UNIVERSITATEA DE ȘTIINȚE AGRICOLE
ȘI MEDICINĂ VETERINARĂ
"ION IONESCU DE LA BRAD" IAȘI

LUCRĂRI ȘTIINȚIFICE

VOL. 53 (12)

MEDICINĂ VETERINARĂ

PARTEA 4

Lucrările Simpozionului
"PROGRESS AND PERSPECTIVES IN
VETERINARY MEDICINE"
Iași, June 10th-11th 2010

EDITURA "ION IONESCU DE LA BRAD"
IAȘI 2010

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Volumul a fost editat cu sprijinul financiar al
Ministerului Educației, Cercetării, Tineretului și Sportului

ISSN: 1454-7406

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INCIDENCE OF HUMAN CRYPTOSPORIDIOSIS IN BUCHAREST BETWEEN 2000 – 2005 PERIOD

Adriana AMFIM, Monica PÂRVU, Violeta –Elena SIMION
Facultatea de Medicină Veterinară, Unversitatea Spiru Haret,
Str. Mașina de Pâine, nr. 7, București, România
adrianaamfim@yahoo.com

Abstract

At present both in Romania and internationally, is a parasitic disease of public health issues carefully monitored because high prevalence, health problems - the lack of diagnosis and correct treatment, problems of differential diagnosis and not least many economic implications.

The purpose of this study is to determine the incidence of cryptosporidiosis in humans dynamics in Romania over a period of time and the category of age at highest risk of contamination Criptosporidium sp.

Setting annual growth rate of new cases of cryptosporidiosis in human populations was done using the database provided by the National Center for Organization and Information System and Information Assurance in Health, Bucharest, Romania. The database is the number of cases of illness occurred annually during 2000-2005, in Bucharest, the ratio of family doctors and hospital doctors in specialty reporting. Epidemiological assessment took account of cryptosporidiosis and age group affected, so that data interpretation was made for four age groups (under one year old, 1-14 years, 15-64 years, over 64 years). Research methodology consisted of retrospective analysis, based on analytical investigations using statistical processing of data from quantitative assessment of epidemiological parameters (incidence).

Thus were established as the age category with the highest degree of contamination Criptosporidium parvum and temporal distribution of new cases of disease occurred during the six years of epidemiological monitoring. Also epidemiological assessment of cryptosporidiosis, given the time and age group, according to the source reporting has shown a consistency of epidemiological information from secondary sources reporting.

Keywords: cryptosporidiosis, zoonosis, incidence

INTRODUCTION

Cryptosporidiosis is a parasitic zoonosis caused by a protozoan of the genus *Cryptosporidium*. By eliminating a large number of oocysts in the environment that can contaminate many animals, cryptosporidiosis is a public health problem. In-developed countries 0.2-0.4% of oocysts excreted healthy and diarrhea sickness oocysts secretion is reported in 2-2.5% of the population (3). Cryptosporidiosis incidence in patiens with BDA, around the world is: 0.6-19.3% in Chile, 13% in India, 9% Liberia, 9.3% in Finland, 4.8% in Australia, 2.8% in the U.S., 0.6% in Canada (3).

The prevalence of cases with *C. hominis* is 62% and it is specific to South America, Australia and Africa. Criprosporidiosis in Europe is produced by *C. parvum* and disease prevalence is 57% (1). It has developed severe disease especially in immunosuppressed persons (HIV patients) (1).

Cryptosporidiosis being a common disease of young animals, especially farm animals, causing high levels of mortality and morbidity, is that it is considered among the most important parasitic zoonoses in nature (4). Highest frequency of cases characterized rural areas. The prevalence in animals is generally established for farm animals, being 55.6% (2).

Although the etiologic agent of disease is included in the O.I.E. List B and "Zoonoses directive 2003/99/EC", cryptosporidiosis is declared only a few countries (Germany and Sweden) and recorded only sporadic reports in the Netherlands and Spain. Surveillance monitoring of water and therefore casuistry with *Criptosporidium sp*., is adopted by United Kingdom law only. Drinking Water Directive 98/83/EC, requires European countries to take measurements of water pollution by *Cryptosporidium sp.,* where the water samples examined were identified *Clostridium perfringens* (Real Decreto 140/2003, BOE 21/02/2003) (5). W.H.O. report for to ouer country 20 children under one year decease/1000 by B.D.A, also 14% of deaths in HIV patients is due to infestation with *Cryptosporidium sp*. (3).

MATERIAL AND METHODS

This study was conducted to monitor the health of human population in Bucharest, within a specified time period (2000 - 2005) in the incidence of cryptosporidiosis, using two sources of data provision.The study was the number of new cases of cryptosporidiosis in humans during 2000-2005, reported by family practitioners and specialist outpatient, Bucharest. Centralization of data was the National Center for Organization and Information System and Information Assurance in Health in Bucharest.

The mode consisted of a survey of descriptive epidemiology using transversal descriptive study, with applications in assessing and monitoring the health of human population between 2000 to 2005. In preparing the database have come following steps:

1.selection of data sources;
2. preparation of the database;
3. establishment of criteria by which to make interpretation of data;
4. analysis and statistical processing of data;
5. dissemination of results.

1. Selection of data sources was consistent with this model in descriptive epidemiological investigation. It took so only data sources provided by secondary care (family offices / outpatient specialist) using medical records. Centralization of data was made by a national body empowered under Art. 108 of the Romanian Constitution, republished, art. 14 and art. 43 para. (2) of Law no. 95/2006, represented by the National Center for Organization and Information System and Information Assurance in Health, Bucharest, Romania.

2.In preparing the data base the following steps have been taken:

- evaluation of data for monitoring the human population in Bucharest criptosporidiosis throughout 6 years;
- data collection;
- entering data into the database;
- data management

3 . The criteria according to which the database have been processed:

- epidemiological index to assess the epidemiological situation (incidence or frequency of new cases of criptosporidiosis in humans);
- sources reporting new cases of disease (family offices and outpatient specialist);
- temporal distribution of disease (time with established scope - 2000 to 2005);
- measurement of disease distribution by age (less than one year, 1-14 years, 15 to 64, over 64.

4. Data analysis was done using descriptive variables such quantity - the incidence of criptosporidiosis in the human population per calendar year, age group and source of data reporting. It was obtained by comparing the incidence of new cases of disease occurring each year from 100,000 during the study. Most convenient summary format and presentation of data was by frequency distribution charts (line graphs, chart structure, Chart, Scatter Diagram, etc.). Epidemiological analysis of criptosporidiosis in humans was done by linking incidence data with calendar period, age group and compared the two reporting sources.

5. Dissemination of results may be in the form of reports aimed to provide knowledge and raise awareness of health problems in humans.

RESULTS AND DISCUSSION

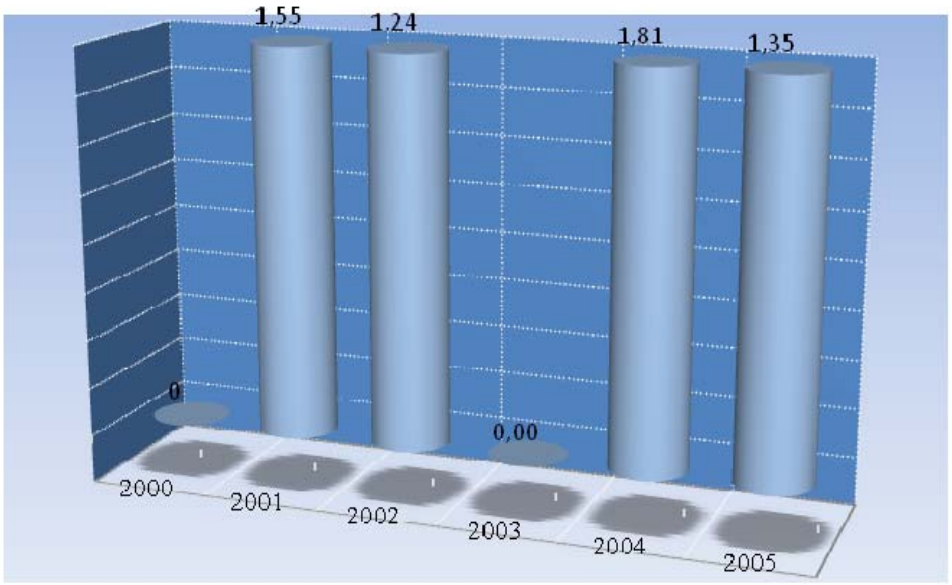
After processing and interpreting statistical database for the period 2000-2005 could make the following observations about the evolution of human cryptosporidiosis in the population measured in terms of temporal distribution of disease and age group, in Bucharest from doctors reporting family (Table 1).

Table 1

Cryptosporidiosis incidence per calendar year and age group (reporting by physicians) (indexed at 100 000 inhabitants)

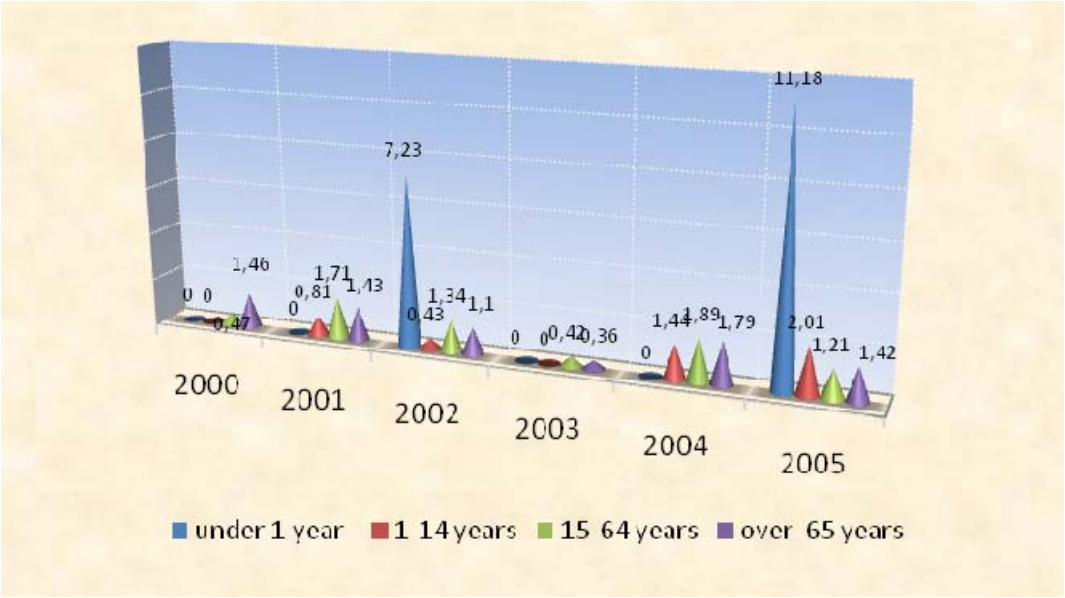
Year	I% (100.000 inhabitams)	Under 1 year	1-14 years	15-64 years	64 years
2000	0.54	0.00	0.00	0.47	1.46
2001	1.55	0.00	0.81	1.71	1.43
2002	1.24	7,23	0.43	1.34	1.10
2003	0.36	0.00	0.00	0.42	0.36
2004	1.81	0.00	1.44	1.89	1.79
2005	1.35	11.18	2.01	1.21	1.42

Between 2000-2005 the incidence of cryptosporidiosis in the human population had values ranging from 0.36 - 1.81%. Highest incidence was recorded in 2004 (I - 1.81%), and the minimum value recorded in 2003 (I - 0.36%). By analyzing the graph is observed that the difference between incidence values throughout the study period is only 0.99% (Chart 1).



Graphics 1. Temporal distribution of the incidence of cryptosporidiosis in Bucharest in the period 2000-2005 (reference - family doctors)

Distribution of new cases of disease occurring in each calendar year in the study according to age of patients is as follows (Chart 2):



Graphics 2 - Measurement of distribution of cryptosporidiosis incidence during 2000-2005, according to age (reporting - family doctors)

- for 2000 the population segment most affected was that of people aged over 64 (I - 1.46%);
- the most affected segment of the population in 2001 was that of persons aged 15-64 years (I-1.71%);
- for 2002 it was observed a lower incidence of between 0.43% for persons aged 1-14 years and 7.23% for children under one year;
- in 2003 the population segment most affected was that of children aged 1-14 years (I-0.42%);

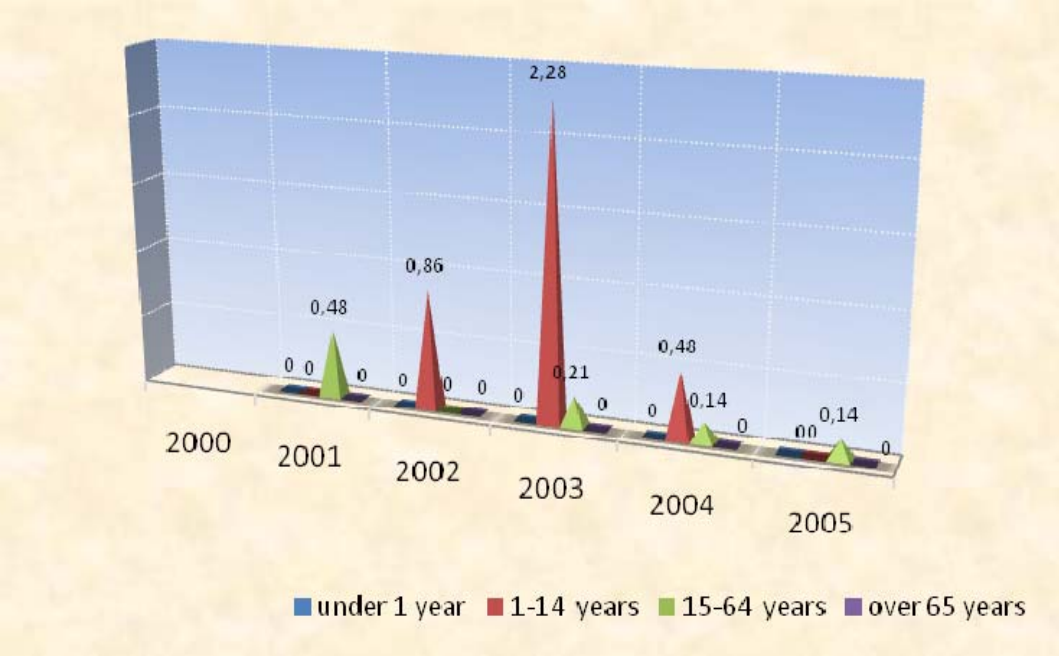
- maximum incidence for 2004 was recorded by age category 15-64 years (I - 1.89%);
- in 2005 in Bucharest, the most affected segment of the population were children aged under one year (I-11.18%).

Health monitoring in the human population in terms of cryptosporidiosis in the period 2000 - 2005 was also achieved by retrospective epidemiological survey of hospital physicians for specialty reporting. The epidemiological analysis were established following on the frequency with which new cases of disease occurred in age category during the six years of study (table 2, schedule no. 3).

Table 2

Cryptosporidiosis incidence per calendar year and age group (reporting - hospital specialist) (indexed at 100 000 inhabitants)

Year	Total	Under 1 year	1-14 years	15-64 years	Over 65 years
2000	-	-	-	-	-
2001	0.35	0.00	0.00	0.48	0.00
2002	0.10	0.00	0.86	0.00	0.00
2003	0.41	0.00	2.28	0.21	0.00
2004	0.15	0.00	0.48	0.14	0.00
2005	0.10	0.00	0.00	0.14	0.00



Ggraphics 3 - Temporal Distribution of cryptosporidiosis cases according to the study group (reporting - specialty hospital)

- in 2000 were not reported new cases of disease contamination with C. parvum, National Centre for Organisation and Information System and Information Assurance in health.
- year with the highest incidence was in 2003 (I - 0.41%);
- children under one year as well as people over 65 were recorded during the entire period of study an incidence of 0.00%;
- age group most affected was that of children aged 1-14 years (I - 0.00 - 2.28%);

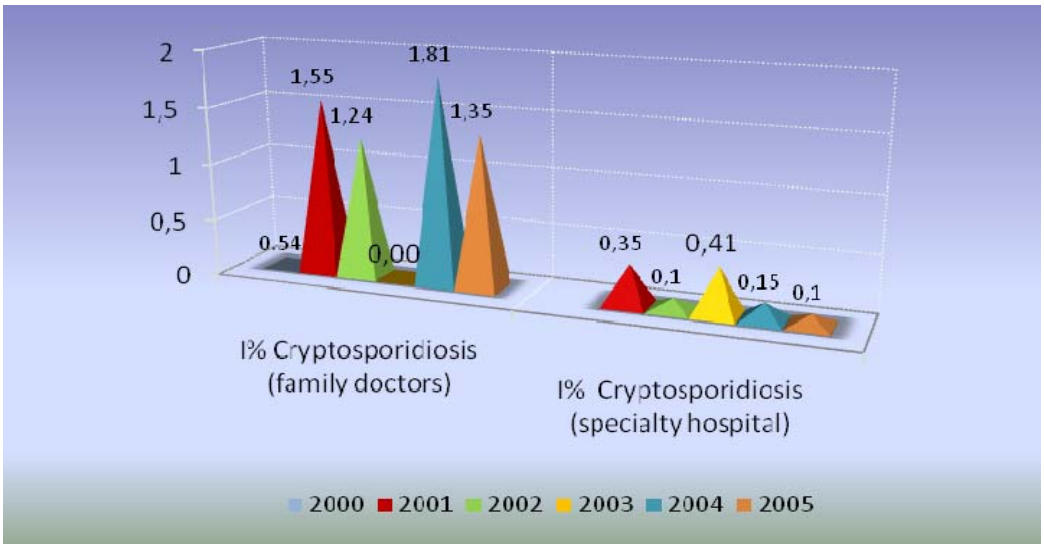
Comparatively analyzing data from the two sources of reporting, can make the following observations (table 3, graph 4):

- frequency of new cases of cryptosporidiosis in Bucharest for the period under study is different depending on the source reporting;
- epidemiological data from family practices indicate a higher risk of contamination with human population *Criptosporidium* sp.;
- outpatient specialty show lower values of incidence during the six years of study and summary reports (in 2000 were not reported new cases of cryptosporidiosis).

Table 3

Cryptosporidiosis incidence during 2000-2005 in Bucharest, according to the source reporting

Year	I% cryptosporidiosis (family doctors)	I% cryptosporidiosis (specialty hospital)
2000	**0.54**	**-**
2001	**1.55**	**0.35**
2002	**1.24**	**0.10**
2003	**0.36**	**0.41**
2004	**1.81**	**0.15**
2005	**1.35**	**0.10**



Graphics 4. Comparative measurement of the distribution of cryptosporidiosis incidence during 2000-2005, according to the source reporting

Conclusions

This study the epidemiological investigations have used such descriptive epidemiological investigation, aimed at establishing the frequency with which new cases occur in humans cryptosporidiosis in Bucharest, in a period of time. Quantifying epidemiological indicator - the incidence - was given the temporal characteristic of the disease, measuring the age distribution, reporting different source.

Statistical processing of the database shows that both the temporal distribution of disease and setting up the contamination risk population is different according to the source reporting. Maximum incidence is different depending on the source reporting having values of 0.41% of ambulatory specialized reporting and 1.81% according to data collected from family practitioners.

Of family physicians reporting cryptosporidiosis is an intestinal parasitosis that affects all age groups (both children under one year and adults 64 years past). Also small differences between incidence values based on age, shows that the disease has an uniform distribution in the human population with an average frequency of occurrence of new cases of disease during the six years is only 1.14%.

Epidemiological surveillance of cryptosporidiosis is having as a source of information and reporting outpatient specialist in Bucharest, established a low frequency of occurrence of new cases of cryptosporidiosis in period 2000-2005 (I - 0.10-0.35%). Regarding the distribution of disease according to age categories, children under one year as people over 65 were recorded throughout the study period an incidence of 0.00%.

In Romania, cryptosporidiosis is a disease of animals included in the “Stock Surveillance Program, Prevention and Control of Animal Diseases, those transmitted from animals to humans (...) ". Knowing its zoonotic nature, it must be monitored in order to achieve “Surveillance and Prevention protocols for Zoonoses of Parasitic Nature”. To this end one can make other kind longitudinal epidemiological survey - retrospective cohort.

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EPIDEMIOLOGICAL RESEARCH ON OUTBREAKS OF AVIAN INFLUENZA IN LETEA AND PLAUR FROM TULCEA COUNTY

M. ARSENE, ARSENE Marinela,TERTIS Marinela, P. BRIAl,
MAFTEI Daniel, BOICIUC Laurenta
DSVSA TULCEA

Studies were conducted in March 2010 in Letea and reed villages in Tulcea county, and followed the clinical course and epidemiology of avian influenza in households in these two localities. The disease was manifested by a trend superacute depression, decreased appetite, polydipsia, adinamie, head and neck edema, cyanosis and swelling ridges and beards, conjunctival congestion and hypersecretion, cyanosis and beards ridge.

Avian influenza virus can be isolated from active outbreaks of avian influenza, of greater or lesser severity, but also from birds with discrete forms of disease or clinically apparently healthy. In the first case we speak of epidemics and outbreaks of avian influenza in the second case we speak only of infection with influenza virus.

Among poultry, avian influenza was reported most frequently in turkey, duck, goose, partridge, pheasant, chicken and various psitacide. Among many species of birds have been isolated from avian influenza viruses, it referred primarily waterfowl, birds that inhabit the shores (seagulls, sea swallows) migratory birds, especially web-footed.

Easy transmission of avian influenza as well as asymptomatic infections, is usually within the same species. In other words, when it is an active outbreak of influenza, a source of infection for birds are all birds, and the source of infection for humans is still a man.

Localities of the Delta system practiced extensive growth of poultry. Although this system is prohibited by law increase veterinary health is difficult to control and imposed a ban growth poultry in open system because the benefits of growth shows that way.

These flocks reared semisalbaticie and those who are allowed to leave the court holding daily household contacts of people have permanent or periodic, regular birds, implicitly wild bird species are incriminated in the epidemiology of the disease (avian influenza).

MATERIAL AND METHODS

Researches were conducted in March 2010 in avian influenza outbreaks in localities Letea and reed, Tulcea County, and presents epidemiological and clinical aspects of disease in terms of the Danube Delta. For diagnosis were collected corpses of cloacal and tracheal samples, serum samples were both serological and virological worked in the Laboratory Veterinary and Food Safety Tulcea and confirmation was made at the Institute of Diagnostic Bucharest.

RESULTS AND DISCUSSION

On 03/11/2010 there were the first clinical signs in two households in the village cycle. Household that was located on the ring canal that connects with the Danube. Letea

village lies between the arms Chilia and Sulina on hill Letea. In this area have their habitat many species of wild birds and migratory wild birds that spend all developmental stages of the Danube Delta territory.

On 14.03.2010 in the household that all birds died, so it was a rapid evolution of disease. Birds of the household were the chickens, and their growth is made, household.

Following implementation of the program to prevent and control avian influenza in the area were examined clinically Letea 1621 to 1561 birds in chickens, 57 ducks, 28 turkeys, 24 geese, a guinea. The examinations performed were identified clinical signs of disease and mortality in three households located near the first outbreak. Clinical examinations were performed in villages located in surveillance zone localities CA Rosetti and Sfistofca. In these places there were reported cases of illness or mortality, and households were not on the bank channel (birds were not directly access to water).

Affected households in five out of 89 hens, a total of 68 died and 21 were killed after the application program to prevent and control avian influenza.

On 25/03/2010 after clinical supervision was found in a household as the total of 15 hens that were still held six live birds not clinical signs and two corpses were collected and sent to the laboratory. Household that is located about 50m from the body of water that place is visited by migratory wild birds. Up to date 26.03.2010 suspicion was confirmed when the remaining birds in the household have died.

All six cases diagnosed as avian influenza in households cities are located on the same street, all with out the pool.

The program of surveillance affected households were harvested samples were examined at LSVSA and confirmed at IDAH as positive in the direction A150 of highly pathogenic H5N1.

