactly as ordinary samples, following all of the prescribed method steps, and the results quantitated and reported in the proper units. It is very important to analyse every matrix separately because it is not possible to estimate the matrices' effects in the MDL of a particular analyt. For the analitycal methods that allow blank subtraction from the sample results, a paired method blank should be analyzed for each set sample and the average blank subtracted from the sample results, as specified by the procedure. Ignoring the contribution of blank variability on sample results can result in an artificially low MDL, and an increased false positive risk. Analyzing only one blank, and subtracting this result from all of the samples does not account for the true contribution of blank variability.

5 The calculation of the MDL

The calculation of the MDL takes into consideration the IUPAC definition of the detection limit:

$$x_L = x_{si} - k * s_{si} \quad (1)$$

where:

x_L - is the method detection limit (MDL)

x_{si} - is the mean of the spiked samples measures,

s_{si} is the standard deviation of the samples measures, and

k is a numerical factor chosen according to the confidence level desired (the Student's value)

It is acceptable to round the calculated value up to the nearest decimal

place. For example, if the calculated MDL is 0.15, it is acceptable to round the MDL to 0.2 if results are only reported to one significant figure. MDLs should never be rounded down, unless the operator demonstrates it can routinely achieve the rounded value (2).

The MDL should be verified from time to time and everytime when something changes in the analithical method or other variable that could alter the calculated MDL (2).

As a conclusion there are three important things when calculating MDL and these are:

1) use the spiked sample standard deviation,
2) use the correct Student's t-value and
3) consider all significant figures.

Conclusions

This procedure is only one of the many ways to calculate method detection limits but it also has its limitations.And it can lead to unrealistic values if it is not well applied or understood.

Performance chacacteristics of different analytical methods should be comparable and this can only be achived if the same validation approach is chosen and if the uncertainty related to performance characteristics is equal. Unfortunately there are no universally accepted procedures for method validation (including detection limits calculation) and this is confusing for both regulators and regulated community.

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