Morphological and clinical aspects found in the bodies of chickens were the following:

- Swelling of face (Fig.3)
- Swelling in connective tissue of the neck and dorsal area of the neck (Fig.6)
- Cyanosis and swelling ridges and beards (Fig.1)
- Hemorrhagic tracheitis (Fig.4)
- Bronchopneumonia
- Liver degeneration
- Bruising and petechiae in the legs (Fig.2)
- Kidney degeneration
- Catarrhal enteritis hemorrhagic.
- Cerebral edema (Fig.5)

On 24/03/2010 there were the first cases of illness in a household of Plaur with the same clinical signs as the city Letea. Plaur is located downstream on the right arm of Chilia. Household that is located near the arm Chilia at 500m which is a flooded area where there are suitable places for wild birds standing. On 25/03/2010 has been confirmed and suspected avian influenza were a total of 80 affected chickens in which 28 died and 52 were killed. The disease was too fast with high mortality. Through monitoring local birds have been recorded cases of death or illness in birds.

Fig. 1- Cyanosis and swelling ridges and beards

Fig.2- Bruising and petechiae in the legs

Fig. 3 – Swelling of face at chicken

Fig. 4 - Hemorrhagic tracheitis at chicken

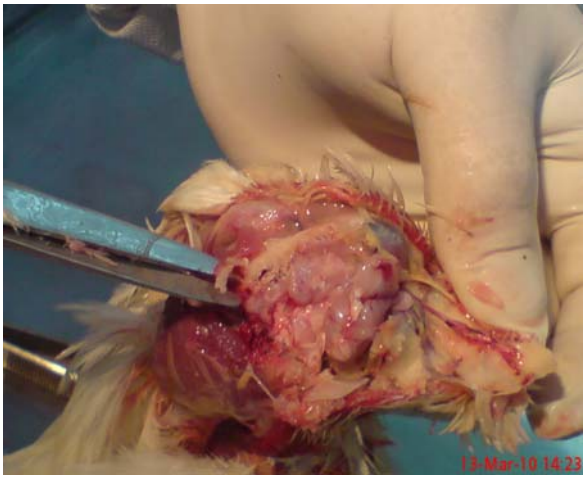

Fig. 5 - Cerebral edema and

Fig. 6 - Swelling in connective tissue of the neck dorsal area of the neck

CONCLUSIONS

1. Existing domestic water birds (ducks, geese), high risk areas for avian influenza is the mode of transmission from wild birds to poultry.
2. An important role in development and disease progression we have species "in touch" as quasi-domestic species to be mixed with water and wild birds while attending human dwellings where poultry are kept.
3. The disease appeared only in households with settlement near shore waters.
4. Because cohabitation poultry with migratory birds avian influenza virus could enter the households. This was possible because of the farming system, particularly web-footed in villages in the Danube Delta.
5. Route of infection was a direct contact with infected migratory birds through habitat and an indirect (body of water that infected migratory wild birds have stopped).
6. Unlike poultry, wild birds typically do not exhibit symptoms of disease, but they may constitute a permanent source of infection in large areas of world.
7. In all cases clinical signs were the same: head swelling, cyanosis and swelling ridges and beards, hemorrhagic tracheitis, bronchopneumonia, and hemorrhagic enteritis etc.

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ETIOLOGY AND PATHOLOGY IN PORCINE RESPIRATORY DISEASE COMPLEX (PRDC) IN FINISHING PIGS

S. BĂRĂITĂREANU [1], Mihaela BEZMAN[2], S. MARINACHE[2], D COBZARIU[1], Gabriela BAGRINOVSCHI[3], Maria OȚELEA[3], M. V. CÂMPEANU[3], Simona IVANA[1], Doina DANEŞ[1]

[1]USAMV Bucureşti – Institutul de Medicină Comparat
Splaiul Independenței 105, Sector 5, Bucureşti
[2]SC IZOCON MC SRL Bucureşti
[3]USAMV Bucureşti – Facultatea de Medicină Veterinară
Splaiul Independenței 105, Sector 5, Bucureşti
doruvet@yahoo.com

Respiratory infections are the most prevalent in the growing pig farms. In this paper it's presented the results of screening carried out on PRDC factors in 11 farms and their economically impact. Blood samples collected from 19 finishing pig herds – 376 specimens - were investigated by HerdChek Pseudorabies Virus gB Antibody Test Kit, CHEKIT APP-ApxIV Actinobacillus pleuropneumoniae Antibody Test Kit, HerdChek Micoplasma Hyopneumoniae Antibody Test Kit, HerdChek PRRSV-Ab Test Kit, HerdChek Swine Influenza Virus Antibody Test Kit – H1N1, HerdChek Swine Influenza Virus Antibody Test Kit – H3N2, and HerdChek Swine Salmonella Antibody Test Kit (IDEXX Laboratories, Inc., USA). For postmortem investigations were collected 20 pigs from two farms, and 38 pigs from one farm were Mycoplasma hyopenumoniae scored in slaughterhouse. The pulmonary lesions registered at the post mortem exam of finishing pigs suggested the presence of several pathogens, and serological investigations revealed the next: IgG H1N1 - 49% positive, IgG H3N2 - 67% positive, IgG PRRSV - 12% positive, IgG PRV - 39% positive, IgG M.hyopneumoniae – 14,6% positive, IgG APP - 19% positive, IgGSamlonella – 49,5% positive. The investigations performed in slaughterhouse showed that the level of pulmonary lesions declined daily growth rate *with 19 g/day/pig (the economical deficit of 5% pulmonary lesions was 2€/pig/production cycle).*

Keywords: porcine respiratory disease complex, swine pathology, PRDC

In professional swine herds the swine respiratory disease complex (PRDC) causes major economic losses [12, 16]. Although PRDC is described in all age groups, this pathology is usually reported in finishing pigs with age between 14 and 22 weeks [17, 13]. The etiology of PRDC are involved bacteria, viruses and zoo-hygiene conditions that in a unfavorable combination, overcome local defense mechanisms and triggers specific respiratory pathology [6].

The most frequent pathogenic viruses associated with PRDC are pig reproduction and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), Pseudorabies virus (PRV), porcine circovirus type 2 (PCV2) and porcine respiratory corona virus (PRCV) [1 5, 11, 15, 17, 18]. These viruses can be isolated with one or more pathogenic bacteria. Depending on the role of bacteria in onset morbid process, they may be primary pathogens (*Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Bordetella bronchioseptica*) or secondary (*Pasteurella multocida, Haemophilus parasuis, Streptococcus suis, Actinomyces pyogenes, Salmonella choleraesuis, Actinobacillus suis*) [1 , 5, 11, 17].

Evaluation of immune status to the main infectious agents (bacterial and viral) as primary or secondary respiratory pathogens is a priority in preventive health management of swine farms [1]. List of pathogens active surveillanced by serological tests should be established according to legal regulations and case history.

This paper mainly aims dominant etiological and pathological aspects associated with PRDC in Romania. In this context it analyzes the data collected from serological investigations of swine farms where clinical examination and necropsy revealed the existence of respiratory pathology in pigs slaughtered or died during the finishing period.

MATERIALS AND METHODS

Pigs (14-22 weeks) were collected from 11 farms, and 19 distinct lots. From September 2006 to December 2009 were collected 376 blood samples. In two farms (X and XI) were additionally collected 20 pigs corpses that showed respiratory disease before death. From a batch of pig (48 pigs) of farm XI have collected data on pulmonary pathology in the slaughterhouse examination (pulmonary lesions).

In the farm I samples were collected 25 (Lot 1: 140 -150 days finishing pig), in the farm II were collected 25 samples (Lot 2: 140-150 days finishing pig), in the farm III were collected 25 samples (Lot 3: 110 -150 days finishing pig), in the farm IV were collected 40 samples (Lot 4: 110-120 days finishing pig - 20 samples, Lot 5: 160-180 days finishing pig - 20 samples), in the farm V were collected 26 samples (Lot 6: 110-125 days finishing pig - 13 samples, Lot 7: 160-170 days finishing pig - 13 samples), in the farm VI were collected 60 samples (Lot 8: 130 days finishing pig - 20 samples, Lot 9: 170 days finishing pig - 20 samples, Lot 10: 185 days finishing pig - 20 samples), in the farm VII were collected 45 samples (Lot 11: 110 days finishing pig - 20 samples, Lot 12: 130-150 days finishing pig - 25 samples), in the farm VIII were collected 40 samples (Lot 13: 110-120 days finishing pig - 20 samples, Lot 14: 160-180 days finishing pig - 20 samples), in the farm IX were collected 20 samples (Lot 15: 140-150 days finishing pig), in the farm X were collected 40 samples (Lot 16: 110-120 days finishing pig - 20 samples, Lot 17: 150-180 days finishing pig - 20 samples), in the farm XI were collected 30 samples (Lot 18: 110 days finishing pig - 15 samples, Lot 19: 130-160 days finishing pig - 15 samples).

To assess immune status of pig herds in this study were used the following ELISA diagnostic kits: HerdChek Pseudorabies Virus gB Antibody Test Kit (IDEXX Laboratories, Inc., USA), APP-Chek ApxIV Actinobacillus pleuropneumoniae (APP) Antibody Test Kit (IDEXX Laboratories, Inc., USA); HerdChek Mycoplasma hyopneumoniae Antibody Test Kit (IDEXX Laboratories, Inc.., USA) Porcine Reproductive and Respiratory Syndrome HerdChek Virus Antibody Test Kit (IDEXX Laboratories, Inc., USA); HerdChek Swine Influenza Virus Antibody Test Kit - H1N1 (IDEXX Laboratories, Inc., USA); HerdChek Swine Influenza Virus Antibody Test Kit - H3N2 (IDEXX Laboratories, Inc., USA); HerdChek Swine Salmonella Antibody Test Kit (IDEXX Laboratories, Inc., USA). Working protocols used were those specific Serology Laboratory of the Faculty of Veterinary Medicine Bucharest, in accordance with the manufacturer's protocol (IDEXX Laboratories, Inc., USA).

Pulmonary and lymph node lesions were assessed and recorded in concordance with interpretation and classification scheme recommended for this species [14]. Pulmonary lesions characterized by the increase in volume, hyperemia and edema were classified as acute.

Pulmonary lesions characterized by moderate edema and bronchial exudates, but no increase in volume and hyperaemia were classified as subacute. Atelectic fibrotic lesions, consolidated lesion with dark-red appearance and dilated bronchi filled with inflammatory

exudates were considered chronic. According to their size lymph nodes were scored as normal = 0, moderate enlargement = 1 or marked enlargement = 2. All lungs examined were with lobular type lesions: clear delineation from lobular level of healthy tissue to the sick.

RESULTS AND DISCUSSIONS

Described as a multifactorial disease, PRDC cannot be managed without close cooperation between the practitioner and the laboratory. Diversifying and improving methods of antemortem diagnosis for both immunological diagnostic and identification of pathogens was beneficial not only for veterinary lab but also for practitioner. Veterinary Laboratory will provide reliable results quickly, and the practitioner will be reduced by the decisions they take economic losses.

However, post-mortem investigations cannot be removed as they provide, along with clinical and zootechnical indicators, information on health and possible infection in swine herds. In our cases the pneumonia lesions were heterogeneous. In addition, there were both acute and chronic pulmonary lesions. This suggests either co-development of at least two infections or an acute injury healing process. Based on morphological characteristics, lung lesions can be classified as embolic, bronchopneumonia and interstitial or bronhointerstitial [4].

In the 20 lungs collected from pig bodies that previously showed signs of respiratory death dominant was lobular bronchopneumonia in the cranial-ventral region (16 lungs). Four lungs showed no macroscopically visible lesions.

Seromucous, purulent or mucopurulent exudates were found in five pigs (25%) with acute bronchopneumonia, eight pigs (40%) with subacute bronchopneumonia and seven pigs (35%) with chronic bronchopneumonia. Seven animals presented fibrotic pleurisy of the diaphragm lobes. Lymph node scores were: score 0 in 4 pigs (20%) (without pulmonary gross lesions), score 1 in 9 pigs (45%), and score 2 in 7 pigs (35%).

To support the swine farmers is necessary to evaluate the immune status against major infectious agents (such as bacterial or viral) that acts primary or secondary on the respiratory system. Undoubtedly, the epidemiological data, clinical signs and pathological lesions provide sufficient information to guide diagnosis to development of a porcine respiratory complex, however many unknown problems it still present in swine industry. Linking the information previously described with serological investigations by ELISA in order to confirm suspicions or assessment of post-vaccination immune status is an activity usually practiced.

Based on epidemiological data, clinical features and lesions attention has focused on evaluation the immune status for folowing infections: Porcine Reproductive and Respiratory Syndrome Virus, Aujeszky's Disease Virus, Swine Influenza Virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Salmonella* spp.

Evaluation by ELISA of IgG anti-H1N1 antibodies to the 376 samples showed that 185 of the pigs tested (49%) were in contact with the virus. ELISA-H3N2 provided positive results in 252 pigs tested (67%). These data highlight the simultaneous circulation of several strains of influenza in pig herds.

In assessing the immune status against PRRSV were obtained 45 positive samples (12%), and for Aujeszky's Disease Virus 39 samples positive (10%).

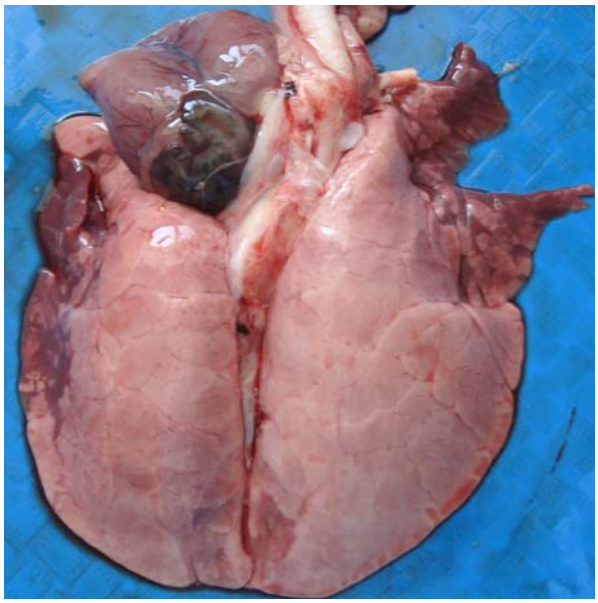
Mycoplasma hyopneumoniae and *Actinobacillus pleuropneumoniae*, the bacteria considered primary etiologic agents in respiratory pathology, were identified in 55 samples (14.6%), and respectively in 71 samples (19%).

Among bacteria that can cause lung damage was investigated as secondary agents only *Salmonella sp*. Antigenic exposure to salmonella was established in 186 specimens (49.5%).

Lot of pig assessed for respiratory lesions (48 lungs) had average live weight at slaughter of 105-120 kg. From 48 lungs 19 lungs were with lesions: 5 lungs with characteristic lesions of swine enzootic pneumonia (*Mycoplasma hyopneumoniae*), and 9 lungs with pneumonia and pleurisies (18.8%); no pigs had pericarditis or lung abscess (Table 2).

These data suggest that the farm revealed 11 lesions were pathognomonic of *Mycoplasma hyopneumoniae* on 10% of lungs examined (five out of 48 lungs), a value similar with result of all serological tests performed for *Mycoplasma hyopneumoniae* in our study.

Each 10% lesion of the total pulmonary respiratory area causes a loss of 37 g gain / day. The pulmonary lesions collected in slaughterhouse suggest a reduction of average daily gain with 19 g / day / pig, and 1.87 kg at the end of 100 days of fattening. Thus, the cycle of fattening will increase with other 2 days.



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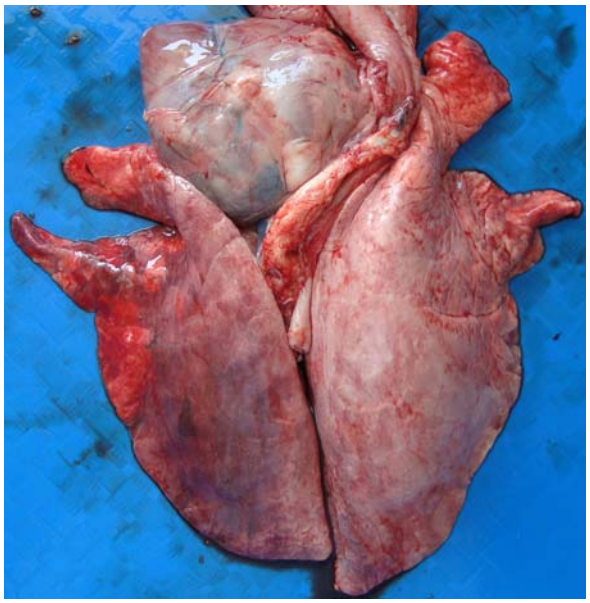

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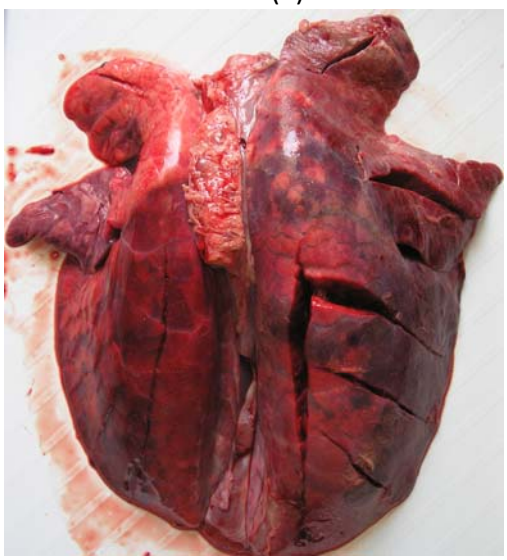

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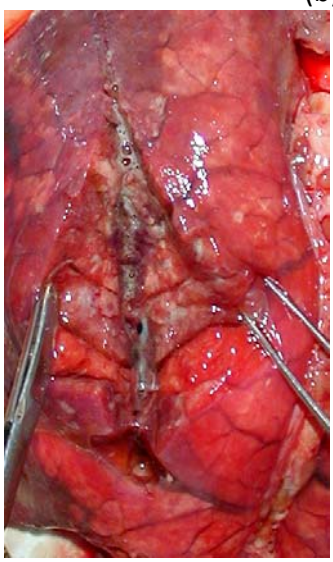

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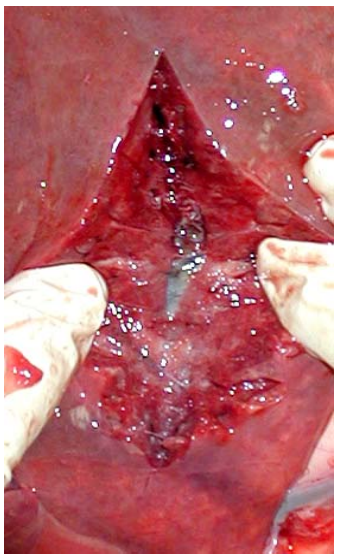

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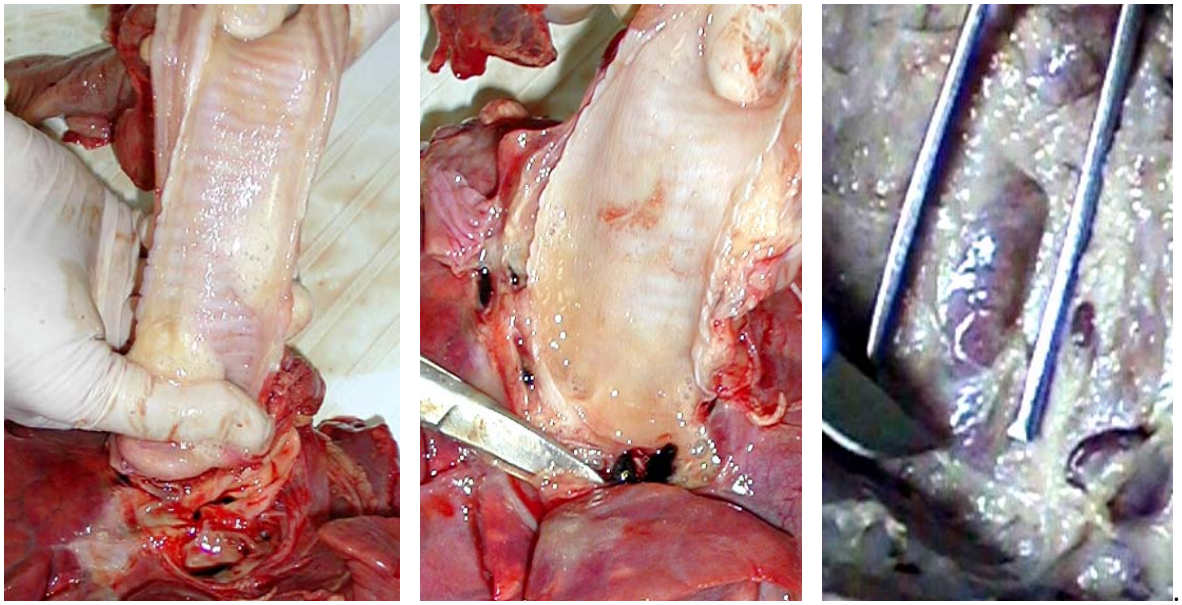


(f) (g) (h)

Fig.1. Respiratory disease associated with PRDC (a, b) cranioventral acute bronchopneumonia, (c) chronic cranioventral bronchopneumonia with pulmonary consolidation and focal fibrotic pleurisy of the diaphragm lobes (d, e) oedematous, hyperaemic and swollen acute lesions (f, g) mucopurulent exudate in trachea and bronchi (h) enlarged lymph volume

Table 1. Serological investigations with ELISA results (14 to 22 weeks age)

ELISA	samples	Negative		Positive		Re-control	
		No.	%	No.	%	No.	%
Actinobacillus pleuropneumoniae (APP) Antibody Tes Kit (ELISA-APP)	376	305	81	71	19	0	0
Pseudorabies Virus gB Antibody Test Kit (ELISA-PV)	376	337	90	39	10	0	0
Swine Influenza Virus Antibody Test Kit - H1N1 (ELISA H1N1)	376	191	51	185	49	0	0
Swine Influenza Virus Antibody Test Kit - H3N2 (ELISA H3N2)	376	124	33	252	67	0	0
Swine Salmonella Antibody Test Kit (ELISA-Salm)	376	190	50.5	186	49.5	0	0
PRRSV Antibody Test Kit (ELISA-PRRS)	376	331	88	45	12	0	0
Mycoplasma hyopneumoniae Antibody Test Kit (ELISA-Mhyo)	376	321	85.4	55	14.6	0	0

Chart 1. Etiology of PRDC in swine farms

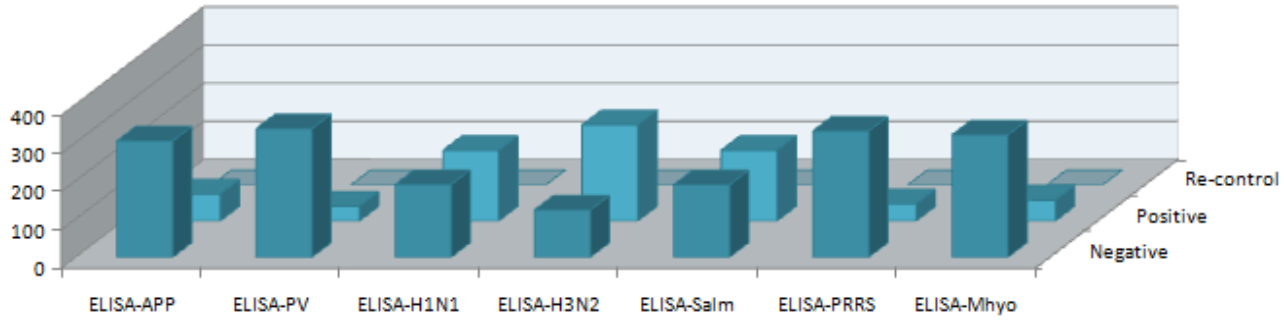
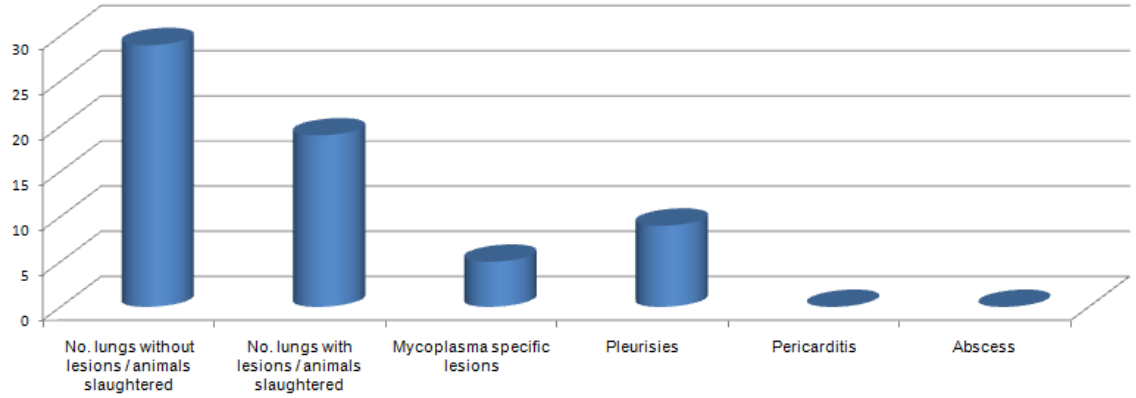


Table 2. Slaughterhouse exam results: assessing lung damage

Cantitate	No. swine	%
No. swine slaughtered	48	4800
Respiratory surface damaged		242
%Respiratory average area / pig		5
No. lungs without lesions / animals slaughtered	29	60
No. lungs with lesions / animals slaughtered	19	40
Mycoplasma specific lesions	5	10
Pleurisies	9	18.8
Pericarditis	0	0
Abscess	0	0

Chart 2. Dominant lung lesion collected postmortem examination at the slaughterhouse



CONCLUSIONS

Of the 376 samples tested 49% were the H1N1 positive anti-IgG, 67% were positive to IgG anti-H3N2, 12% were positive to IgG anti-PRRSV, 10% were positive to IgG anti-prv, 14 , 6% were positive to IgG anti-*Mycoplasma hyopneumoniae*, 19% were positive to IgG anti-*Actinobacillus pleuropneumoniae* and 49.5% were positive to IgG anti-*Salmonella* sp.

Circulation of influenza strains in swine farms suggest the monitoring of their progress through regular serological investigations. It recommends immunoprophylactic actions or adjustment of already schemes of vaccination implemented in livestock of sows and youth. Even if the specific prevention programs implemented in farms included immunoprophylactic products from some of the pathogens, adverse effects caused by the actual movement of viruses or other pathogenic bacteria are being felt through reduced feed conversion rate and average daily gain.

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SIGNS AND LESIONS OF THE SPONTANEOUS ACUTE PNEUMONIC PASTEURELLOSIS IN PET RABBITS

S.BĂRĂITĂREANU [1], D. COBZARIU[1], Maria OȚELEA[2], M. V. CÂMPEANU[2], Simona IVANA[1], Doina DANEŞ[1]

[1]USAMV Bucureşti – Institutul de Medicină Comparată

[2]USAMV Bucureşti – Facultatea de Medicină Veterinară

Splaiul Independenței 105, Sector 5, Bucureşti, doruvet@yahoo.com

Pasteurellosis is one of the most serious diseases of rabbits, with considerable economic loss in large production units and unfavorable prognosis for sick rabbits. However, *Pasteurella* is thought to live in the throats and nasal passages of some rabbits without causing any signs of disease. Our case study is focused on pet rabbits that were diagnosed with acute pneumonic pasteurellosis. The rabbits can develop health problems caused by *Pasteurella*, especially if they are unwell or stressed, but the pet rabbit husbandry is much different from that of rabbit farm, and therefore the risk factor are others. The identification and quantification of risk factors are difficult and dependent by subjectivity of pet owner, and for a complete disease history we recommend a detailed discussion with all family members. In this outbreak the major stress factor was: phonic stress, excessive manipulation, transport stress and improper feeding. Clinical and lesion data collected in this study support the heterogeneity of rabbit *Pasteurella* infections.

Keywords: rabbit pasteurellosis, rabbit respiratory pathology

Pasteurellosis or haemorrhagic septicaemia is one of the most commune diseases of rabbits [5, 6, 7, 9, 11]. Pasteurella sp. are ubiquities in conventional farms of domestic rabbits, and its implications in leporidae pathology was discussed and detailed in several scientifically papers [2, 4, 5, 7, 9]. Infection most likely occurs immediately after birth, and the prevalence of pasteurella carrying rabbits increase to over 90% at 5 months of age [2, 7].

The pathology in rabbit farms pasteurella infected is maintly represented by rhinitis, pneumonia, otitis media, metritis, conjunctivitis, orchitis, subcutaneous abscess and septicemia [4]. The stress and immunodeficiency play a major role in the onset of auto-infection for the pasteurella carrier rabbits [5]. This required the development of health management programs in all holdings of domestic rabbits, adapted to the particularities of each unit in a manner that meets all the requirements of welfare. The pasteurella outbreaks can benefit from therapeutically intervention, but for the economical reasons usually all ill rabbits are eliminated from the farm herds [2, 5, 9]. The Veterinary medical approach of pet rabbits is different. The economical value is a low mark in health management of rabbit pets, and the therapeutically decision can be considerate if the owners want this.

Unfortunately there are several cases where the veterinarian intervention is delayed, in context of numerous peracute and acute septicemia infections. For this reasons, the preventive medicine is an activity that mast be included on the rabbit growth in non-professional houses.

MATERIALS AND METHODS

In this paper we focus on the relationship between pasteurella pathology and rabbits as companion animals. We present a clinical case of rabbit pasteurellosis in two Angora rabbit

bred with 5 weeks of age. The methods of investigations used were described previously [8, 12, 13].

RESULTS AND DISCUTIONS

Rabbits are particularly sensitive to environmental stressors and handling. For this reason, in the leporidae health management housing and feeding conditions are priority. In addition, contention and handling should be limited to that necessary. These objectives are not respected especially in pet rabbits. In the context of pasteurella portaj, the carrier pet rabbits stressed by an excessive handling and an improper accommodation (noise, poor housing, frequent transport, poor diet) have a great risk to develop a grave form of pasteurellosis by auto-infections. If those stressors are associated with the early removing from the rabbit nest, the risks are significantly increased. Health problems of pet rabbits acquire new facets in the context of the heterogeneous care and feeding conditions, tailored to each owner and major influenced by the level of owner education in interrelation with the pet rabbit.

The case history is a key in analyzing of each pathological event in rabbit pasteurella carriers. However, the identification in disease history of events that constitute the risk factors is usefully, but not sufficiently in etiological diagnosis. In our cases, unfortunately, the etiological diagnosis was obtained after death of rabbits. The evolution of disease was peracute/acute and the therapeutically actions were limited. Usually, rabbit pasteurellosis is clinically manifested by rhinitis. Symptomatology consists in sneezing, coughing, nasal serous, white or purulent discharges and noisy breathing. These secretions can be passed by rabbit grooming comportment to front paws and head (fig. 1), and can contaminated the sinuses, eye and tear duct and cause sinusitis, conjunctivitis and dacriocistitis. Since rabbits are mandatory nasal breathing, any obstruction of the nasal passages will result in respiratory compromise [1]. Although described in rhinitis, mouth breathing in rabbits is rather a sign prior to death, under which the vital prognosis is unfavorable.

Peracute septicemia with sudden death is not characterized by nasal discharge; even more the single element that can be identified is the bleeding diathesis [9]. This type of pasteurella infection is frequently described in young pet rabbits.

In acute form the pasteurellic pneumonia is associated with fatigue, lethargy, depression, anorexia and progressive weakness. In our study this was associated in one case with rhinitis. Frequently the respiratory pathology is associated with digestive disorders expressed by catarrhal (fig. 2) or hemorrhagic diarrhea. Bacteraemia is most often associated with the more virulent strains of P. multocida [10].

Usually, the diagnostic of lower respiratory tract disease is based on clinical exam and X-rays. Often the trachea and lungs listening permit the identification of the place where the airflow is obstructed. X-rays confirm the thoracic disease, and can differentiate the origin of respiratory signs. Thoracic ultrasounds can determine if abscesses or densifications are present. Blood tests are not useful in determining the etiology, but may be made in any context to determine the metabolic profile and evaluating the presence of concurrent disease. Serological testing of pasteurella can be performed, but results are difficult to interpret [1].

In lower respiratory tract the acute/subacute forms of pasteurellosis, with a history of over 5 days, are associated with marked pulmonary consolidation, often with hemorrhage and fibrin-purulent exudates in affected areas. Pulmonary lesions are frequently accompanied in sub-acute forms by pleuritis, fibrin purulent pericarditis and rarely with empyema [8, 10].

Marked pulmonary congestion was described in individuals who died 18 hours postinfection experimental intravenous [10], but this situation was described in young rabbit with natural infections (fig. 4). Symptoms and lung lesion picture are not always correlated. Thus, experimental infections have been described in animals have remained asymptomatic for 14 days, but at necropsy showed areas of consolidation or diffuse pulmonary nodules. However, there is a direct correlation to the duration of the evolution of infection and degree of lung damage. The asymptomatic rabbits, sacrificed at 14 days post-infection showed diffuse alveolitis with pneumocitic proliferation, leucocyte infiltration and obliteration of normal lung architecture. Acute-subacute forms are histopathological characterized by heterophilic and macrophage infiltration, and a marked alveolar interstitial infiltrat, but the airways are relatively free of inflammatory exudate.

In some cases the cell population that invade lung can be represented by polymorphonucleated, this cellular infiltration is associating with pleural fibrosis. The rabbits that will die at 7 days post-infection develops fibrin purulent bronchopneumonia with varying degrees of enlargement, while those that die at 9 days post-infection showed a marked congestion of alveolar capillaries and pulmonary edema with protein material [10].

Fig. 1. Rabbit, Angora bred, 4 weeks, male. Purulent rhinitis. Pasteurellosis

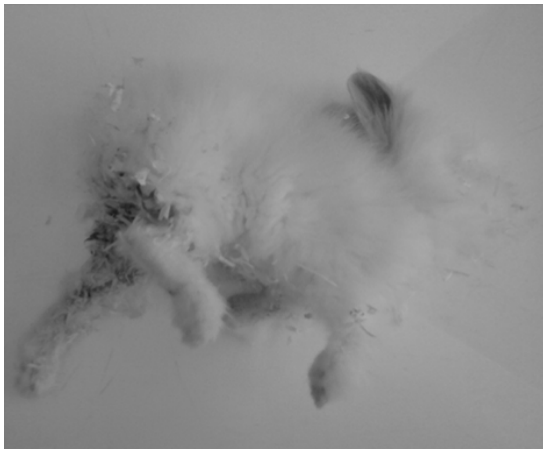

Fig.2. Rabbit, Angora bred, 4 weeks, male. Enteritis. Pasteurellosis.

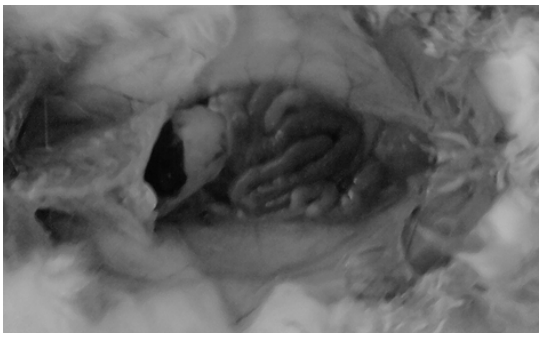

Fig. 3. Rabbit, Angora bred, 4 weeks, male. Visceral congestion. Pasteurellosis

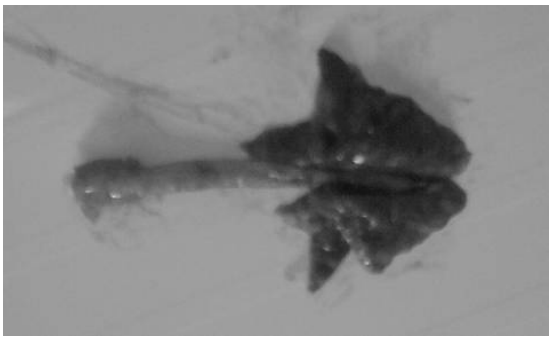

Fig. 3. Rabbit, Angora bred, 4 weeks, male. Pulmonary congestion. Pasteurellosis

Clinical and lesion data collected in this paper, and the information provided in many previous works with same subject supporting the heterogeneity of leporidae pasteurella infections [1, 2, 4, 5, 7, 9, 10].

Special problems in diagnosis are found in peracute/acute pasteurellosis of young pet rabbits. The small number of pet rabbits, without or with moderate signs often required

extensive investigations for confirmation, and that can delay therapeutic intervention and thus reduce the chances of recovering the animal. The lesions are not always helpful, often those have septicemia character. In addition, the pasteurella is not the single leporidae respiratory pathogen. *Bordetella bronchiseptica* is also involved in respiratory pathology of rabbits, *Klebsiella pneumonia*, pneumococcal and virus infections may have respiratory symptoms.

CONCLUSIONS

The young pet rabbit with peracute pasteurellosis don't have prodromal signs before sudden death. In the acute form the signs can consist in dyspnoea, misconduct, rhinorrhea, sneezing, and death in few days. The pet rabbits identification and quantification of pasteurellosis risk factors is difficult and dependent of data provided by the owner. Because the transportation to veterinary clinics, medical manipulation and therapy are stress factors, all young pet rabbits with possible signs of pasteurellosis need to be approached with caution. When the medical care without pet rabbit transportation is possible, this option can be considered to protect the patient.

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TREATMENT EFFICACY IN UDDER LOCALISATION OF CONTAGIOUS ECTHYMA IN SHEEP AND GOATS

R. BIȚA[1], G. RĂPUNTEAN[2], S. RĂPUNTEAN[2]
e-mail: bita.romulus-bn@ansvsa.ro
1. DSVSA Bistrița-Năsăud; 2. USAMV Cluj-Napoca

ABSTRACT
In Bistrita Nasaud County are affected by contagious ecthyma approximately 30-40% of lactating herd of sheep and goats leading to significant economic losses through decreased milk production and weight loss. Disease evolves throughout the year, but is prevalent in summer, especially if the pastures are not changed annually. Flocks are mixed, consisting of sheep and goats, predominantly mostly sheep. The disease has a higher incidence in the population of goats, which are more sensitive. Clinically the dominant locations are on the udder, with the formation of lesions on the teats. Diseased animals were treated locally for 3-5 days using drugs based on the following formula: a) methylene blue + oxytetracycline powder + honey or vaseline, b) methylene blue + oxytetracycline powder + osmatin c) methylene blue + oxytetracycline powder + grease. By using these treatment formulas good results were achieved, after 5 days the healing rate was over 90%. About 5% of treated animals showed complications with different pathogens, resulting mastitis and effectively requiring the removal of such animals.
Key words: sheep, goats, contagious ecthyma, udder, treatment.

INTRODUCTION

Contagious ecthyma of sheep and goats is widespread worldwide, causing major economic losses particularly in lambs and kids by mortality and to youth and adults with weakness and decreased milk production. The disease has health importance (zoonosis) as it passed from animals to humans, so safety measures must be taken (1, 2, 9, and 15). The disease is caused by a specific virus (Parapoxvirus) epiteliotrop, which has a high resistance in the external environment, thus ensuring greater importance of the secondary sources of infection and the perpetuation of disease outbreaks (1, 3, 14, 17).

Clinically, the disease is manifested by the formation of vesicles, pustules, and finally thick crusts on the lips, nostrils, face, teats, udder, legs and rarely in the mouth (2, 5, 8, 9, 13). In suckling, lambs and kids, there are frequent lesion locations in the mouth and around the mouth, and in lactating sheep and goats the dominant development lesions site is mammary gland skin, often complicated with mastitis. Less commonly found locations are seen at the ends of limbs (the crown and interdigital space), genital (vulva, foreskin, scrotum, and penis) and eyes (1, 2, 5, 7, 13).

Ecthyma causes major economic losses through mortality in young animals (lambs and kids), or complications that arise due to secondary bacterial infections (4, 14, 17). Morbidity in some populations may reach 70-80% even 100%, but mortality is low, less than 5-15% (3, 11, 13). Fatalities are more common in lambs and kids with severe form of disease, due to peribuccal injuries they refuse sucking and may die from starvation (7, 8). The young sheep and goat with oral and peribuccal lesions have difficulties to consume feed, so that economic losses are due to decrease in weight. The location on the udder is common in

lactating period; the economic losses are due to weakening and decreased milk production. To this adds costs of treatments and preventive nonspecific or specific measures (3, 12, and 15).

MATERIAL AND METHOD

In Bistrita Nasaud County approximately 30-40% of the flocks of dairy sheep and goat households are affected by contagious ecthyma. The paper presents results in disease treatment in sheep and goats dairy farms belonging to three veterinary districts: Bistrita Bargaului, Rodna and Zagra. Note that usually the flocks are mixed, composed of both sheep and goats, sheep are predominant. From each district one herd was chosen (where the disease was diagnosed), and the treatment groups comprises 100 sheep and 50 goats, which were monitored during treatment.

Sick animals were treated locally for 3-5 days, using the following drug formula:

a) methylene blue + oxytetracycline powder + honey or vaseline;

b) methylene blue + oxytetracycline powder + osmatin;

c) methylene blue + oxytetracycline powder + grease.

Animals were heading back into contention, then a clinical examination was performed by inspection and palpation, and udder and teats were washed with water to remove coarse impurities, then medication was topically applied. Treatment was performed daily for 3-5 days. Sick sheep and goats from the flock, were caught and handled daily during milking. They were separated and treated, then were allowed back in the fold. Milk from these animals was collected in separate vessels and destroyed or used in dog food after boiling.

RESULTS

On examination it was found that the lesions were located on the teats, as in one or both. Initially is noticed a red area (macula), which soon turns into vesicle or bleb, white-gray, which is covered with a brown crust, adhering and with an irregular outline. Lesions were usually singular and affecting only one teat (Fig. 1), rarely were more and smaller (Fig. 2). Sometimes lesions had the appearance of thick crust covering almost entirely the teat.

Fig. 1 – Lesions on the teats

Fig. 2 – Multiple lesions on the teat

In the farm of Bistrita Bargaului district (Bargaului Valley) the following formula was used for treatment: aqueous solution comprising 100 ml of methylene blue, oxytetracycline powder 50 g, 500 ml distilled water and honey or neutral vaseline. Before carrying out the treatment bee honey or grease is applied to udder for dipping crusts, then they are removed

and oxytetracycline and methylene blue aqueous solutions were topically applied. Treatment was carried out by topical applications, daily for 3-5 days.

In the farm of Rodna district (Someşului Valley) the following formula was used for treatment: 100 ml of methylene blue, oxytetracycline 100 g, 800 g osmatin. Osmatin was heated until it became slightly liquid and was mixed with oxytetracycline and methylene blue, obtaining a suspension that has been used for local administration for 3-5 days. Neocaf spray was used in goats, 250 ml spray containing oxytetracycline and methylene blue, by topical and spray for 3-5 days.

In the farm of Zagra district (Tibleşului Valley) the following formula was used for treatment: 100 ml of methylene blue, oxytetracycline 50 g and 800 g of grease. Grease was gently heated to form a uniform mixture with oxytetracycline. The first phase was methylene blue brushing and then the mixture of grease and oxytetracycline was applied daily, usualy for 3-5 days.

Contagious ecthyma incidence is higher in herds of goats than in sheep.

Images of treated animals are shown in the figures below (Figs. 3 and 4).

Fig. 3 – Teat during treatment

Fig. 4 – Teats during treatment

Number of animals treated and cured the number and percentage values obtained in the three farms are shown in Table. 1.

The result of treatment obtained in 3 days and after 5 days

Table no. 1

District in wich farm is located	The total number of treated animals	After 3 days of treatment		After 5 days of treatment	
		No.	%	No.	%
CSV Bistriţa Bârgăului (Bargaului Valley)	150	120	80,0	134	89,33
CSV Rodna (Someşului Valley)	150	123	82,0	136	90,66
CSV Zagra (Ţibleşului Valley)	150	126	84,0	139	92,66
Total	450	369	82,0	409	90,88

Analyzing the data from Table. 1, it appears that after the treatment performed on sheep and goats with contagious ecthyma (mammary form) were obtained satisfactory results. After 3 days of treatment, from the total of 450 animals treated, in 369 (82.0%), there was an

improvement in the development of lesions and after 5 days the situation improved even more, so that were considered as cured 409 animals (90.88%). Differences in the improvement and healing of lesions observed among the three herds and the different formula applied for treatment are insignificant, which means that effective treatment was established. We appreciate that the mixture that included an antiseptic (methylene blue), a broad-spectrum antibiotic (oxytetracycline) and an emollient substance (honey, osmatin, grease or neutral vaseline), meet the qualities of an complex product that favored both healing and prevention of secondary bacterial complications. In 4-5% of animals included in the treated groups some complications were found with diverse microbial flora, which resulted in suppurative and necrotic lesions, which led to the compromise of udder and actually requiring slaughtering.2

Young lambs and those belonging to households, after weaning are kept in separate flocks from sheep in lactation, but they have just rarely serious disease, clinically presenting peribuccal lesions and mouth problems. When presented serious forms they were treated locally with methylene blue and applications of tetracycline suspensions or ointments.

Contagious ecthyma disease has an endemic trend in sheep and goat farms in the area of Bistrita Nasaud. Illness affects both young lambs and fattening farms, in which clinical dominates peribuccal and oral forms, but the disease is also present in private backyards in milking sheep and goats, and we appreciate that they are affected in 30-40%. Sheep and goats heard are usually mixed, approximately 400 heads, prevailing sheep. Goats are more sensitive making injuries more serious and favored by the greater length of nipples (1, 5, 14, and 17). The disease is endemic and outbreaks occurs dominant in summer, during milking, the appearance is enhanced by producing more frequent injuries on the teats, and transmission as a result of milking, and by flying insects, which fulfill the role of vectors (4, 9).

Therapeutic formulas generally seek achievement of an emollient effect, local asepsis with broad spectrum antibiotics combat secondary bacterial infections (7, 11). This treatment generally does not affect the evolution and pathogenic immune response caused by viral infection, but prevents bacterial infections that worsen lesions (7, 8). Crusts from the mammary gland should be kept flexible, the purpose being applied by different local applied ointments, iodinated glycerol solutions (5, 11).

Treatment conducted in sheep and goats in this study was based on local applications of therapeutic formulas that have had their composition with antiseptic effect (methylene blue, oxytetracycline antibiotic with broad-spectrum antibacterial action and an emollient substance represented by honey, neutral grease, or osmatin).

Methylene blue is recommended because it is moderate and lasting antiseptic, penetrating the lesional tissue (wounds, burns, gall, pressure sores, frostbite, glossitis etc.) and by repeated applications have an analgesic effect, promoting healing. In view of these properties with an antiseptic effect this substance was used in all three forms of treatment (6, 10).

Oxytetracycline is a broad-spectrum antibiotic (tetracycline group), with bacteriostatic action against many Gram-positive and Gram-negative bacteria, contained in numerous products that are in the form of injections (solutions or suspensions) or ointments, powders, for local administration. The combinations used in cases with ecthyma had a decisive impact in preventing secondary bacterial infection, promoting healing.

Emollient substances are necessary to achieve softening crusts, having protective effect against moisture, sunlight, cold and prevent the formation of crevasses. Vaseline is a mixture of saturated hydrocarbons with emollient effect, cheratoplastic and against itching, but has a lower power of penetration and could not become rancid. It can be used as such (*per se*) or in other ointments. Osmatin contains unsaturated aliphatic amine, paraffin and vaseline

and it have bactericidal effect against pathogens that are transmitted by milkers hands (9, 12). Pork grease have an emollient effect, but can become rancid very easy and to prevent this we can add preservative (6, 10). Honey is recognized for its beneficial effects.

Other authors recommend the use of iodine solutions, as Betadine, iodine sprays, povidone iodine (1, 5). It is considered that an iodine solution inactivates Gram-negative and Gram-positive bacteria, fungi, viruses and protozoa. Their application forms a film that protects tissues from contamination with bacteria, not irritating, ensure full coverage and good penetration and can be used in many animal species and in different age categories. Should also considering flying insect control (4, 9). Whatever treatment is aimed at protecting tissues and prevent secondary bacterial infections.

CONCLUSIONS

1. In Bistrita Nasaud County are affected by contagious ecthyma approximately 30-40% of backyard lactating herd of sheep and goats (mixed flocks); infection evolving in the summer and is more common in goats.

2. 450 animals were treated, each 150 heads of three different flocks, and in three different areas (Bistrita Bargaului, Rodna and Zagra), which clinically showed eruptive lesions on the teats; with stadial development in which highly expressed was the crust stage.

3. Sick animals were locally treated daily for 3-5 days with a medication consisting of an antiseptic substance (methylene blue), a broad-spectrum antibiotic (oxytetracycline) and an emollient substance (osmatin, grease, honey, pork lard).

4. Following treatment was noted an improvement of lesions after 3 days and healing after 5 days, in more than 90.0% of animals; a small number of animals showed complications and mastitis appeared, not sensitive to treatment, requiring their removal.

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EFFECTIVENESS OF TREATMENT FORMULA IN CONTAGIOUS ECTHYMA IN LAMBS FROM INTENSIVE BREEDING SYSTEM

R. BIȚA[1], S. RĂPUNTEAN[2], G. RĂPUNTEAN[2]
e-mail: bita.romulus-bn@ansvsa.ro
1. DSVSA Bistrița-Năsăud; 2. USAMV Cluj-Napoca

ABSTRACT

Livezile farm from Bistrita Nasaud county, is growing Țurcană breed lambs for meat production, by its own resources and through acquisitions from neighboring counties. Housing is performed in open shelters on metal grates floors. The lambs aged between 3-4 months showing lesions developed on the skin of the lips and frequently extended to the mucosa of the mouth. 150 lambs were treated, divided into three lots of 50 head. Local treatment consisted of manual removal of crusts, followed by methylene blue brushing and spraying antibiotics packed in spray bottles for 5 days. In most of the treated animals was found an improvement in disease development after 3 days of treatment, and after 5 days to produce healing in more than 90% of treated animals.

The best efficiency expressed as the percentage of healing at after 5 days was found in the product Terramycin (98.0%), followed by Alamycin (94.0%) and Donarom (92.0%). We appreciate that the product Donarom was better supported by the fact that dexamethasone reduced the inflammatory phenomena and anesthesine analgesic effect attenuates the pain. Antimicrobial efficacy was favored by ensuring an appropriate diet, consisting of soft food and water ad libitum and improving hygiene conditions.

Key words: contagious ecthyma, lambs, treatment

INTRODUCTION

Contagious Ecthyma of sheep and goats is widespread worldwide, causing significant economic damage, particularly in lambs and kids and in the youth adult population mortality, weakening and declining production. Disease has important health importance being placed in group zoonoses and easily transmitted from animals to humans, so that staff to examine sick animals should wear protective equipment (1, 2, 3).

The disease is caused by a specific virus, epiteliotrop (Parapoxvirus), acute and characterized clinically by a vesicular rash with scabs forming on lips, nostrils, face, eyes, teats, udder, feet and mouth (2, 3, 4, 6, 9). During weaning young animals are most susceptible, compared with other age groups (6).

Contagious Ecthyma cause significant economic damage by mortality, especially in youth, as a result of complications that may occur consecutively secondary bacterial infections. Morbidity may reach 70-80% even 100%, but mortality is low, less than 5-15% (3, 8). Fatalities can be found in lambs and kids with severe form of disease due to lesions of the mouth, they refuse the sucking and may die by starvation. Oral and around the mouth lesions in young, will reduced weight due to lower feed consumption leading to economic losses. In the location on the udder, mothers refuse to breastfeed the lambs/kids because of the pain, and they may weaken and die, and economic losses are due to both weaken and decrease weight and reduce milk production. This is added to costs of nonspecific or specific treatments and prevention measures (7). It is known that disease tends to be permanent in some farms,

due to the fact that the virus has a high resistance to temperature and dryness, which can survive in dried scabs for years.

In Bistrita Nasaud County contagious ecthyma develops in lambs between 3-4 months, in breeding and fattening farms with oral location or around the mouth and mammary location in adult ovine and goats from households. The paper presents results from treatment in an intensive farming that has developed endemic contagious ecthyma.

MATERIAL AND METHOD

The experiment was conducted on three groups of sick lambs, each batch consisting of 50 subjects, using different formulas for treatment. Lots of sick animals were separated from the rest of the herd. Local treatment consisted of manual removal of crusts, followed by methylene blue brushing and antibiotic application by spraying:

- Group 1 was treated with Terramicine spray (Pfizer) 150 ml bottles containing 4 g of Oxytetracycline hydrochloride and the green marker substance;
- Group 2 was treated with Alamycin spray (Norbrook) 140 g vials containing 5 g of Oxytetracycline hydrochloride and the blue marker substance;
- Group 3 was treated with Donarom spray (Romvac) 150 ml bottles containing 10 g of oxytetracycline and dexamethasone, crystal violet, anesthesine and excipients.

Support staff has been trained on the risk of contamination and have taken safety measures (use of gloves, gowns, rubber boots and provide antiseptic substances). To reduce environmental contamination, crusts removed manually and collected in the containers were burned, and accommodations have been cleaned and repeated disinfection was made with Virkon and Virocid solution.

RESULTS

Sheep breeding and fattening farm (Țurcana race) is in the area of Livezile in the county of Bistrita, is owned by a private breeder. Fattening young sheep population is formed from its own sources and through purchase of sheep from different owners in the county of Bistrita and in neighboring counties (Maramures, Satu Mare, Cluj, Salaj, Mures, Suceava). Growth is housed (open sheds), over the metal gratings on a total of 10 lines, each line having two sections (Fig. 1 and 2). Feeding is done with alfalfa, natural hay and concentrates and the watering is automatic with constant level troughs.

Fig. 1 & 2 - Aspects of the accommodation sections of young animals

In the flock of young sheep for fattening, at the age of 3-4 months is evolving contagious ecthyma; illness is most common in the months from July to August, the occurrence of red by poor hygienic conditions. Transmission shall directly benefit from the close contact between animals due to the growth system.

Clinically ill animals show more frequently lesions around the mouth and oral sites, rarely other sites. Vesicular rash appears on the lips, corners, nose tip of the snout and rarely on the cheeks, eyelids, ears or proliferative lesions appear in the mouth. Evolutionary stages very quickly succeeded each other, often the disease was seen in the scabs stage. Following the opening of vesicles occur bleeding lesions, painful, becoming scabs, leading to thick formations, light gray brown, irregular, adherent to underlying tissues (Fig. 3 and 4).

Fig. 3 & 4 – Young sheep with ecthyma: crust located around the mouth and nasal

Most animals have lesions located around the mouth, lips being comprised entirely or only some areas more frequently lip corners. Sometimes extending stage is observed, starting from the nose and catching the mouth. In cases with more severe forms, swollen lips and muzzle were processed in a large necrotic wound, which bleed easily if detached (Fig. 5 and 6). Lesions were very painful, preventing animals consume feed.

Fig. 5 & 6 - Content and application of medication by spraying

After manual removal of the crusts the methylene blue brushings were made, taking into consideration the whole area from which the crusts have separated, after which proceeded to spray the antibiotic to the affected areas. Spraying was done from a short

distance (10-15 cm) for a few seconds until it covered the whole affected area. Treatment was given daily for 5 days.

Fig. 7 & 8 – Animals with lesions around the mouth in the treatment phase

Feeding sick animals was made with soft and liquid feed, and improved hygiene. Accommodation was subjected to repeated disinfection using Virkon and Virocid solutions and clean bedding was provided.

Treated animals were daily observed and assessments being made of efficacy at after 3 days and 5 days. The assessing focuses on the healing, regression of lesions and restored ability to feed properly. The results obtained for each of the products used are listed in Table.1.

Treatment outcome in the three groups

Table 1

No.	Groups	Antibiotics (spray)	Results			
			After 3 days		After 5 days	
			No.	%	No.	%
1	Group 1	Terramycin (Pfizer)	39	78,0	49	98,0
2	Group 2	Alamycin (Norbrook)	35	70,0	47	94,0
3	Group 3	Donarom (Romvac)	32	64,0	46	92,0

Analyzing the table data can be seen that treatments have proved effective in all three applied formulas. In most of the treated animals was found an improvement in disease development after 3 days of treatment and after 5 days the cure occurs in over 90.0% of treated animals. Those who were not cured were still treated. Best efficiency, expressed as the percentage of healing after 5 days was found in the product Terramycin (98.0%), followed by Alamycin (95.0%) and Donarom (92.0%). We appreciate that Donarom was better supported by the fact that dexamethasone reduced the inflammatory phenomena and anesthesine attenuates the pain by analgesic effect.

DISCUSSIONS

To treat infectious ecthyma many and varied therapeutic formulas were tested (brushings with iodine solution, iodized glycerine, potassium permanganate, methylene blue,

spraying with sulfa and antibiotics etc.). (3, 6, 7, 11). In the treatment of around mouth and nostrils forms, generally good results are obtained, but in oral form, which usually occurs in lambs and kids, the therapeutic efficacy is lower (6).

The principle is accepted that in cases of benign forms of disease no treatment is necessary, the animals heal in about 2-3 weeks, achieving a satisfactory immunity to new infections (2, 3, 4). In severe forms, which may occur in lambs and young animals, treatment must be done to prevent secondary bacterial infections, which can sometimes cause serious complications. For this purpose, most often is being made a local treatment (spray, ointments) with broad spectrum antibiotics in combination with different antiseptics (3, 4).

Pop M, et al., (1975) performed treatments by using one of the following formula: local applications of lotagen, lotagen in saline solution (1/1), bromocet, 2% methylene blue solution, iodized glycerine, ramcemic chloramphenicol ointment 10%, 5% terramicine ointment. In all peribuccal form cases before medication the crusts scrape was made (6).

Methylene blue is recommended because it is moderate and lasting antiseptic, penetrating the lesional tissue (wounds, burns, gall, pressure sores, frostbite, glossitis etc.) on which, by repeated applications have an analgesic effect, promoting healing. Given these properties, this substance was used for its antiseptic effect in all three forms of treatment, preceding administration of the antibiotic spray.

Antibiotics in a spray form has the advantage of easy application and sprayed from a convenient distance provides a uniform distribution on the affected area. Small size of particles splashed ensure penetration into the smallest crevices, without any further maneuvers, benefiting in smooth and rapid wound healing. Spraying should be done with the bottle upright at a distance of 10-15 cm and a length of at least 5 seconds until the product evenly cover the entire area affected.

All three sprays (Terramycin, Donarom and Alamycin) that have oxytetracycline as active substance in combination with marker coloring substance and some excipients, have bactericide effect knowing that oxytetracycline hydrochloride is a broad-spectrum antibiotic that act as bacteriostatic on many Gram positive and Gram-negative bacteria, the rickettsia, and spirochaetes. After spraying a protective film is formed having a protective effect over lesion tissue, bacterial complications are avoided and healing is favored.

To prevent disease will avoid feeding coarse feed, which can cause the nose or mouth lesions (5) and will combat flying stinging insects (3, 5, 7, and 10). Antimicrobial efficacy may be enhanced by ensuring adequate diet consisting of soft food and water ad libitum, and improving hygiene conditions (11).

CONCLUSIONS

1. In Livezile farm from Bistrita Nasaud county, young Turcana breed sheep are reared for meat production. In young animals aged between 3-4 months is evolving oral and peribuccal forms of contagious ecthyma.

2. A total of 150 lambs, divided into three batches of 50 heads were initially treated topically doing manual removal of crusts, followed by methylene blue brushing and spraying antibiotics for 5 days.

3. Efficacy of products used resulted in gradual healing of lesions after 3 days of treatment and complete recovery after 5 days, the best performance was found to Terramycin spray (98%), followed by Alamycin (94%) and Donarom (92%).

4. Antimicrobial efficacy was enhanced by ensuring adequate diet consisting of soft foods, water ad libitum and better accommodation.

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CLINICAL AND ANATOMOPATHOLOGICAL RESEARCH IN PORCINE CIRCOVIRUS TYPE 2 INFECTION IN YOUNG SWINE

N. CĂTANA, V. HERMAN, IONICA, FODOR, V. PETRUSE
Facultatea de Medicină Veterinară Timişoara
epirovet@yahoo.com

Abstract
Porcine circovirosis is a disease of viral etiology, with special significance in the intensive farming of pigs. The investigations were conducted during 2007-2009 in three swine farms, in the Western part of country, where it was pursued the presence of PCV2 infection by clinical and anatomopathological examinations. During the research, in the herds of these three farms it was reported only the post weaning multisystemic wasting syndrome (PMWS), the other syndromes not being reported. Observations of clinical trials confirm that based on clinical examination may be detected the 6 fundamental symptoms which may create the suspicion of PMWS syndrome. We found these symptoms described above, except the presence of jaundice, in the herds from the three farms. Gross lesions encountered most frequently were: enlarged inguinal lymph nodes and necrotic-hemorrhagical tonsillitis, catarrhal and hemorrhagic bronchopneumonia, hyperplastic splenitis, enlarged mesenteric lymph nodes and pallidity of mucosae and tissues.

Key words: swine, PCV 2, symptoms, anatomopathological lesions.

Swine pathology in intensive farming has changed significantly due to the emergence of nosological entities, especially with viral etiology, that is currently a dominant component of infectious pathology.

Porcine circovirosis is a disease of viral etiology, with special significance in the intensive farming of pigs. First described in Canada in 1991, that disease has spread worldwide, being also reported and studied by a multidisciplinary team in Romania. In many countries, given the economic importance of the disease and some unexplained aspects of epidemiology, pathogenesis, anatomoclinical signs, diagnosis and prevention of this disease, several collectives have done large and diverse studies in this area (1, 4, 6).

The research of this work were made in order to obtain epidemiological and anatomoclinical data, in three swine farms in Western part of the country where it was suspected the presence of infection with *PCV2*, respectivelly PMWS syndrome evolution.

MATERIALS AND METHODS

The investigations were conducted during 2007-2009 in three swine farms, in the Western part of country, where it was pursued the presence of PCV2 infection by clinical and anatomopathological examinations.

Farm A of pigs' breeding, in Arad county, has organized activities in three areas: maternity, young swine and fattening pigs, and the breed effective is the hybrid PIC, Large White breed and Kahib hybrid. In the farm is practiced only the artificial mounting. In the beginning of the research the herd structure was as follows: 1442 sows, 363 young sows, 12 boars, 1719 infant piglets, 6315 young pigs and 5477 fat pigs.

Farm B, in Bihor county, is also organized in three sectors: maternity, young swine and fattening pigs, and the breed effective is the hybrid PIC, Large White and Duroc breeds, from the import.

From the beginning of the experiment at this farm herd structure was as follows: 1200 sows, 405 young sows, 15 boars, 1520 piglets infant, 5800 young pigs and 6200 fat pigs.

Farm C, situated in Caras-Severin county, is a fattening farm for young swine designed to be slaughtered. Several times a year, for that purpose they are imported effectives of young pigs aged of 2,5-3 months. Average the imported effectives are around 3000 heads per series.

Research on the presence of *PCV2* infection was made in these farms, because the existing swine herds, PMWS syndrome was suspected.

Clinical examination. Clinical examinations were performed on age groups, seeking to the presence of syndromes caused by *PCV2* virus. In the three farms, in terms of clinical signs, only PMWS syndrome was suspected.

Anatomopathological examination. Anatomopathological examinations were performed only on young swine corpses in which PMWS syndrome was suspected. From these corpses were sampled organs (lungs, spleen, kidneys and inguinal lymph nodes). These samples were intended for laboratory tests.

Histopathologycal examination. It was performed on the organs sampled in the necropsy of corpses, being used the conventional methodology, by fixing in absolute alcohol, including into the paraffin, cutting and sections' coloring by trichromic-HEA (Hematoxiline-Eosine-Metil Blue) stain. This examination was conducted in order to highlight some characteristic lesions of *PCV2* infections (4).

Cytological examination. This examination was performed on fingerprints smears from the inguinal lymph nodes. For the evidence of changes in cytology May Grundwald Giemsa stain was used (5).

RESULTS AND DISCUSSIONS

Research conducted in the three farms confirmed the presence of *PCV2* infection, which has evolved during that period as PMWS syndrome. At the same time these results confirmed the observations of other researchers who consider that to certainty specify the diagnosis there are required more laboratory tests and by corroborating the obtained results may be confirmed the presence of the disease.

Results of clinical examination. Clinical examination was performed on age groups being recorded all detected symptoms. During the research, in the herds of these three farms it was reported only the post weaning multisystemic wasting syndrome (PMWS), the other syndromes not being reported.

In the sick pigs first signs began to appear after the age of 6 weeks. The dominant symptom was progressive weakening of the affected pigs. Along with that symptom they also have been reported: dyspnea, diarrhea, pallidity of skin and mucous membranes and enlarged superficial lymph nodes, especially of the inguinal lymph nodes. In many animals these symptoms have been masked by symptoms of some associated diseases usually with respiratory tracking.

Observations of clinical trials confirm that based on clinical examination may be detected the 6 fundamental symptoms which may create the suspicion of PMWS syndrome. We found these symptoms described above, except the presence of jaundice, in the herds from the three farms. In farm A the clinical examination confirmed that there were the most clinical cases of PMWS syndrome that developed constantly, in every series of piglets after six weeks of age, but records of morbidity and mortality were not kept clearly in the farm.

In the farm B and farm C the number of sick animals was lower but the development of symptoms was identical to that of farm A.

These findings are similar to existing data in the literature on the prevalence of PMWS syndrome which is more frequent compared with other syndromes and the morbidity of this syndrome is variable (2, 6).

Clinical observations have confirmed the presence of so-called associated diseases PCVAD (Porcine Circovirus Associated Disease) which in the three farms were as follows: pasteurellosis, contagious pleuropneumonia, enzootic pneumonia and dysentery with *Brachyspira*.

Results of anatomopathological examination. Anatomopathological examinations conducted on the corpses suspected of PMWS have revealed characteristic gross lesions more or less associated with lesions caused by associated infections. Gross lesions encountered most frequently were: enlarged inguinal lymph nodes and necrotic-hemorrhagical tonsillitis, catarrhal and hemorrhagic bronchopneumonia, hyperplastic splenitis, enlarged mesenteric lymph nodes and pallidity of mucosae and tissues. In the three farms there were not found characteristic lesions of dermatitis-nephritis syndrome of pigs to the necropsy of corpses.

In other organs that liver, kidney, stomach, small intestine and large intestine, the observed lesions were specific to other diseases, usually bacterial infections or dystrophic injuries consecutive of some nutritional diseases or mycotoxicosis. In more corpses, perhaps with longer clinical evolution, specific lesions of PCVAD diseases were found, such as: fibrinous pleuritis, fibrinous or hemorrhagic bronchopneumonia, poliserositis and necrotic-hemorrhagical colitis.

The results of anatomopathological exam conducted in the three farms are linked to existing literature data regarding the frequency and the significance of characteristic lesions of this syndrome and of the lesions caused by PCVAD (3, 4, 6).

Results of histological and cytological examination. Characteristic histological lesions were found in the lymph nodes and spleen and lesions of viral and bacterial associated infections have been revealed in other organs.

In **inguinal lymph nodes** by histological examination were highlighted the following lesions: rendered lymphocytic depletion, histiocytes massive infiltration and the presence of giant cells (fig. 1). These cells confirm the presence of granulomatous type inflammation, respectively the granulomatous lymphoreticulitis characteristic to *PCV2* infection.

In the **spleen** was found also a marked lymphocytic depletion until the disappearance of lymphoid follicles and the presence of giant cells characteristic to granulomatous splenitis. In the case of that organ also the found lesions are characteristic to *PCV2* infection, having a special meaning the inflammation of granulomatous type.

Cytological examination performed on fingerprints smears of inguinal lymph nodes as the methodology recommended by LUPPI and all. (2008) highlighted a small number of lymphocytes and a large number of histiocytes. Multinucleated giant cells have also been highlighted. This exam has the advantage of speed on the diagnosis of PMWS syndrome, it could be performed in any laboratory and the results provided help to specify the diagnosis, not to replace the histological and serological examination and the PCR test (5).

According to the results obtained by LUPPI and all (2008), depending on the number of giant cells and on the infiltration with histiocytes it can appreciated the evolution of *PCV2* infection. These authors consider that the changes found, depending on their intensity, may be of grade 1, grade 2 and grade 3 (5).

The obtained results in the research carried out by this examination revealed granulomatous inflammation of lymph nodes but has not allowed us to appreciate the intensity of existing cytological changes in lymph nodes, respectively the moment of infection.

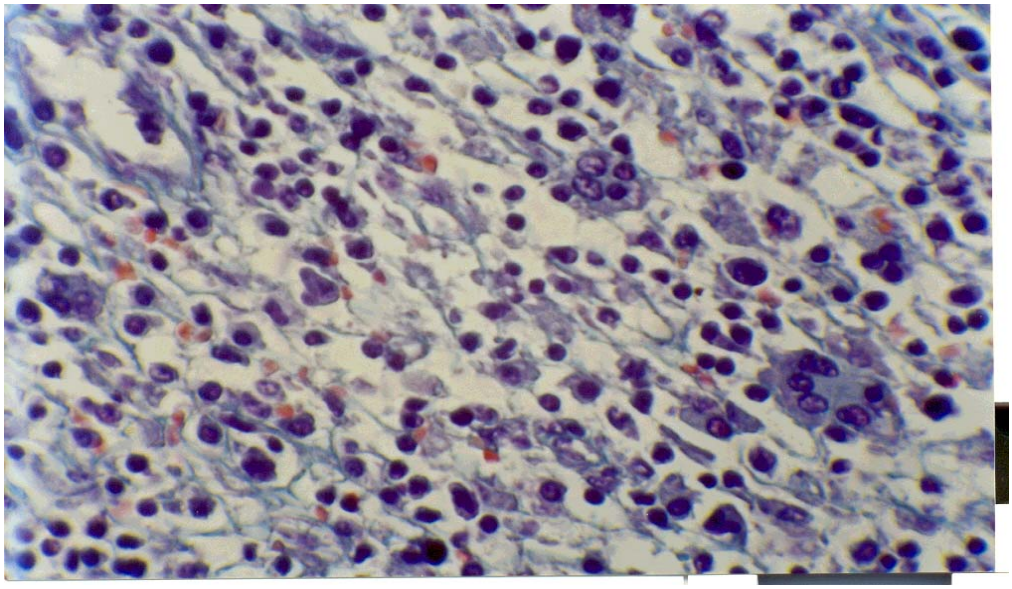

Fig. 1 Giant cells in inguinal lymph nodes

CONCLUSIONS

In the three farms, *PCV 2* infection was introduced by the bought and uncontrolled swines that were virus carrying.

Clinical examination confirmed the development of PMWS syndrome with dominant symptoms, less jaundice, in the three farms.

Anatomopathological examination constantly revealed enlarged inguinal lymph nodes and the presence of gross lesions produced by PCVAD.

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SEROLOGICAL AND PCR RESEARCH DIAGNOSIS OF THE INFECTION WITH PORCINE CIRCOVIRUS TYPE 2 IN YOUNG SWINE

N. CĂTANA[1], VIRGILIA POPA[2], V. HERMAN[1], IONICA FODOR[1]
[1] Facultatea de Medicină Veterinară Timişoara
[2]S.N. Institutul Pasteur S. A.
epirovet@yahoo.com

Abstract
In european countries PCV2 infection evolves epidemic, with the starting point of western countries, from where it permanently expanded to central and eastern Europe.To confirm PCV2 infection in the existing swine herds of the three farms were made the following laboratory tests: immunoassay test, Polymerase chain reaction.By PCR reaction viral DNA was revealed, respectively ORF2 gene, in samples of lymph nodes and spleen, being confirmed the presence of PCV2 virus, by a reliable diagnosis method.Analyzing the results obtained in the three farms, regarding the seroprevalence by age groups and type of infection we can note that PCV2 infection was present in all production groups from farms with the exception of infant piglets, these infections being classified in all 3 categories according to the evolution moment..
Key words: *PCV2* infection, granulomatous inflammation, seroprevalence.

In european countries *PCV2* infection evolves epidemic, with the starting point of western countries, from where it permanently expanded to central and eastern Europe. Thus, there are european countries where the disease develops severe, with large economic losses, countries where the disease is not a major problem or is less monitored as Romania and countries where the disease has not a recognized status (2,3).

In this context it has been included the research conducted in a multidisciplinary research program of C.E.E.X. type, coordinated by SN Pasteur Institute SA, the Department of Infectious Diseases of FMV Timişoara being foreign partner.

The research covered the work were made in order to confirm the presence of *PCV2* infection by laboratory testing in three swine farms from the western part of the country.

MATERIALS AND METHODS

To confirm *PCV2* infection in the existing swine herds of the three farms were made the following laboratory tests.

Immunoassay test. A variant of catch was used to determine antibody titres (IgM and IgG type) specific to *PCV2*. To this purpose it was used the commercial kit Ingezim Circovirus IgG / IgM, made by Ingenasa Madrid - Spain, who also supplied the software for interpretation.

Polymerase chain reaction. It was done to detect ORF2 gene that is present in the viral DNA (5). To detect that were used gene primers ORF2 and amplicons 967 bp. This technique was performed in the Laboratory of Molecular Biology of SN Pasteur Institute S.A Bucharest. The reaction was carried out on samples of organs taken from corpses with necropsy (2).

RESULTS AND DISCUSSIONS

Results of polymerases chain reaction. By this reaction viral DNA was revealed, respectively ORF2 gene, in samples of lymph nodes and spleen, being confirmed the presence

of *PCV2* virus, by a reliable diagnosis method. This reaction is recommended to confirm diagnosis of *PCV2* infection as virusological examination, respectively the isolation and cultivation of this virus, cannot be implemented in all laboratories (1, 4).

Serological examination conducted by the reaction of immunoassay, catch variant highlighted the presence of specific antibodies in serum collected from animals in the three farms. The values of these titres expressed in OD (optical density) were processed through its program for the interpretation of the used commercial kit. The program for the interpretation grouped the obtained results in positive samples and negative samples based on OD values of the investigated sera compared with OD values of control sera.

This program allows in the positive sera, depending on the optical density for IgM and IgG, the classifying of infections in three categories, as follows: active infection, recent infections, old infections.

Table 1

The serological test results

Category	Results	Farm A		Farm B		Farm B	
		No.	%	No.	%	No.	%
Young pigs	Negative	5	9,6	13	32,5	-	31,5
	Pozitive	6	11,5	12	30	-	68,4
Sows	Negative	8	15,3	1	2,5	6	-
	Pozitive	16	30,7	4	10	13	
Fat pig	Negative	-	-	2	5	-	-
	Pozitive	13	25	8	20	-	-
Boars	Negative	2	3,85	-	-	-	-
	Pozitive	2	3,85	-	-	-	-
Centralization results	Negative	15	28,8	16	40	6	31,5
	Pozitive	37	71,1	24	60	13	68,4
	Active infection	2	3,8	6	15	13	68,4
	Recent infection	8	15,4	8	20	-	-
	Old Infection	27	52	10	25	-	-

PCV2 infection was confirmed serologically in all age groups under this test.

In **farm A**, the most positive samples (37.7%) were from sows followed by fat pigs (25%) and young pigs (11.5%).

In **farm B** the most serologically positive samples were from young pigs (30%), followed by fat pigs (20%) and sows (10%).

In **farm C**, in the imported young swine, 68.4% of samples were positive.

Dynamics of positive samples, whose titres were expressed in OD is reproduced in table 1.

The interpretation program of the commercial kit also established the results of the infection type, respectively the three categories (active, recent and old).

In **farm A**, 71.1% of samples were positive, 52% being old infections and 15.4% recent infections.

In **farm B**, 60% of samples were positive, of which 25% with old infection, 20% with recent infection and 15% with active infection.

In **farm C**, 68.4% of samples were positive, all being active infection.

Analyzing the results obtained in the three farms, regarding the seroprevalence by age groups and type of infection we can note that *PCV2* infection was present in all production groups from farms with the exception of infant piglets, these infections being classified in all 3 categories according to the evolution moment.

Although initially most researchers argued that serological examination is not sufficiently decisive, currently by using enzyme immunoassay test with kits and performantes programs for the interpretation of results, it can be determined with certainty so *PCV2* infection seroprevalence and type of infection.

These results completed the results of other exams and they are interrelated with existing data in the literature (3, 4).

CONCLUSIONS

Characteristic histological lesions (lymphocytic depletion, infiltration with histiocytes, giant cells) were consistently present in inguinal lymph nodes.

Inflammation of granulomatous type, represented by multinucleated giant cells, was confirmed by cytological examination, this examination being recommended as a guidance and rapid test for PMWS diagnosis.

Serological examination conducted by the capture enzyme immunoassay test confirmed the presence of *PCV2* infection and allowed the classification of infections in three categories: active, new and old.

By using performing kits the enzyme immunoassay test can be used in epidemio-surrveillance of *PCV2* infection in swine farms.

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RESEARCH CONCERNING THE ANTIBACTERIAL ACTIVITY OF HONEY AGAINST COAGULASE-NEGATIVE STAPHYLOCOCCI WITH USE IN VETERINARY MEDICINE

C.CEAUŞI, I. ȚOGOE,
ANCA MARIA GALIŞ, L. TUDOR, I.L. ILIE,
* Faculty of Veterinary Medicine Bucharest

Abstracts
Honey is considered to have a great potential to treat infected wounds, but for the moment the details in the research field are not so concluding and still not enough as number. The apparition and development of antibiotic-resistant strains of coagulase-negative staphylococci is considered a very complicated and important problem in the medical field.
The objective of this study was the to determine the optimal dilution of 3 types of honey, for 25 clinical cases with isolates of coagulase-negative staphylococci. In order to determine the active dilution, it was used a technique of agar incorporation, with dilution steps of 5 %. The plates were inoculated with 25 ml of culture, from the isolated coagulase-negative staphylococci. Honey presented an inhibitory effect at dilutions from 4,2 to 1,8 % for acacia honey, 3,7 to 1,2 % for linden honey and 3,9 to 1,1 % for polifloral honey. The main conclusion was that these types of honey may have beneficial activities when treating different types of wounds, especially when coagulase-negative staphylococci are present.
Keywords: coagulase-negative staphylococci, honey, infection, wounds.

Regarded as saprophytic commensals of low pathogenicity that normally inhabit human skin and mucosal membranes, coagulase-negative staphylococci were not considered but until now opportunist pathogens, being now among the five most frequently isolated causative agents of hospital-acquired infection (2, 5, 7), frequently associated with the use of temporary and permanent invasive medical devices (intravenous catheters, dialysis catheters, urethral stents, endotracheal tubes, etc.). The most commonly isolated coagulase-negative staphylococcus is the S. epidermidis, but there were reports including other species too (1, 10). The apparition of a methicillin-resistant strain of these coagulase-negative staphylococci and also of the other species which reveal a multiple antibiotic resistance, has made this problem a very important and difficult one (4, 8, 9). Until now, coagulase-negative staphylococci were not tested sufficiently, so this study had the objective of investigating their susceptibility to honey. Not all the honey types present an antimicrobial activity which could influence precisely the coagulase-negative staphylococci activity, therefore honey with median levels of activity was used.

MATERIALS AND METHODS

The three natural honeys used were selected to be close to the median antibacterial activity for each type of activity and tested against Staphylococcus aureus: acacia honey, linden honey and polifloral honey. The isolated cultures of coagulase-negative staphylococci

were obtained from the veterinary clinic, in number of 25. The cultures were isolated from peritoneal fluid, cerebrospinal fluid and breast aspirate. The isolates were identified using the classic biochemical and morphological techniques. The media used for this investigation included: TSB, nutrient agar and blood agar (with defibrinated sheep blood).

Before the initiation of the tests, each isolate was introduced to culture in TSB, and after incubation at 37°C for 16 hours, the agar incorporation technique was used. Nutrient agar was made up at double strength, measured out into 25 mL aliquots and autoclaved. To prepare the plates it was melted and tempered in a 50°C water-bath until poured. The honey samples were prepared in sterile de-ionized water, and poured into duplicate Petri dishes, forming a mixture with the agar.

A dilution series with honey concentrations in the range 5–20% (v/v) final honey concentration, in 5% increments, was used for the susceptibility assays for the natural honeys. The inoculated plates were incubated at 37 ^{o}C for 16 h, and the results were recorded on each plate.

RESULTS AND DISCUSSION

In Table 1, it can be seen that the growth of all 25 coagulase-negative staphylococcus isolates was inhibited by acacia, linden and polifloral honey in different degrees and at different concentrations (dilutions). Honey presented an inhibitory effect at dilutions from 4,2 to 1,8 % for acacia honey, 3,7 to 1,2 % for linden honey and 3,9 to 1,1 % for polifloral honey. Acacia honey presented values of 4.0, 4.1 and 4.2 % (samples no. 20, 21 and 22) of dilutions which inhibited the S. epidermidis growth, while, linden honey had values of 3.5, 3.6 and 3.7 % (samples no. 15, 14 and 9) and polifloral honey 3.5 and 3.7 % (samples no. 9 and 21), for the same species. In what concerns S. haemolyticus, the highest values were registered for polifloral honey (3,8 %, sample no. 24), while linden honey had a value of 3,7 % (sample no. 16) and acacia honey only 2.9 % (sample no.1).

Table 1 - The average values for the dilutions (%) of different types of honey on the species of Staphylococcus spp. isolated from the 25 clinical cases

No.	Species	*Types of honey*		
		Acacia	Linden	Polifloral
2	***Staphylococcus epidermidis***	1,8	2,8	2,0
5	***S.epidermidis***	2,0	3,1	2,9
9	***S.epidermidis***	1,8	3,6	3,5
10	***S.epidermidis***	2,2	2,0	2,4
11	***S.epidermidis***	2,6	1,5	1,5
12	***S.epidermidis***	3,6	2,7	3,0
14	***S.epidermidis***	2,8	3,7	1,7
15	***S.epidermidis***	3,4	3,5	1,1
20	***S.epidermidis***	4,0	2,9	2,2
21	***S.epidermidis***	4,1	1,2	3,7
22	***S.epidermidis***	4,2	3,1	1,8
25	***S.epidermidis***	3,9	2,8	2,8
1	***Staphylococcus haemolyticus***	2,9	1,9	3,2
13	***S. haemolyticus***	3,1	3,3	2,4

16	***S. haemolyticus***	2,7	3,7	1,6
23	***S. haemolyticus***	1,9	1,8	2,7
24	***S. haemolyticus***	2,0	2,9	3,8
3	***Staphylococcus simulans***	3,5	2,2	2,8
4	***S. simulans***	4,0	3,0	3,1
7	***S. simulans***	3,8	2,8	1,1
6	***Staphylococcus capitis***	3,6	3,1	1,8
8	***S. capitis***	3,7	2,2	3,4
19	***S. capitis***	4,1	1,9	2,5
17	***Staphylococcus warneri***	4,2	1,2	3,9
18	***S. warneri***	3,3	1,6	3,7

Sample no. 4, presented the highest values for S. simulans, 4.0 % for acacia honey, 3.0 % for linden honey and 3.1 % for polifloral honey. S. capitis was inhibited at 3.1 % concentration for linden honey (sample no.6), at 3.4 % for polifloral honey (sample no.8) and 4.1 % for acacia honey (sample no.19). S. warneri was inhibited at 4.2 % for acacia honey, 3.9 % for polifloral honey and only 1.6 % for linden honey (samples no. 17 and 18).

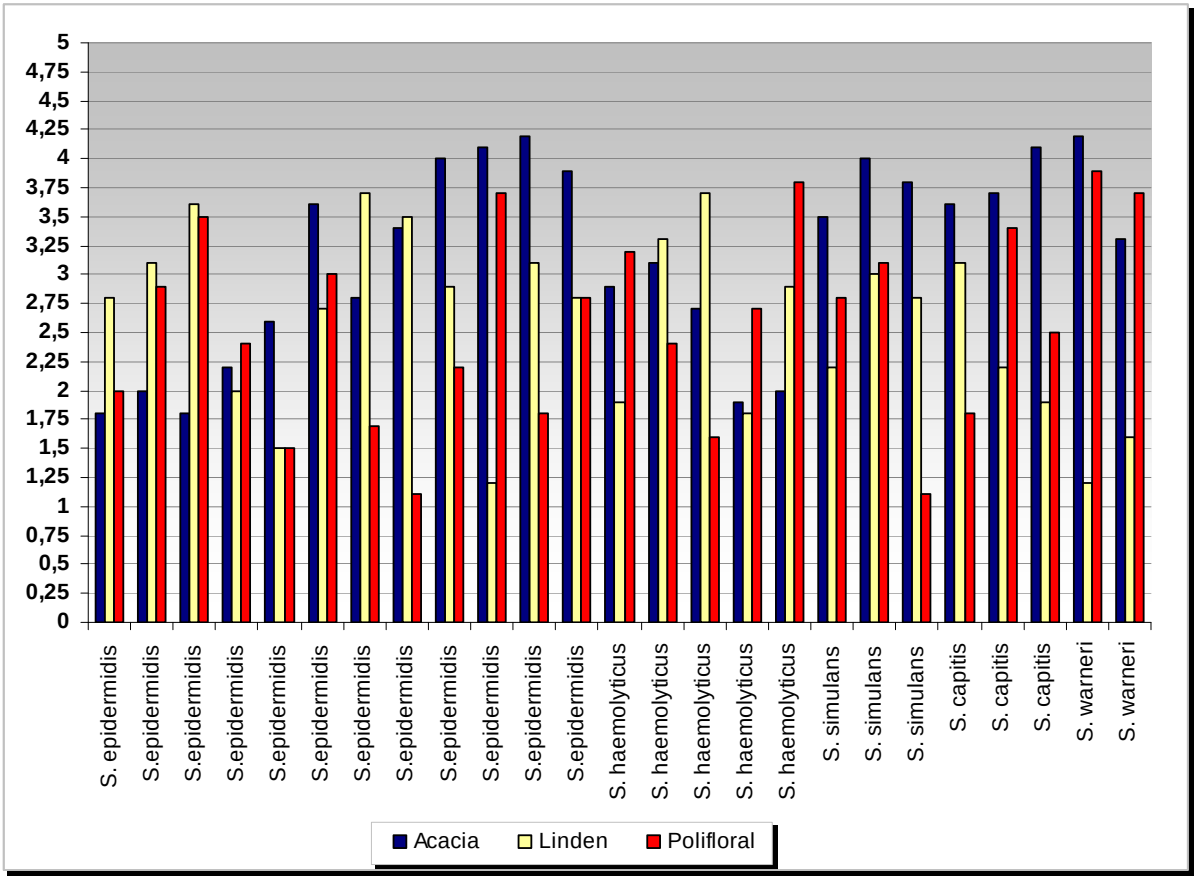

Fig. 1 - The average values for the dilutions (%) of different types of honey on the species of Staphylococcus spp. isolated from the 25 clinical cases

CONCLUSIONS

The main conclusion after performing this study, is that acacia honey presents the highest anti-microbial activity concerning coagulase-negative staphylococci, with values of the concentration of 4,2 %, and in the future, this may be used with success when clinical cases appear in medical institutions with veterinary profile.

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RESEARCHES REGARDING THE EFFICIENCY OF PLANTS ESSENTIAL OILS EXTRACTS ON SOME BACTERIAL STRAINS ISOLATED FROM MASTITIS COW MILK

F. CHIRILĂ, N. FIȚ, S. RAPUNTEAN, G. NADĂȘ, O. NEGREA
University of Agricultural Science and Veterinary Medicine, Cluj-Napoca,
Faculty of Veterinary Medicine, e-mail: nfit@usamvcluj.ro

Abstract: *Between January 2009 - May 2010 in the Laboratory of Microbiology of the Faculty of Veterinary Medicine in Cluj-Napoca, 17 samples collected from 16 dairy cows and buffaloes with acute and recurrent clinical mastitis have been examined by classic bacteriological examination and mycological examination. From the examined samples were isolated the following bacterial species: 12 strains of Staphylococcus spp., one strain of Streptococcus spp., one strain of Bacillus spp., one strain of Klebsiella spp., a strain of Candida spp. and from buffalo milk one strain of Streptococcus agalactiae.*

Sensitivity of these strains was tested by „aromatograma" on Mueller-Hinton medium for bacteria and Sabouraud medium for Candida to the following essential oils: pine, mint, lavender, St John's wort, basil and thyme, as a possible alternative to current antibiotics. Aromatograma diffusimethric technique was performed, each essential oil was applied diluted 1/10, 20 ml/well.
Were used as controls the following international reference strains: Staphylococcus aureus ATCC 6538P, Bacillus cereus ATCC 14579, and Candida albicans ATCC 90028. Of the 12 Staphylococcus strains tested, 10 strains (83.33%) were sensitive to pine oil, 9 strains (75%) for oil of thyme, 7 strains (58.33%) for peppermint oil and three strains (25%) for lavender oil. Control strain of Staphylococcus aureus ATCC 6538P was very sensitive to thyme oil and moderate sensitive to pine oil, being resistant for the other tested oils.

Keywords: clinical mastitis, large ruminants, essential oils from plants, bacteria, yeasts.

INTRODUCTION

Widespread use of antibiotics in animal breeding, particularly intensively, lead to selection of resistant strains of organisms with increasingly higher resistance to most of these products (2, 3, 4, 8; 9, 10, 11). Their retention in the body, their elimination in milk, which is a staple food for children and the elderly, lead to restrictions in using this product, in significant economic losses for the farmer, the public health risks and limit the use of antibiotics.

For this reason, the world is seeking alternatives to using antibiotics, one of which being represented by aromatherapy. Antibacterial and anti-inflammatory properties of essential oils are known for some time but they are still little used in veterinary medicine, although they may represent a future alternative (7, 12, 13, 14).

Aromatherapy used in animal health is reglemented with Annex 2 of Regulation EEC 2377/90 showing that 19 essential oils can be administered to all food producing species without the neessity to set maximum residue limits. This information led us to „in vitro" test of the effect of plant essential oils on some species of microorganisms isolated from clinical mastitis in large ruminants (cows, buffalo).

MATERIALS AND METHODS

Our investigations were performed on 17 microbial strains isolated from milk from cows with acute and recurrent clinical mastitis in Mures and Cluj counties and three international reference strains used as controls. Isolation of microbial strains was done by classic bacteriological and mycological exam, 50 ml of each sample was inoculated in broth on blood agar and Sabouraud medium. Tubes were incubated at 37° C for 24 hours, prolonging the incubation to 48-72 hours if Sabouraud environment, with daily examination of cultural characters. Genus identification was made based on morphological characters on Gram stained smears, cultural character, complemented by selective or differential biochemical properties tests. Microbial strains isolated were then sowed in soft agar tubes and incubated 24 hours at 37° C.

International reference strains, kept in soft agar in the laboratory's own collection and other strains of interest isolated from cases of mastitis were resowed in broth for 24 hours before the aromatograma was made and incubated at 37° C.

Aromatograma was made by a technique similar to that used in antibiogram diffusimetrical method. Bacterial strains were sowed on Mueller-Hinton medium and yeasts of the genus *Candida* on Sabouraud medium. In each Petri dish with a diameter of 5 mm, a radial pattern was made, for the six essential oils used for testing. 24 hours cultures of the bacterial and yeast strains were diluted 1/1000 in tubes with sterile nutrient broth and inoculated by flooding the Petri dishes in which previously wells were practiced. After coating the surface of the culture media evenly, excess culture of wells and surface Petri dish was removed with a sterile Pasteur pipette, the dishes were then left in a laminar flow hood to dry for 10-15 minutes.

After drying the surface environment of Petri dishes sowed with the test strain, each well was filed by 20 µl of test oil, diluted 1/10, then they were incubated 24 hours at 37°C.

In the test were used six essential oils, made in the plate in the following order: pine, peppermint, lavender, St John's wort, basil and thyme. Reading and interpretation of results was performed the next day, based on the presence or absence of microbial culture lysis areas.

RESULTS AND DISCUSSIONS

Of the 17 milk samples collected from cows with clinical mastitis the following microorganisms were isolated: 12 strains of *Staphylococcus spp.*, two strains of *Streptococcus spp.*, and one strain of *Bacillus spp.*, *Klebsiella spp*. and *Candida spp*. In nutrient broth both Gram positive and Gram-negative bacteria caused an ntense turbidity with a low deposit, easy homogenized. On blood agar, staphylococci formed S-type colonies 2-3 mm in diameter, surrounded by a zone of 1-2 mm of α or α and β haemolytic while streptococci presented small colonies of up to 1 mm in diameter surrounded by a reduced zone of β haemolysis type. Morphological characters exam on smears stained by Gram method, staphylococci were presented as Gram positive cocci, grouped as irregular clusters of cocci while streptococci form Gram-positive ovoid-shaped, group in the chain lengths variable depending on the strain and species.

Effect of six essential oils from plants on the strains isolated from mastitis milk samples and the three international reference strains used as controls are presented in Table 1.

Table 1: Effect of six essential oils from plants on pathogenic micro flora isolated from clinical mastitis in cows

Tested strain	Pine essential oil	Pine essential oil	Lavander essential oil	St John's wort essential oil	Basil essential oil	Thyme essential oil
115 Staph.	13 mm	15 CR	10 CR	R	R	11 mm
0753M+ Staph.	8 mm	8 mm	6 mm	R	R	12 mm
0753R Staph.	13 mm	11 mm	8 mm	R	R	13 mm
2675 Staph	20 mm	15 mm	8 mm	R	R	11 mm
8626 Staph	22 mm	20 mm	7 CR	R	8 mm	R
8674 Staph	17 mm	17 mm	13 mm	R	R	23 mm
2060 Staph.	15 mm	12 mm	10 mm	R	10 mm	13 mm
Staph. 3	15 mm	R	6 mm	R	R	12 mm
Staph. 5	13 mm	7 mm	7 mm	R	10 mm	15 mm
1231 Staph.	10 IP	R	10 IP	R	10 IP	8 mm
3056 Staph.	13 mm	15 mm	11 mm	R	R	12 mm
8556 Staph.	13 mm	10 IP	10 mm	R	R	10 mm
3057 Str.	R	R	R	R	R	R
64 L Str.	13 mm	17 mm	9 mm	R	R	18 mm
Bacillus spp.	R	R	R	R	R	12 mm
Klebsiella spp.	R	R	R	R	R	R
Candida spp.	R	R	R	R	R	8 IP
S. aureus ATCC 6538P	9 mm	R	R	R	R	22 mm
B. cereus ATCC 14579	11 mm	12 mm	11 mm	R	R	14 mm
C. albicans ATCC 90028	R	R	R	R	R	10 IP

Legend: CR = resistant colonies present in the inhibition area;
IP = partial inhibition;
R = total resistance.

Strain of *Klebsiella spp.* and *Bacillus spp*. were developed as large colonies 4-6 mm in diameter, smooth, free of haemolytic activity. *Candida spp.* strain was developed as small colonies of 1-2 mm after 24 hours of incubation which subsequently grew to 4-5 mm size, matte white. On morphological characters exam, *Klebasiella* were observed as Gram-negative bacilli isolated or grouped diplo, the genus *Bacillus* form of rods with rounded ends, Gram positive, isolated or as short chains while yeast of the genus *Candida* were as oval formations characteristic aspect of keg, isolated piles as many cells are burgeoning.

Aromatograma analysis results performed on the 17 microbial strains isolated from clinical mastitis in milking cows and three international reference strains used as controls showed that from 12 strains of *Staphylococcus spp*., 10 strains (83.33%) were sensitive to pine oil, 9 strains (75%) to thyme oil, 7 strains (58.33%) to mint oil and (25%) to lavender oil. For the other strains tested, belonging to other genres can not yet draw meaningful conclusions regarding their effect because of the small number of strains tested.

It was noted however that „in vitro" effect of essential oils on pathogen strains isolated from clinical mastitis in cows is similar to antibiotics. Some strains that are sensitive to one or more oils and others are moderately sensitive or has a total resistance to all the essential oils used.

The results we obtained by testing in vitro are consistent with those obtained by other authors (1, 6, 7, 15, 16) and may constitute an alternative to current antibiotic therapy. But note that, to have the desired efficiency in this approach is mandatory testing of essential oils by aromatograma sensitivity of isolates from cases of mastitis. Figures 1, 2, and 3 are presenting the effect of essential oils from three different types of microorganisms: *Staphylococcus, Streptococcus* and *Bacillus*.

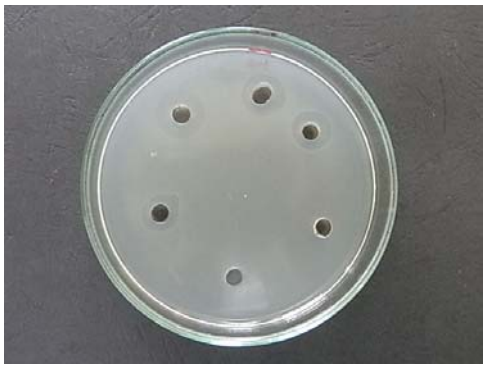

Fig. 1 *Staphylococcus* spp.

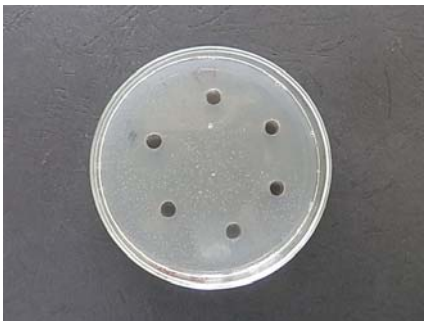

Fig. 2 *Streptococcus spp.*

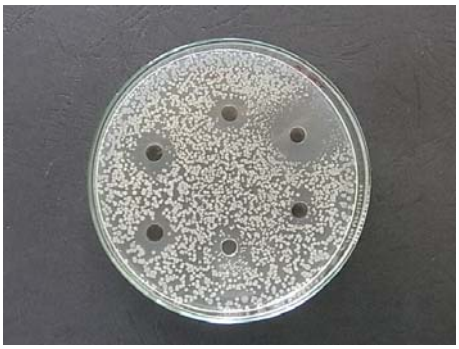

Fig. 3. *Bacillus cereus*

The first three genera, Gram-positives group are sensitive to pine oil, mint and thyme while *Klebsiella* strain has a total resistance to the six essential oils tested.

CONCLUSIONS

1. Bacteriological examination allowed us to isolate the following types of microorganisms in 17 samples of milk from cows with mastitis: *Staphylococcus*, *Streptococcus*, *Bacillus*, *Klebsiella* and *Candida*.
2. In vitro tests by aromatograma, indicate that strains of *Staphylococcus spp*. are sensitive to essential oils in 4 of 6 situations in the following percentages: 10 strains (83.33%) to essential oil of pine, 9 strains (75%) to essential oil of thyme, 7 strains (58.33%) to essential oil of mint and 3 strains (25%) to essential oil of lavender.
3. The first three essential oils can be an alternative to current therapy of staphylococcal mastitis with antibiotics, but only after in vitro susceptibility testing isolates by aromatograma.

4. In case of bacterial strains belonging to *Staphylococcus* and *Bacillus*, the sensitivity is variable from one strain to another.
5. Strain of *Klebsiella spp*., isolated from mastitis in cows was resistant to all six essential oils used in aromatograma test.

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RESEARCH ON THE FREQUENCY OF *BACILLUS CEREUS* IN MILK AS RAW MATERIAL

C. CIOTĂU[2], E.V. ŞINDILAR[1]
[1]Facultatea de Medicină Veterinară Iaşi
[2]Direcția Sanitar Veterinară şi pentru Siguranța Alimentelor Suceava

Abstract
The contamination of milk as raw material for processing purposes with Bacillus cereus *is important under sanitary as well as technological aspects. Studies performed by the authors showed an average frequency of contamination of the whole raw milk with* Bacillus cereus *5,02%. Other species by Bacillus genus that was identified from the samples:* B. cuagulans *5,02%,* B. mycoides *3,86%,* B. firmus *2,32%,* B. circulans *1,44%,* B. licheniforms *1,16%,* B. thuringensis *0,77% and* B. subtilis *0,77%.*

Key words: raw milk, *Bacillus cereus*, microbiological quality.

Bacillus genus includes a great number of species, 22 of them being better known. (2, 11). This genus is made of Gram-positive bacilli, aerobic or optional anaerobic, with development temperatures between 3^0 and 75^0C, optimal temperature of 25^0-37^0C. All the species are sporogeneous, some of them are mobile and some have capsules. (2, 3, 4, 11)

Bacillus genus is a heterogeneous genus, aspect that reflects the large variety of environments in which these microorganisms live (2, 3, 4, 10). This heterogeneity and the fact that G+C from the DNA varies between 32%-69% suggests that some species included in this genus could form different taxa (7, 12, 13).

Bacillus genus includes both saprophytes and pathogenic bacteria for humans, animals and insects. This genus includes *bacillus cereus*, able to produce food poisoning and occasionally other infections (3, 11, 12). Like all taxa included in this genus, *bacillus cereus* is widespread in nature, usually in soil, dust, plant materials, human and animal faeces.

Overall the food and milk contamination is made by unsanitary conditions of production, food processing and storage (3, 8, 9, 14).

Bacillus cereus is a bacterium morphologically similar with *Bacillus anthracis*, to which is antigenic related, but forms shorter chains and the ends appear rounder. It is noncapsulogenic, mobile with numerous peritrich cillia, with the oval sporum in the middle or subterminal, which doesn`t change the cell shape, aerobic or optional anaerobic, haemolytic, licithinolytic, catalase-positive, oxidation-negative (1, 2, 3, 4, 9, 11, 12).

Growth and development temperatures varies, depending on the strains, and for others is between 15^0-50^0C (4, 8, 11, 12). Optimal temperature for growth and multiplication is $28^{0-}35^0$C when the generation time is shortest (18-27 minutes). pH limits in which *B. cereus* can multiply are 4,9 and 9,3 and the minimal *aw* value is 0,95. *B. cereus* grows and multiplies in saline media with NaCl concentration less than 7.5% and it is inhibited by nisin and ascorbic acid. Most *B. cereus* strains have a protease and licithinase shown on egg yolk medium, forming a white-yellow precipitate (3, 8, 12, 15).

The biochemical features of *B. cereus* are: glucose fermentation, casein and starch hydrolysis, gelatin liquefaction, reduction of nitrates in nitrits, use of amonium citrate as only carbon source.

Bacillus cereus can live both in liquid and solid media, but for growth, isolation, identification and quantification are special media that tend to the bacterial properties not to ferment mannitol, insensitive to polymyxin, to produce lecithynase and haemolysin (2, 3, 9, 10, 12).

After a latency period in food, *B. cereus* germinates and multiply, inducing enzymatic changes of the substrate, and produces vomiting- and diarrhea-causing exotoxins which cross the cell membrane (3, 6, 10, 11, 13).

The presence of the bacterium in food has sanitary importance when their number exceeds 10^5/g or ml10^5/g or ml.

MATERIAL AND METHOD

The study analyzed 259 milk samples collected by the processor on two collection routes. Of the total samples, 128 milk samples were from route 1 and 131 samples were from route 2.

Investigations were aimed at determining the number of Bacillus cereus / ml milk, and the number of other bacteria of the genus Bacillus / ml milk found in the examined samples.

Procedure

The preparation of samples and dilutions of raw milk was done according to SR EN ISO 8261/2005, and the method of analysis was done according to EN ISO 7932/2005, using the technique of counting colonies at 30 ° C.

Initial inoculum of product and decimal dilutions were inoculated 1 ml of the original product and 0.1 ml of decimal dilutions on the surface substrate MYP (mannitol agar and phenol red), with extra egg yolk and polymyxin B in 2 Petri dishes (140 mm) and the following dilutions in 2 Petri dishes (90 mm). The Petri dishes were incubated at 30 ° C for 24 hours. After the incubation period the dishes that had between 15 and 150 colonies on two successive dilutions were kept. For identification and confirmation the colonies suspected of being *Bacillus cereus* were selected. They were pink and almost always surrounded by a zone of precipitation, indicating the production of lechitinase. Certain strains of *B. cereus* produce little or no lechitinase at all. These colonies were subjected to biochemical confirmation test.

Isolation and selection of colonies for confirmation of B. cereus

From each selected Petri dish 5 suspected *B. cereus* colonies were subjected to confirmation test. Where some samples have many colonies and it was not possible to select well isolated suspect colonies, the replication of 5 colonies on selective substrate containing MYP was conducted, to obtain isolated colonies.

Incubation was done at 30 ° C for 18-24 hours. From each dish well isolated colonies were selected and subjected to confirmation test on the following substrates:

- glucose agar medium for fermenting glucose. The coloring in yellow of the seeding indicated a positive reaction;
- medium for Voges-Proskauer reaction with potassium hydroxide solution, α Naphtol and creatin crystals, which highlighted the production of acetylmethylcarbinol, observed because of the pink-eosin color of the medium, meant the positive test for *B. cereus;*
- nitrate medium for highlighting nitrite in the presence of reactives of 5-2 ANSA, sulfamidic acid and zinc powder. The reducing of the nitrate to

nitrite marked by the change of color from yellow to red in the presence of the reactives meant the positive test for *B. cereus.*

Method of calculation

The number of *Bacillus cereus*, confirmed in at least 80% of colonies selected from the dishes which contained between 15-150 colonies on two successive dilutions was calculated with the formula, according to ISO 7932/2005:

$$N = \frac{\Sigma a}{(n_1 + 0{,}1n_2)xd}$$

in which:

Σa – sum of *B. cereus* colonies from all the selected dishes;

n_1 – number of dishes selected on first dilution;

n_2 – number of dishes selected on second dilution;

d – dilution rate level corresponding to the first dilution.

If from the selected dishes with 15-150 colonies on the 2 successive dilutions were confirmed less than 80% of selected colonies, the calculation of the number of *B. cereus* was made percentage, depending on the number of confirmed colonies. If the selected dishes had less than 15 *B. cereus* colonies, the average of the number of colonies from the 2 dishes was made.

Expression of the result on the number of B. cereus per ml or per gram product was made by a number between 1.0 and 9.9, multiplied by 10x, where x is the corresponding power of 10.

Identification of Bacillus cereus species from the cultures obtained on the selective isolation medium MYP, by the method according to ISO 7932/97 was made with the mini API microbiological analyzer using API_{50}CH gallery, with suspension medium API_{50} CHB/E.

Preparation of the inoculation

The characteristic colonies from the selective medium MYP was made a culture on nutritive agar to obtain a sufficient number of colonies for the preparation of the bacterial suspension. The bacterial suspension was prepared on API_{50} CHB/E medium for the identification on Bacillus bacteria, which allows the observation of the fermentation of 49 carbohydrates from the microtubes of the API_{50} CH gallery.

Gallery inoculation was done by pipette, filling the microtubes carefully to the level indicated by full, avoiding the appearance of too full. Adding paraffin oil in microtuburile tests allow better visualization of color. Galleries Incubation was at 30 ° C for 24-48 hours.

The reading of API_{50}CH galleries was done in 2 stages: one at 24 hours and second at 48 hours. The biochemical processes occurring during incubation in glucose catabolism, producing organic acids which change the pH indicator (phenol red in the environment), to yellow. This change indicates a positive result. Also tested in microfuge 25 aesculin color turns from red to black, indicating a positive result.

For reading the galleries the reading program was typed. Analytical module called reader records the color reaction from each microtube and transmits the information to the computer where it is interpreted with the identification software.

RESULTS AND DISCUSSION

The study for isolation and identification of *Bacillus* species from the milk samples of route 1 shows the fact that there were 24 positive samples, representing 18.5%, from which after the confirmation test 5 samples were positive for *Bacillus cereus* (3.91%), and 19 samples (14.84%) proved to be other species of *Bacillus*.

On route 2, after the analysis of the 131 milk samples we found 29 positive samples for species of *Bacillus*, representing 22.14%. From the positive samples, 8 confirmed to have *B. cereus* representing 6.11%, and 21 milk samples (16.03%) had other species of *Bacillus*.

From the total of 2596 milk samples investigated on routes 1 and 2, resulted 53 positive samples (20.46%) for *Bacillus spp*, from which in 13 samples was confirmed the presence of *B. cereus* (5.02%), and in the other 40 samples (15.44%) was confirmed the presence of other species of *Bacillus.*

Table 1 *The incidence of the Bacillus cereus in raw milk collected from two routes*

Route	Nr. of samples	Frequency of *Bacillus spp* in milk samples							
		Normal samples (negatives)		Positive samples for *Bacillus spp*		Positive samples for *B. cereus*		Positive samples for other species of *Bacillus*	
		A	R	A	R	A	R	A	R
I V.D.	128	104	81,25	24	18,75	5	3,91	19	14,84
II C.M.	131	102	77,86	29	22,14	8	6,11	21	16,03
Total	259	206	79,54	53	20,46	13	5,02	40	15,44

A – absolute frequency; R – relative frequency.

In the second work stage we identified the other species of *Bacillus* found in the investigated milk samples. The results are presented in Table 2.

Table 2 *The identification of the species of Bacillus cereus in raw milk collected from two routes*

Species	Frequency of identified species					
	Route I = 128 samples		Route II = 131 samples		Total = 259 samples	
	A	R	A	R	A	R
Bacillus cereus	5	3,91	8	6,11	13	5,02
Bacillus cuagulans	6	4,68	7	5,34	13	5,02
Bacillus micoides	5	3,91	5	3,82	10	3,86
Bacillus firmus	3	2,34	3	2,29	6	2,32
Bacillus circulans	2	1,56	2	1,3	4	1,44
Bacillus licheniformis	1	0,78	2	1,53	3	1,16
Bacillus thuringiensis	1	0,78	1	0,76	2	0,77
Bacillus subtilis	1	0,78	1	0,76	2	0,77
Total	24	18,74	29	22,11	53	20,46

A – absolute frequency; R – relative frequency.

Analyzing the data from Table 2 reveals that, in addition to B. cereus were still identified seven species of the genus Bacillus identification with a decreasing rate, as follows: *B. cuagulans* 5.02%, *B micoides* 3.86%, *B. firmus* 2.32%, *B. circulans* 1.44%, *B. licheniformis* 1.16%, *B. subtilis* and *B. thuringensis* each 0.77%.

Data analysis shows some aspects that require further discussion. In this context it is noted that the milk collected on the two collection routes is contaminated with several species of bacteria of the genus Bacillus.

From the total number of 259 milk samples investigated, 206 samples (79.54%) were free of bacteria of the genus *Bacillus*, and a number of 53 samples (20.46%) were contaminated with various species of bacteria of the genus *Bacillus*. This indicates deficiencies regarding compliance with hygiene rules to harvesting, handling and cooling the milk immediately after milking.

Of all species of Bacillus found in investigated milk samples, Bacillus cereus is involved in producing food poisoning by consumption of milk. Isolation percentage of 5.02% of investigated samples with values <10 CFU / ml of milk is a major risk to public health.

CONCLUSIONS

- In the socio-economic conditions in the mountainous area from which the milk samples were collected and the investigation of the presence of B. cereus involved in producing of food poisoning shows that:

- Collected milk is contaminated with bacteria of the genus *Bacillus* in a percentage of 20.46%, of which with *Bacillus cereus* only 5.02% and 15.44% difference with other species of the genus *Bacillus*;

- The situation resulting from the investigations made on milk samples from the two collection routes imposes taking measures for improving the hygiene conditions by implementing rules of good practice in production, and good practice especially in obtaining and conditioning of the milk in dairy farms.

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OBSERVATIONS REGARDING THE HISTOLOGICAL STRUCTURE OF THE FOOT MUSCLE OF THE FOOD SNAIL *H. pomatia*

Andreea-Flavia CIRLAN, **E. SINDILAR**,
University of Agricultural Sciences and Veterinary Medicine
"Ion Ionescu de la Brad" Iasi, Faculty of Veterinary Medicine, Mihail Sadoveanu Alley no. 8,
700489 Iasi e-mail: flavia_cirlan@yahoo.com

ABSTRACT
Vineyard snails (H. pomatia) meat has always been highly valued for its dietetic and nutritive properties. Given the fact that very few studies have been made on this snail, the aim of the present research was to study the histological structure of the vineyard snail's foot muscle, which is the main part used for consumption . The muscles have been sampled from 15 snails found in a garden in the city. After removing the shell and the organs, we isolated the muscle. This was processed by the common paraffin technique and then for the hematoxyline-eosin (HE) and the Alcian blue staining. We examined them microscopically and observed the histological structure of the foot muscle.

Key Words: *Helix pomatia*, food snail, muscle, histological structure

INTRODUCTION

Food snails *H. pomatia* meat has always been highly valued for its dietetic and nutritive properties.Lately, in our country, many people began to organize farms in which they raise this specie. Even a fabric for snail products has been made and most of these products are exported. Given these facts, we decided that the food snail Helix pomatia should be more studied and we began by revealing the histological structure of the main part used for consumption: the foot muscle.

MATERIAL AND METHODS

The research took place in four consecutive steps:
Step 1 – Twenty adult snails from the specie *Helix pomatia* were collected from a garden and then introduced into a bowl with ethanol for the anesthesia.
Step 2 – We removed the shell and then we dissected the snail's body. We made an incision from the mouth and cut the snail body and then we removed the organs.
Step 3 – We divided the muscle into three parts (antherior, middle and postherior) which were used for the histological analysis. In order to do this, the samples were fixed in formaldehyde 10%, dehydrated in 3 alcohol baths, cleared in acetone, rinsed in benzen, embedded in paraffin an sectioned at 7 µm thickness. Paraffin sections were dewaxed in xylene, hydrated in 4 alcohol baths and in one distilled water bath.
Step 4 – Finally, the sections were treated for hematoxyline-eosin (HE) staining and for Alcian Blue staining and then microscopically analyzed.

RESULTS AND DISCUSSIONS

The histological exam of the foot, the mobile organ of the snail, revealed its muscular structure. The muscle is composed of smooth muscle cells intermingled with large spaces represented by haemocoelic capillary sinuses (Fig. 1).

The smooth muscle cells are surrounded by collagen fibrils in the subepithelial stratum of integument. The muscle fibres are dispersed beneath the epithelium and have a longitudinal, transverse or radial orientation with respect to the major body axis.

The Alcian Blue staining revealed the presence of the colagenic connective tissue under the surface epithelium (Fig. 2).

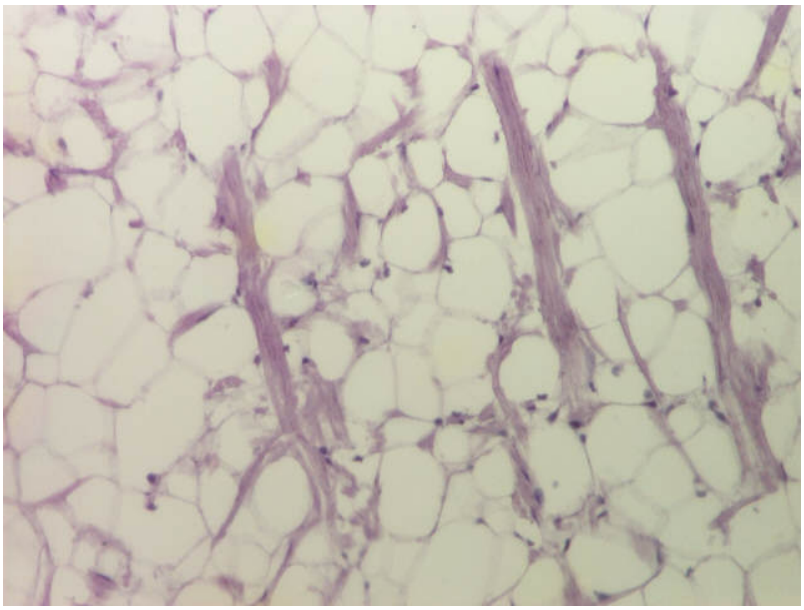

Fig. 1. Smooth muscle fibres and haemocoelic capillary sinuses. Col. HEA x100

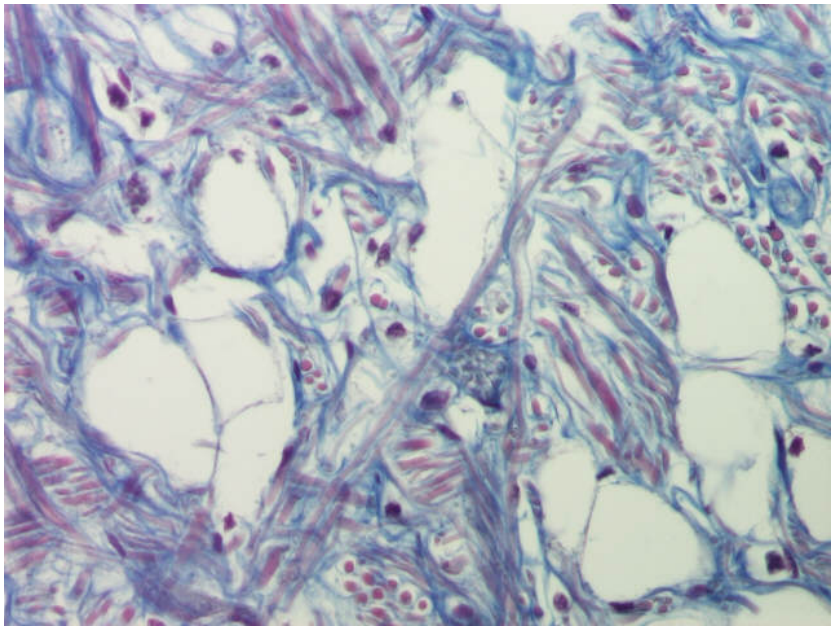

Fig. 2. Connective tissue and capillary sinuses in the structure of the foot. Col. AB x200

Using the Alcian blue staining and the microscope with polarized light we could observe the shining collagen fibrils (Fig. 3).

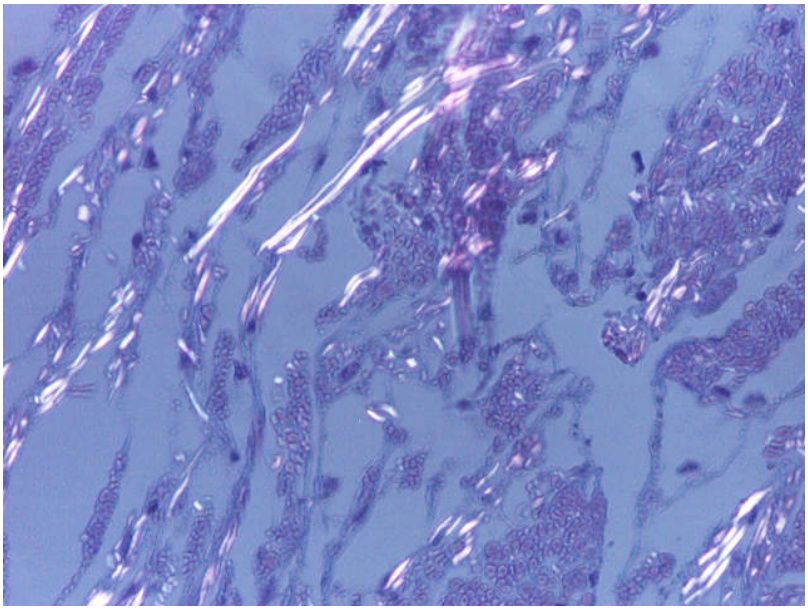

Fig. 3. The collagen fibrils in the structure of the foot muscle, observed with the microscope with polarized light. Col. AB x200

Near the surface epithelium we could distinguish mucus-secreting and mucus-storing cells. They are surrounded by smooth muscle cells and by connective tissue (Fig. 4).

In the ventral zone, the tegument is covered with abundant mucus. Unlike the dorsal zone, we could notice here the uniformity of the epithelial structure and of the mucus. This is probably connected with the existence and the form of the shell in the dorsal zone, as concluded by some authors: Bariati and Comazzi (2001) and Tomar and Markos (2003).

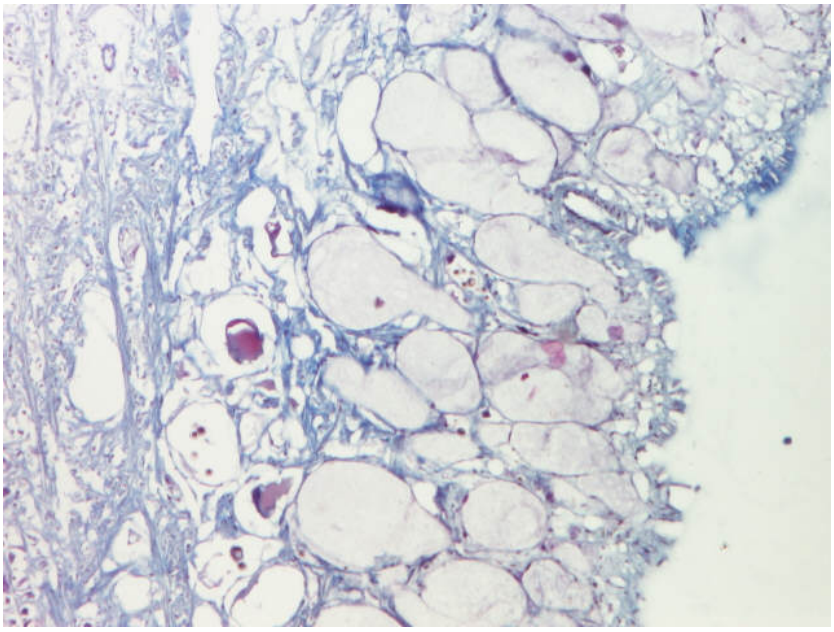

Fig. 4. Many mucus cells, muscular tissue and connective tissue under the surface epithelium. Col. HEA x100.

CONCLUSIONS

1. In the structure of the snail's foot are smooth muscular tissue, connective tissue and haemocoelic capillary vessels.
2. Using the microscope with polarized light we could notice the numerous collagene fibrils in the snail's foot.
3. In the ventral zone of the foot we could observe an abundency of the mucus-secreting and mucus-storing cells, the tegument being covered with a thick layer of mucus.

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OBSERVATIONS REGARDING THE HISTOLOGICAL STRUCTURE OF THE GENITAL SYSTEM OF THE GARDEN SNAIL *H. POMATIA*

Andreea-Flavia CIRLAN, **E. SINDILAR**,
University of Agricultural Sciences and Veterinary Medicine
"Ion Ionescu de la Brad" Iasi, Faculty of Veterinary Medicine, Mihail Sadoveanu Alley no. 8,
700489 Iasi e-mail: flavia_cirlan@yahoo.com

ABSTRACT

The aim of our study was to reveal the histological structure of the genital system of the garden snail Helix pomatia. We used in this research 15 snails from which we have isolated the 3 parts of its genital system: the ovotestis (the hermaphrodite gland), the female part and the male part. These were processed by the common paraffin technique and then for the periodic acid Schiff (PAS), the hematoxyline-eosin (HE) and the Alcian blue staining. We examined them microscopically and observed the complex histological structure of the following: the ovotestis, the sperm channel, the penis, the oviduct and the albumen gland.

Key Words: *Helix pomatia*, snails, histological structure, genital system

INTRODUCTION

Helix pomatia is a hermaphroditic snail with a complex genital system, composed mainly of three parts: the ovotestis, the male part and the female part. Given the fact that in our country very few studies have been made on this snail, we decided to carry some researches on its histological structure, mainly on its complex reproductive system structure.

MATERIAL AND METHODS

The research took place in four consecutive steps:

Step 1 – Twenty adult snails from the specie *Helix pomatia* were collected from a garden and then introduced into a bowl with ethanol for the anesthesia.

Step 2 – We removed the shell and then we dissected the snail's body. We made an incision from the mouth and cut the snail body. Then we removed the genital system.

Step 3 – We divided the genital system into its composing parts which were used for the histological analysis. In order to do this, the samples were fixed in formaldehyde 10%, dehydrated in 3 alcohol baths, cleared in acetone, rinsed in benzen, embedded in paraffin an sectioned at 7 µm thickness. Paraffin sections were dewaxed in xylene, hydrated in 4 alcohol baths and in one distilled water bath.

Step 4 – Finally, the sections were treated for periodic acid Schiff (PAS) staining, for hematoxyline-eosin (HE) staining and for Alcian Blue staining and then microscopically analyzed.

RESULTS AND DISCUSSIONS

Helix pomatia is a hermaphroditic snail, which has three important parts in his reproductive system: the ovotestis (the hermaphrodite gland), the female part and the male part.

The ovotestis consists of four lobes, each of them represented by a large number of follicles intermingled with loose connective tissue. Inside the follicles we could notice: male germ cells that occupy the lumen of the follicles, female germ cells observed on the walls of the follicles, follicle cells and Sertoli cells (Figure 1).

The male elements were represented by the spermatogonia, which were large, round cells with a narrow basophilic cytoplasm around the nucleus. We could also notice primary spermatocytes, similar to the spermatogonia, but with an eosinophilic cytoplasm, secondary spermatocytes and elongated spermatids.

Examining the hermaphroditic channle, we revealed in its structure a pseudostratificated cylindrical epithelium and loose connective tissue.

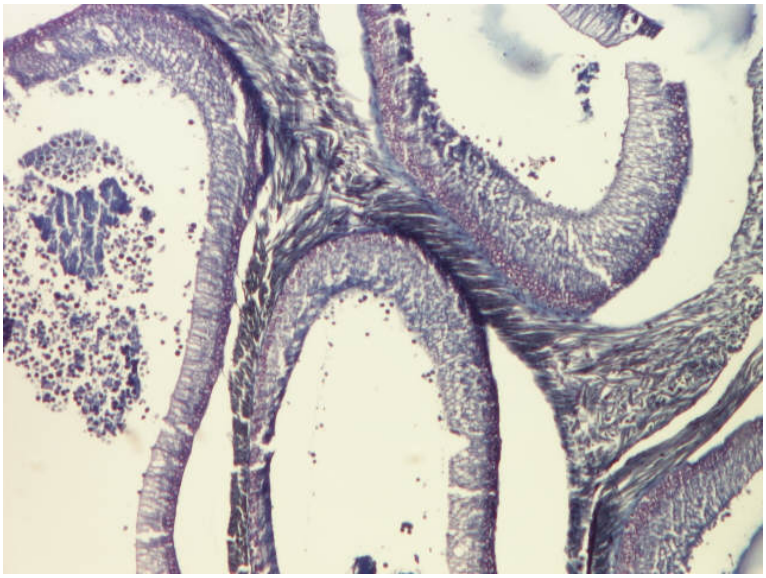

Fig. 1. The ovotestis lobes intermingled with loose connective tissue. The lumen is occupied by the spermatogonia and in the walls are the oocytes. Col. HEA x40

The sperm channel, through which the sperm is transported to the penis, is a functional duct with a narrow lumen outlined by folded epithelial crests surrounded by a few muscle and connective fibres.

The penis is a muscular organ with circular, longitudinal, radial and oblique muscle fibres. It is outlined by a pseudostratificated cylindrical ciliated epithelium (Fig. 2).

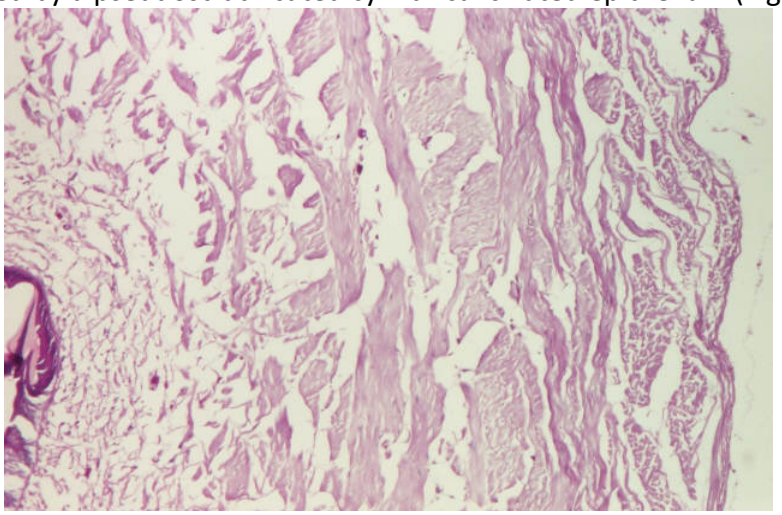

Fig. 2. The circular, longitudinal, oblique and radial muscle fibres in the structure of the penis. Col. PAS x40

The oviduct is a muscular duct with simple prismatic epithelium. We noticed here two types of cells: ciliated and secretory cells (Fig. 3). The epithelium forms various longitudinal folds.

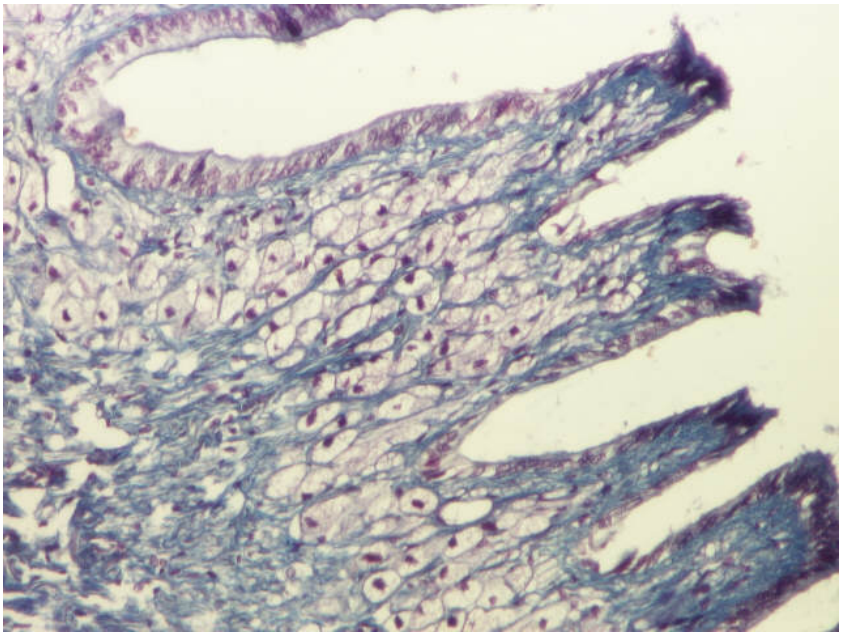

Fig. 3. The simple prismatic epithelium of the oviduct, with big secretory cells. Col. AB x100.

The albumen gland is involved in the formation of the envelopes surrounding the fertilized oocytes and it opens in the oviduct. In the parenchymal glandular mass we could observe albumen secretory cells and labyrinthic cells (Fig. 4).

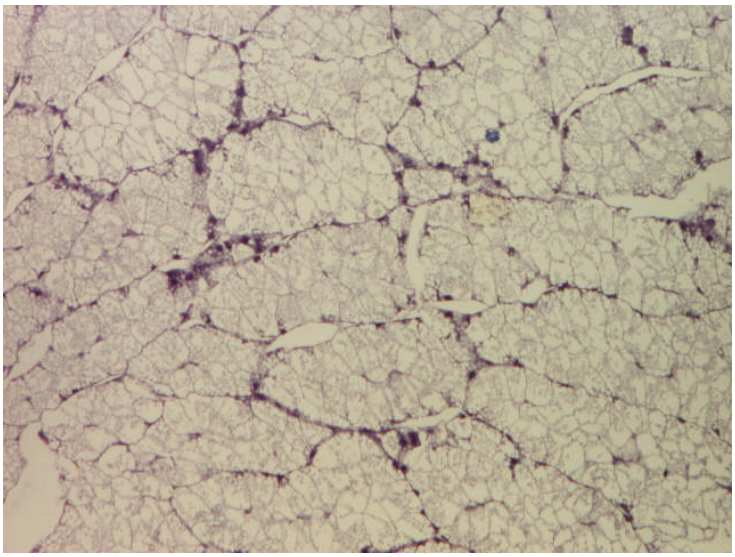

Fig. 4. In the parenchymal mass of the albumen gland, most of the cells are big secretory cells with an ovoid form. Col. HEA x40

CONCLUSIONS

1. The hermaphrodite gland consist of 4 lobes, each of which is represented by a large number of follicles containing male germ cells, female germ cells, follicular cells and Sertoli cells.
2. The penis is a muscular organ and its fibres are circular, longitudinal, radial and oblique.
3. In the structure of the oviduct are two types of cells: cilliated cells and big, secretory cells.
4. In the structure of the albumen gland, involved in the formation of the eggs, we could also observe two types of cells: secretory and labyrinthic cells.

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THE CONCORDANCE BETWEEN THE TOTAL GERMS NUMBER (TGN) FROM THE SHELL AND THE TOTAL GERMS NUMBER FROM THE FOOT OF THE FOOD SNAIL *HELIX POMATIA*

Andreea-Flavia CIRLAN, E SINDILAR,
University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, The Faculty of Veterinary Medicine, Mihail Sadoveanu Alley no. 8, 700489 Iasi e-mail: flavia_cirlan@yahoo.com

ABSTRACT

Letely, in our country, the food snail Helix pomatia is very appreciated. Many people began to raise this specie in organized farms. The snails are processed or exported. Our purpose was to identify the TGN from the shell and from the foot of this snail and to see if there are bacteria involved in the human digestive pathology. The methods we used are: the serial decimal dilutions method, the inoculation on several media and the Gram-stain. The values of the CFU/cm^2 obtained from the shell surface were significantly higher than the values of the CFU/cm^2 obtained from the foot surface. This fact may be related to the quantity and quality of the mucous produces by the snail's glands. On the Sabouraud medium, two species of fungi have grown: Cladosporium spp and Chrysosporium spp. No specie has grown on the selective media for pathogenic bacteria that we have used. The identification of the Gram-negative bacilli was made using the API galleries. Further identifications are going to be made.

Key words: total germs number, food snail, *Helix pomatia*

INTRODUCTION

Food snails *H. pomatia* meat has always been highly valued for its dietetic and nutritive properties.Lately, in our country, many people began to organize farms in which they raise this specie. The snails' meat is usually exported. Still, in our country, the microbiology of the food snails has not been properly studied. Consequently, the aims of the present paper are: to determine the total germs number from the shell; to determine the total germs number from the foot; to see if there is a concordance between these counts; to isolate some bacteria involved in the human digestive pathology.

MATERIAL AND METHODS

The research took place in the following steps:

Step 1 – Ten adult snails from the specie *Helix pomatia* were collected from a garden at the end of september (the begining of their hibernation period).

Step 2 – The shell and the foot were examined separately, using the following technique: moistened cotton-wool swabs have been used for the whole surface sampling. After swabbing, the head of the swab was broken off into diluant (Brain Heart Infusion broth), which is shaken to release the organism. Serial decimal dilutions were then prepared. The dilutions 1/10 and 1/100 were incubated at 37°C, for 24 hours.

Step 3 – Plates with the following media were then inoculated with 10 µl of dilution: Agar with sheep blood, MacConkey with Crystal Violet, XLD Agar, Brilliant Green Agar, Yersinia Selective medium, Bile Esculin medium for isolation of Listeria and Sabouraud medium for the isolation fo fungi.

The media were incubated at 37°C for 24 hours and at the room temperature for other 24 hours. The Sabouraud medium has been studied for 7 days.
Step 4 – The values were expressed in log CFU/cm^2 of the shell surface and log CFU/cm^2 of the foot surface.

RESULTS AND DISCUSSIONS

The values obtained from the dilution 1/10 are presented in the tables 1 and 2.

Snail's number	Media – CFU/cm^2							
	Brain Heart Infusion (BHI)	Blood Agar (BAP)	MacConkey with Crystal Violet	Bile Esculin Agar (BEA)	Yersinia selective medium (CIN)	Brilliant Green Agar	XLD Agar	Sabouraud
1	+	6.6	0	0	0	0	0	0
2	+	37	37	0	0	0	0	0
3	+	12.9	0	0	0	0	0	0
4	+	30.8	29.6	0	0	0	0	0
5	+	13.6	8.6	0	0	0	0	0
6	+	23.4	11.6	0	0	0	0	0
7	+	18.5	11.1	0	0	0	0	0
8	+	25.3	7.4	0	0	0	0	0
9	+	24.8	13.5	0	0	0	0	0
10	+	11.1	2.22	0	0	0	0	0

Table 1. The number of CFU/cm^2 isolated from the shell surface.

Snail's number	Media – CFU/cm^2							
	Brain Heart Infusion (BHI)	Blood Agar (BAP)	MacConkey with Crystal Violet	Bile Esculin Agar (BEA)	Yersinia selective medium (CIN)	Brilliant Green Agar	XLD Agar	Sabouraud
1	+	0.8	0	0	0	0	0	0
2	+	16	4.4	0	0	0	0	0
3	+	4	1.2	0	0	0	0	0
4	+	4	0	0	0	0	0	0
5	+	6	0	0	0	0	0	0
6	+	4	0	0	0	0	0	0
7	+	4	0	0	0	0	0	0
8	+	4.4	0	0	0	0	0	0
9	+	60	30	0	0	0	0	0
10	+	6.8	0	0	0	0	0	0

Table 2. The number of CFU/cm^2 isolated from the foot surface.

After an incubation at the room temperature for 48 hours, we could notice that the CFU doubled their number in most cases, which proves that the isolated bacteria grow well at temperatures below 37°C.

On the non-selective, but very rich medium Blood Agar, we obtained both a large number and density of the colonies isolated from the shell. The CFU/cm^2 was between 54 and

confluent cultures. The number of CFU/cm^2 isolated from the shell on Blood Agar was between 8 and 210.

Morphology on Blood Agar	Morphology on MacConkey Agar	Morphology on Gram-staining	Identification
Very small, α-haemolytical colony	-	Gram-positive bacilli, arranged in V or Y forms	-
Small, white colony, with a narrow haemolytical zone	-	Gram-positive cocci, arranged in clusters	Coagulase-negative
Yellow-orange, non-haemolytical colony	-	Gram-positive diplococci and cocci arranged in clusters	Coagulase-negative
Big, yellow-brown colony	-	Gram-negative, polymorphous bacilli	Pseudomonas mendocina
Citrin yellow colony	-	Gram-positive bacilli, arranged in V and Y forms and in pallisades	Rataibacter spp.
Viscous, salmon pink colony	-	Gram-negative, polymorphous bacilli	Pseudomonas putrefaciens
Big colony with double haemolysis	-	Gram-positive, sporulated bacilli	
Colony with a narow haemolytical zone, with a brown-green pigment and irregular edges	Lactose ±	Gram-negative, polymorphous bacilli	Pseudomonas pseudoalcaligenes
Grey, not haemolytical colonies, with a mucous aspect	Lactose-negative	Gram-negative, bipolar stained bacilli	Pseudomonas pseudomallei
Small, white-grey colonies	Lactose-negative	Gram variable, thick coccobacilli	
Grey, mucous colonies, from small to very big ones	Lactose-negative		Pseudomonas putida
White, fluffy colony, with a brown base	-		Cladosporum spp
Black, fluffy colony, appeared after 7 days	-		Chrysosporium spp.

Table 3. The colonies aspects on Blood Agar and on MacConkey Agar

On the low selective medium MacConkey Agar, the number obtained was between 0 and semi-confluent cultures, while on the medium and high selective media for the isolation of the human pathogen bacteria, no colony was observed.

After 5 days, on the Sabouraud-gentamicin-cloramphenicol agar plates we could notice at only one snail, the appearance of two colonies (a black, fluffy one and a white, fluffy one) on the culture made from the shell and of one white, fluffy colony, which had a brown base after 7 days and which was intensively adherent to the medium.

The dimensions of the colonies on the Blood Agar varies from punctiform colonies to big, grey colonies with a mucuos aspect.

Some of the colonies present α-hemolyze, other colonies present a small area of hemolyze and others present β- or double hemolyze.

Some colonies are pigmented in citrin yellow, in brown-green, salmon pink or red.

On the MacConkey Agar, most of the colonies are lactose negative after 24 hours. After 48 hours, some of them have a yellow aspect which proves their slow capacity for lactose fermentation.

We used the Gram-stain for all the types of colonies. The results are in table 3.

The identification of the Gram-negative bacilli has been made on API galleries with automatic reading.

CONCLUSIONS

1. The number of CFU/cm^2 is significantly smaller on the foot surface compared to that on the shell surface.
2. No bacteria implicated in the human digestive pathology were isolated.
3. The number of CFU/cm^2 is signifanctly smaller on the foot surface compared to that on the shell surface, with the exception of one snail: the snail no. 9.
4. The small number of CFU/cm^2 at 9 of the 10 studied snails cand be related to the quantity and the quality of the mucous produced by the snail's glands.

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MAIN ANTIBIOTICS USED IN THE TREATMENT OF UDDER INFECTIONS IN HUMAN CONSUMPTION MILK SUPPLIER ANIMAL SPECIES FROM SOUTH-EASTERN SIBIU COUNTY

M. CÎRSTEA[1], R. TRIF[2], S. MORARIU[2], Florica MORARIU[3]
[1]DSVSA Sibiu, Calea Șurii Mari, 12; cirstea.marius-sb@ansvsa.ro
[2]Faculty of Veterinary Medicine Timișoara, Calea Aradului 119;
[3]Faculty of Animal Sciences and Biotechnologies, Calea Aradului, 119.

Abstract
Antibiotics are widely used in treatment of any infectious disorder, including mastitis also. Their improper use could seriously harm the consumer of meat, milk, eggs and other derived products obtained from treated animals. This paper emphasized the use of antibiotics in five localities from Sibiu County in three species of animals (cows, buffaloes and ewes) which provide milk for human consumption. The most used antibiotic was a combination of Penicillin and Streptomycin (31.84%), followed by Enrofloxacyn (20.72%) and Oxytetracycline (18.77%).
***Key words**: antibiotics, milk, Sibiu County.*

Antibiotics are vital drugs, used as last strategy in infection treatments. But their efficacy is threatened by the improper and exaggerated use of them, not only in medicine but also in agriculture. In veterinary practice, antibiotics are used mostly in infection treatment or prevention (2).

The presence of antibiotics in milk is due, in most cases, to the improper use of various drugs against mastitis or other infectious diseases (1).

The antibiotic contaminated milk is considered compromise by the Human Health Authorities and represents a potential danger for consumer health. The presence of drug residues in food over the admitted maximum level is considered harmful to human health by many international public authorities (4).

Also, some pathogens (i.e. *Staphylococcus aureus*) can produce several toxins which exacerbate their pathogeny. Some of these exotoxins could selective destroy the polymorph nuclear cells and monocytes. These leucotoxins belongs to a family with two components, being composed by two proteins: S (slow-eluted) and F (fast-eluted), which are acting synergistically to produce veritable “holes” into the membrane of phagocyte cells. Panton-Valentine leucocydine (LukS-PV + LukF-PV), γ-haemolysine (HlgA + HlgB and HlgC + HlgB) and recently described LukM (LukM + LukF`-PV) and LukE/D (LukE + LukD) (3; 5; 7; 8; 10).

MATERIALS AND METHODS

During 2009 five localities from south-eastern Sibiu County (Arpașu de Jos, Porumbacu de Jos, Avrig, Turnu Roșu and Tălmaciu) were monitored concerning the antibiotic use in various affections, but mainly for mastitis of economic interest animals.

Only animals which produce milk for human consumption were selected from consultation registers.

RESULTS AND DISCUSSIONS

Only cows, buffaloes and ewes were selected for this study.

Because the lack of treatment can cause serious injuries both to the health of the animal and to the milk production, the drug treatment is the first option. The antibiotics used in the five localities of Sibiu County are listed in the Table 1.

Table 1.

The antibiotics used in treatment of mastitis in animals from the five investigated localities.

Crt. No.	Antibiotic	No. of cases (%)	Waiting time for milk
1.	Amoxicillin trihydrate	414 (16.85%)	3 days
2.	Ampicillin trihydrate	24 (0.97%)	3 days
3.	Penicillin + Streptomycin (PenStrept)	782 (31.84%)	5 days
4.	Enrofloxacyn	509 (20.72%)	5 days
5.	Erythromycin thiocyanate	63 (2.56%)	7 days
6.	Gentamicin sulfate	174 (7.09%)	7 days
7.	Oxytetracycline	461 (18.77%)	3 days
8.	Tylosin tartrate	29 (1.18%)	4 days

Out of the 2456 cases of mastitis in all 3 monitored species it can be observed that 3 antibiotics or their combinations were intensely used, with more or les satisfactory results: PenStrept, Enrofloxacyn and Oxytetracycline. These 3 antibiotics represented more then 2/3 from all used antibiotics (71.33%). The most used combination was Penicillin-Streptomycin (31.84%) in almost 1/3 of cases, due probably to its lowest cost comparing with benefits.

It is obvious that the mastitis can not be totally eliminated from a herd, but it is also obvious that their evolution could be controlled irrespective of breeding system. Thus, in 2006, Jones (6) considers that therapeutically approach in these situations must be the same of a surgeon before starting the surgical treatment.

The i.m. injection of the antibiotic had a 90% efficacy, while the local treatment healed only 50% of cases. In the same time the combination between systemic and local treatment cured all cases, even those considered compromised (9).

The prevalence of antibiotics used in the treatment of mastitis in all five investigated localities is shown in Figure 1.

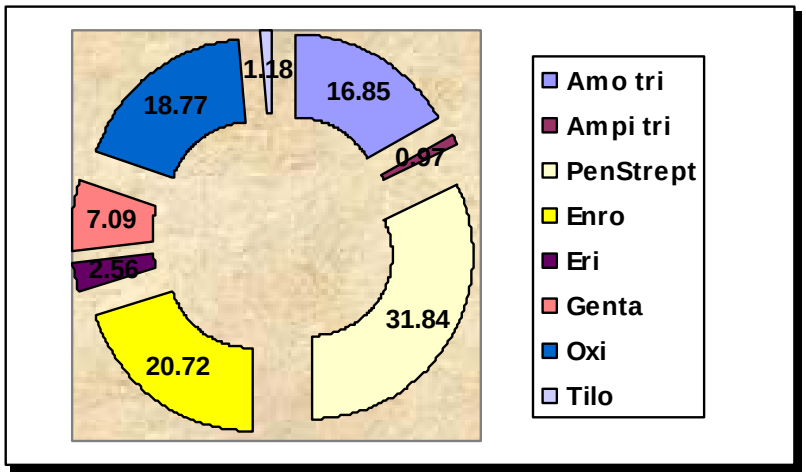

Figure 1. The prevalence of antibiotics used in mastitis treatment.

CONCLUSIONS

The most used antibiotic was the combination Penicillin-Streptomycin (31.84%), followed by Enrofloxacyn (20.72%) and Oxytetracycline (18.77%).

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PREVALENCE OF MASTITIS IN COWS, SHEEP AND BUFFALOES IN FIVE LOCALITIES FROM SIBIU COUNTY

M. CÎRSTEA[1], R. TRIF[2], S. MORARIU[2], Florica MORARIU[3]
[1]DSVSA Sibiu, Calea Șurii Mari, 12; cirstea.marius-sb@ansvsa.ro
[2]Faculty of Veterinary Medicine Timișoara, Calea Aradului 119;
[3]Faculty of Animal Sciences and Biotechnologies, Calea Aradului, 119.

Abstract
Mastitis is a widespread inflamation of udder all over the world. This paper describes the prevalence of mastitis in cows, sheep, and buffaloes from five localities of south-western Sibiu County. Mean mastitis prevalence in cows was 3.84%, 0.84% in buffaloes, and 5.44% in sheep, respectively.

***Key words**: Sibiu County, mastitis, prevalence.*

Mastitis is the udder inflammation. The diagnosis is accomplished by some criteria, which implies both the clinical part (they are recognized after inspection or palpation) and para-clinical exams (milk detection of bacteria or somatic cells).

Cows' mastitis represents a serious problem, because of its devastating economic effects. Annually, approximately 2 billion dollars losses are recorded in USA, and over than 500 billion dollars are recorded in India (1; 8; 12).

The aim of this study was to investigate the prevalence of mastitis in some localities from south-eastern Sibiu County.

MATERIALS AND METHODS

During the 2009 year five localities from Sibiu County (Arpașu de Jos, Porumbacu de Jos, Avrig, Turnu Roșu and Tălmaciu) were monitored concerning the prevalence of clinical and subclinical mastitis (Figure 1).

There were investigated 47744 animals from households as follows: 4549 cows, 41658 ewes, and 1537 buffaloes, respectively.

Figure 1. Geographical distribution inside the County of the five investigated localities.

Milk samples were collected in sterile recipients from all affected quarters, after a thoroughly disinfection of the tits and only after the first drops were discarded. Samples were immediately transported to bacteriology laboratory of DSVSA Sibiu where they were processed. The suspected colonies were macroscopically, morphologically and biochemical identified, on the basis of selected literature (2; 9; 13).

RESULTS AND DISCUSSIONS

Table 1 shows the data concerning the prevalence of clinical and subclinical mastitis in the five monitored localities from Sibiu County.

Table 1.The prevalence of cow mastitis in five localities from Sibiu County in 2009.

Crt no.	Locality	Cows		Buffaloes		Ewes	
		Total	Mastitis (%)	Total	Mastitis (%)	Total	Mastitis (%)
1.	Arpașu de Jos	763	29 (3.8%)	282	1 (0.35%)	4550	273 (6%)
2.	Porumbacu de Jos	1564	47 (3%)	665	7 (1%)	12695	698 (5.5%)
3.	Avrig	1100	55 (5%)	309	3 (1%)	14685	734 (5%)
4.	Turnu Roșu	486	19 (3.9%)	281	2 (0.7%)	2070	126 (6.1%)
5.	Talmaciu	636	25 (3.9%)	-	-	7658	437 (5.7%)
TOTAL		4549	175 (3.8%)	1537	13 (0.8%)	41658	2268 (5.4%)

Investigating the distribution of mastitis it can be observed that this is most prevalent in sheep. Localities with the highest prevalence were: Avrig for cows and buffaloes, Porumbacu de Jos for buffaloes (together with Avrig) and Turnu Roșu for sheep.

Next graph shows the mastitis prevalence on species in the five localities, based on the data from Table 1.

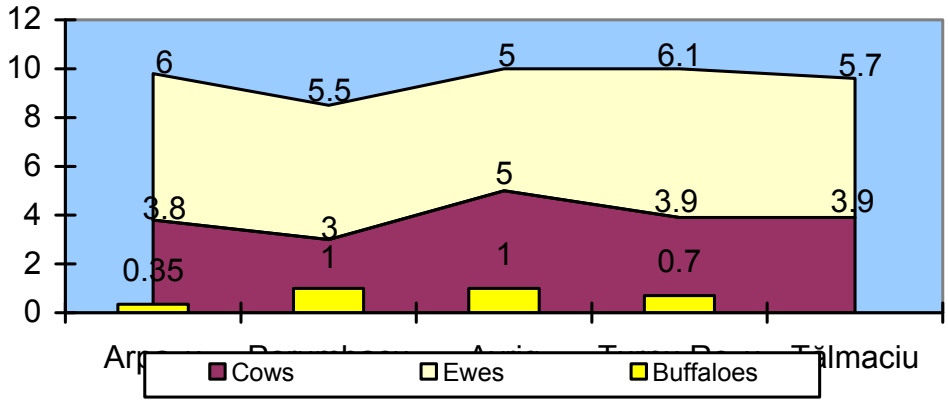

Figure 2. The prevalence of mastitis in cows, sheep and buffaloes in the five localities of Sibiu County.

A similar study carried out in 2008 in Egypt revealed an increased prevalence of mastitis in cows (22.5%), six times greater than registered in the five localities investigated in Sibiu County. In the same time, the prevalence was lower in sheep (2.63%) and goats (4.63%) than in the monitored localities (10).

This fact could be due to the improper hygienic conditions of cows' benefit of, comparing with the cattle from Romania. Talking about sheep, the related prevalence of mastitis expresses the same inconvenient in the breeding systems from both countries.

Indeed, the prevalence of clinical and subclinical mastitis is different from country to country, since it is due to differences between breeding technologies, management, and, not the last, in the approaching methods in this concern.

So, in cows, the prevalence was high in USA (27.3%), Kenya (28.7%) and Ethiopia (38.2 % - 60.8%), while in sheep, this ranged between 12% and 37% in USA, Israel, Greece, England, Wales, and Spain, respectively (3; 5; 6; 7; 11; 14). Concerning buffaloes mastitis, a study conducted in 2009 in India reported 3.1% prevalence, namely out of 1000 examined quarters, only 31 were affected by a clinical or subclinical mastitis (4).

CONCLUSIONS

The prevalence of mastitis was greater in sheep (5.44%) and lower in buffaloes (0.84%) in the five investigated localities from Sibiu County.

The highest prevalence of mastitis in cows was noticed in Avrig (5%).

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RESEARCH RELATED TO THE REFINEMENENT OF THE POULTRY AS PRODUCTS WITHOUT PROTECTIVE MEMBRANE

Lilica COCLEA[1], Angela GAVRILAŞ[2], D. SIMEANU[2], Cristina SIMEANU[2]
[1] – SC Tabco-Campofrio SA Tulcea, [2] – USAMV Iaşi

Abstract
The present work has the purpose of testing the quality of the raw chicken meat and that of a product without protective membrane manufactured in the framework of SC "Tabco-Campofrio" SA – Tulcea. The analysis carried out has been in the range of organoleptic, physical, chemical and microbiological tests. Following these tests we have noted that the raw materials utilized were of good quality, thus leading to final products well valued by consumers, especially since all links in the manufacturing technology have been followed. For the two analyzed products high protein content has been detected; at the same time, fat content was low. Microbiologically, the studied products did not endanger the consumer's health. In the reported context, the products manufactured by the above mentioned company have enjoyed a good appreciation of consumers from all categories. For the future, the company where the research was carried out plans to diversify more the chicken meat product range, so as to cover the growing consumer requirements. In the same time the natural flavors range will be extended for the manufactured products, so as to satisfy consumers requirements in this respect as well.

Key word: pastrami, , products, organoleptic, physical, chemical

In the supply population with food, with high biological value, a particularly important role rests to the animal origin products, and among these, meat and meat derivatives are prevalent. Thus, poultry occupies a special place, whereas it features characteristics that makes it not only very nutritious, but dietary as well.

Concerns for superior refinement of poultry as products are a growing concern induced by a more open market for such products.

The area in which research has been carried out, North Dobrogea respectively, the problem addressed is very topical, since here is recorded a lesser consumption of chicken meat and products, due various reasons like:

- lack of tradition in poultry consumption
- limited range of chicken meat products in the grocery stores and high price of the existing ones, not meant to stimulate public interest in their consumption
- lack of meat poultry farms, ensuring a supply of processing units with rhythmic material and to determine the lowest production cost for finished products

To those shown we believe that our research results will lead to an increase in chicken meat processing in the area and will provide incentives for business to invest in poultry.

MATERIALS AND METHODS

This work has analyzed the product "Chicken legs smoked pastrami" in regards with organoleptic, physical, chemical and microbiological characteristics, which allowed us to make a proper assessment of the product's quality, in conformity with the quality of food required by current Romanian legislation as well as by EU regulations.

Organoleptic characteristics were related to: product appearance, color, it's consistency and smell, respecting the provisions of the STAS 7031/83.

pH value

The acidity is assessed *objectively* (by electrometric measurement, using a pH-meter or a iono-meter).

Easily hydrolysable Nitrogen

Nitrogen from amino groups is released by hydrolysis with a weak base and together with the free ammonia, is driven by steam distillation in an acidic solution quantitatively and qualitatively known. Excess acid is determined by titration with an equivalent alkaline solution.

Hydrolysable Nitrogen derives both from amino groups and free ammonia. Free ammonia is only a part of the easily hydrolysable nitrogen, so it should not be confound.

Hydrogen sulfide

Hydrogen sulfide in the sample to be analyzed forms in reaction with the lead acetate the lead sulfide (dark-brown compound).

Water test was done by the oven drying method. Principle of the method consists in drying the sample to be analyzed in the oven at a temperature of +105 ^{o}C.

Minerals test

To determine the ash, calcinations method in the electrical furnace was used. Principle of the method consists in calcinations of the meat sample at +550 ^{o}C in an oven.

Protein test was carried on using Kjeldhal method.

The principle of the method: combinations of organic nitrogen, by heating with concentrated sulfuric acid, in the presence of a catalyst, are converted into ammonium sulfate. By adding a strong base (NaOH 33%), ammonia is released, and through distillation, captured into a fixed amount of sulfuric acid with known normality. The excess acid was titrated with an alkaline solution of same normality, and the difference was the total amount of N fixed. Chemical reactions that occur are:

$$N\ (protein) + H_2SO_4 \rightarrow (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2\ NaOH = H_2SO_4 + 2\ NH_4OH$$

$$2\ NH_4OH + H_2SO_4 = (NH_4)_2SO_4 + H_2O$$

Fat test was carried out using Soxhlet method. Principle of the method consists in fat extraction with organic solvent.

Microbiology

N.T.G.M.A. test is based on the inclusion of an amount of meat to be tested in a suitable nutrient environment, poured in Petri plates. Following incubation, each group of germs or each microorganism will develop a colony.

For this determination the following culture environments are used: yeast extract agar – glucose – gelatin (Frazier); agar tryptone – yeast extract – glucose (Plate count agar)

Principle of the method. Presence of Salmonella is detected by seeding the sample in an pre-enriching non-selective liquid environment, incubation at 37 ^{o}C, then seeding in two enriching selective liquid environments using cultures from the pre-enriching environment on selective solid media, which after incubation at 37 ^{o}C are checked for the presence of Salmonella suspected colonies and confirmed by identification test.

The main experimental data were statistically processed, calculating: arithmetic average, variance, average standard deviation, variation coefficient, and the homogeneity of the experimental variances were tested by *Fisher test* (*Sandu Gh., 1995*).

RESULTS AND DISCUSSIONS

Organoleptic characteristics

For the fabrication of the sort "Chicken legs smoked pastrami" are used chicken thighs, trimmed, weighing between 350-450 g. The raw material is chilled, but it comes from both fresh and frozen meat. Organoleptic characteristics of the raw chicken legs used in the manufacturing of the sort "Chicken legs smoked pastrami" are presented in *table 1*.

Table 1. **Organoleptic characteristics of the raw chicken meat**

Peculiarity	Presentation	STAS 7031-83 A1: 1997 pct. 4
Meat appearance	The meat surface was dry, with shiny and elastic tendons. Fat composition was normal. Synovial fluid was clear	Meat surface has a dry film. Fat, color, consistency and taste are normal. Tendons are shiny, elastic and strong. Articulated surface is smooth and glossy. Synovial fluid is clear.
Color	The meat had a pink to red color, moist without being sticky, and the muscular juice was difficult to obtain by pressing.	On the surface the meat is pink to red; on the cross cut it is glossy, slightly moist, without being sticky. Muscle juice is difficult to obtain and is clear.
Texture (elasticity)	When pressing a finger grooves were not formed on the surface, having an elastic texture.	The meat is firm and elastic; it doesn't keep grooves after pressing by fingers; it is compact in the cross cut.
Smell	Fragrant, both on the surface and deep, without showing abnormal smells.	On the surface, in cross cut and inside the carcass the smell is pleasant, aromatic, typical for the fresh meat, without abnormal smells.

We note in the table above tat the chicken legs, used as raw material, match the requirements of STAS 7031-83 A1: 1997 pct. 4, thus being fit for human consumption.

Physical characteristics

Measurment of the pH value on the raw materials used for the fabrication of the Chicken legs smoked pastrami was made using the pH measuring apparatus.

In *table 2* the pH values are shown, acquired for both fresh meat and frozen and stored meat as well. The values recorded were normal; thus, for the fresh meat the values ranged between 6.13-6.28, while for the frozen meat the values recorded are i the interval 6.33-6.26, these being typical to the poultry meat stored for some time.

Regarding the variance coefficient, this highlights the homogeneity of the studied characteristic (V%<10).

Values obtained by laboratory analysis on the chicken legs to determine the easily hydrolysable nitrogen content are recorded in *table 3.* These values were 20.52 mg NH_3/100 g meat for the chilled meat and grew and up to 23.31 mg NH_3/100 g meat for the frozen raw material.

Table 2. **pH value of the raw chicken meat** (n=25)

Type of raw meat	Storage									
	24 h		7 days		24 h		3 months		6 months	
	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%
Refrigerated chicken meat	6,13±0,11	4,12	6,28±0,14	4,25	-	-	-	-	-	-
Frozen chicken meat	-	-	-	-	6,33±0,09	4,91	6,19±0,07	3,87	6,26±0,08	4,19

** In fabrication it was used only 3 months stored frozen meat*

The recorded values are lower than the maximum admissive stated by the STAS 9065-7/2007.

The variance coefficients recorded values bellow 10%, proving a very good uniformity of the studied characteristic.

Table 3. **Easily hydrolysable nitrogen content of the raw meat (**n=25)

Statistical estimators	Raw meat state		STAS 9065-7/2007
	Refrigerated meat	***Frozen meat***	
$\bar{x} \pm s_{\bar{x}}$ NH_3 mg/100 g product	20,52±0,24	23,31±0,32	25
V%	3.87	4.08	

Hydrogen sulfide. Hydrogen sulfide was not revealed into the raw material under review, demonstrating once again the degree of freshness of the raw material.

Chemical characteristics

Water content. Refrigerated raw material had a water content of 69.6%, while the frozen one recorded a water content of 67.8%. In comparison with the literature consulted, which provided a water content value of 67.5% (*Vacaru-Opriş I. şi col., 2004; 2005*), we note that our results are slightly higher for the refrigerated raw material.

In calculating the variance coefficient, values were small (V%<10), which shows the homogeneity of the studied characteristic.

Dry matter content. Laboratory analysis to determine the dry matter content of the raw chicken legs revealed comparable results with the specialized literature data (32,5 - 27,9%), *(Vacaru-Opriş I. şi col., 2004, 2005).*

Calculated variance coefficients are bellow 10%, which demonstrates the uniformity of the raw material batches analyzed.

Protein content. For protein content the following results were obtained: 22.7% for the refrigerated meat and 22.96% for the frozen one. These values don't differ to the ones in the specialized literature.

Variance coefficients did not exceed 10%, correlated with a very good homogeneity of the characteristic studied.

Fat content. On the fat content parameter laboratory results indicated values in a range between 5.53%-6.16%, The values we have obtained are comparable with those found in the literature consulted.

Calculated variance coefficients bellow 10% certify that the batches are homogenous.

Mineral content. When determining the mineral content, their amount in the raw material ranged between 1.99% to 2.12%, exceeding the 1.3% as is reflected in data from the literature consulted *(Vacaru –Opriş I. şi col., 2005, etc).*

Values set for the variance coefficients were very small, (V%<10), which shows a high degree of homogeneity for this characteristic.

The overview of the chicken legs raw materials chemical characteristics are presented in *table 4.*

Table 4. **Chemical characteristics of the raw material meat (**n=25)

Chemical characteristics	Statistical estimators	Raw meat state	
		Refrigerated meat	Frozen meat
Water content (%)	$\bar{x} \pm s_{\bar{x}}$	69,6±0,71	67,8±0,86
	V %	6.18	6.37
Dry matter content (%)	$\bar{x} \pm s_{\bar{x}}$	30,4±0,32	32,2±0,39
	V %	5,29	4,37
Protein content (%)	$\bar{x} \pm s_{\bar{x}}$	22,7±0,18	22,96±0,16
	V %	6,27	4,08
Fat content (%)	$\bar{x} \pm s_{\bar{x}}$	5,53±0,06	6,16±0,08
	V %	5,39	5,41
Minerals content (%)	$\bar{x} \pm s_{\bar{x}}$	1,99±0,04	2,12±0,05
	V %	3,84	3,49

Microbiological characteristics of the raw material

Following laboratory tests, no aerobe mezofile germs were found in the meat depth, while on the surface their presence was very low (*table 5*).

The values obtained, of 10.11 germs/g meat, found on the surface of the refrigerated meat samples and 11.58 germs/g frozen meat are considered normal and bringing insignificant changes to the used raw material, given that the maximum allowed germ density on a microscope field is 30 (*Professional Standards Collection for the poultry meat, 1997*).

Table 5. **Total number of aerobe mezofile germs in the analyzed meat (**n=25)

Specification	Raw meat state	
	Refrigerated meat	Frozen meat
NTGMA/g meat in depth	absent	absent
NTGMA/g meat on surface	10,11±0,25	11,58±0,28
V%	4,35	6,42

Calculated variance coefficients were bellow 10%

Organoleptic, physical, chemical and microbiological characteristics of the studied sort: *"Chicken legs smoked pastrami"*

Organoleptic characteristics

In accordance with the internal fabrication norms, the finished product has the appearance of chicken thighs well shaped, with no fringes, with well developed muscles, orange-brown colored, weighing ~300 g. Taste and smell were pleasant, boiled and smoked like, specific to the flavors and spices used. Meat tenderness was high, while juiciness was the one particular to the chicken legs.

In analyzing the final product organoleptic characteristics compliance with the above requirements was found, the sort being characterized by a pleasant taste and smell, without foreign odors, its surface was uniformly dry, showing small breaks of the skin, but without affecting the commercial value of the product.

Physical characteristics

pH value. Determination of the pH value was performed on the fresh product, product at mid-term warranty, after 10 days, and on the product at the end of its warranty, 20 days after.

One may note from the acquired results the gradual decrease of the pH value from 6.58, value on the fresh product, to 6.28 for the product at mid warranty term, after 10 days storage, for at the end of warranty, after 20 days storage, pH value to reach 6.06

The values obtained, considered in the light of the variance coefficients values, indicate a very good homogeneity of the studied characteristic (V%<10).

Easily hydrolysable nitrogen. Easily hydrolysable nitrogen tests on the product "Chicken legs smoked pastrami" revealed values of 20.48 mg NH_3/100g product on the fresh one and 28.96 mg NH_3/100g product after 20 days storage.

Given that the law imposes a maximum of 45 mg NH_3/100g product, we can say that our product is complying with the outstanding regulations.

Small values of the calculated variance coefficient (V%<10) demonstrates the homogeneity of the characteristic watched.

Hydrogen sulfide. Laboratory analysis did not revealed the presence of hydrogen sulfide in the samples studied.

Salt content. This product range is required to have a maximum salt content of 3.5%, but, as all chicken meat based products, salt content was a low one. In our case, salt content varied between 1.8%, value recorded on the fresh product, to 2.1% value at the end of warranty term.

Batches homogeneity is confirmed by the small values obtained when calculating the variance coefficients.

Nitrites content. For the time being the maximum allowable nitrites content on the meat based products is 10 mg $NaNO_2$/100 g product. This limitation implies testing all meat products to verify this characteristic.

We have obtained values ranging from 4.52 to 4.63 mg $NaNO_2$/100 g product, these being well bellow the maximum admissible given by the law in force.

Viewed in terms of variance coefficients, the batches were the analyzed products originate from are homogenous.

Table 6 presents the laboratory analysis results for the physical characteristics of the sort "Chicken legs smoked pastrami".

Chemical characteristics

To determine the chemical characteristics we have appealed to determinations of water, protein and fat content of the finished product.

Water content. Fresh product water content was 77.12%, but decreased during the period of validity, so that after 20 days reached a value of 74.48%.

The variance coefficients recorded small values, bellow 10%, demonstrating the consistency of product batches where the samples analyzed were taken from.

Protein content. Analyzes revealed 17.76% protein content in the fresh product, 18.19% in the product after 10 days of storage and 19.01% at the product after 20 days storage. We can say that our product meets legislative requirements, given the values above 15% recorded, which is minimum required by law for protein content.

The variance coefficients values were bellow 10%.

Table 6. **Physical characteristics of the sort "Chicken legs smoked pastrami" (**n=25)

Physical characteristics	Statistical estimators	Fresh product	Product after 10 days storage	Product after 20 days storage
pH value dynamics	$\bar{x} \pm s_{\bar{x}}$	6,58±0,08	6,28±0,06	6,06±0,08
	V %	5,08	4,67	5,13
Easily hydrolysable nitrogen content (NH_3 mg/100 g product)	$\bar{x} \pm s_{\bar{x}}$	20,48±0,11	24,14±0,17	28,96±0,23
	V %	2,56	3,08	4,24
Hydrogen sulfide reaction	Negative			
Salt content (%)	$\bar{x} \pm s_{\bar{x}}$	1,8±0,03	1,9±0,08	2,1±0,10
	V %	3,29	3,97	4,06
Nitrites content ($NaNO_2$ mg/100g product)	$\bar{x} \pm s_{\bar{x}}$	4,52±0,11	4,6±0,10	4,63±0,12
	V %	4,08	4,61	4,27

Fat content. Values resulting from fat content determinations ranged between 3.38% and 3.62%. Although it's not a limitative factor, for this product range it is taken into account in fabrication.

Variance coefficients remained bellow 10%.

În continuare în *tab7.* sunt prezentate rezultatele determinărilor caracteristicilor chimice ale sortimentului analizat.

Next, in *table 7*, we are presenting the results of chemical characteristics test for the analyzed product.

Microbiological characteristics of the sort "Chicken legs smoked pastrami"

Determinations to show any contamination with Salmonella and Listeria shown us that the analyzed products are considered appropriate, not having contamination that may render the product unfit for human consumption.

Table 7. **Chemical characteristics of the studied sort "Chicken legs smoked pastrami"** (n=25)

chemical characteristics	Statistical estimators	Fresh product	Product after 10 days storage	Product after 20 days storage
Water content (%)	$\bar{x} \pm s_{\bar{x}}$	77,12±0,73	76,27±0,70	74,48±0,76
	V %	5,39	6,08	6,47
Protein content (%)	$\bar{x} \pm s_{\bar{x}}$	17,76±0,12	18,19±0,11	19,01±0,13
	V %	4,38	5,27	4,35
Fat content (%)	$\bar{x} \pm s_{\bar{x}}$	3,38±0,11	3,56±0,16	3,62±0,17
	V %	6,27	4,71	4,61

CONCLUSIONS

From our findings clearly emerges that in our research was used a high quality raw material, which led to obtain finished goods valued by consumers, especially since all fabrication technological steps have been followed.

All finished products analyzed had high protein content, while the fat content was low. Microbiologically, the studied products did not endanger the health of consumers.

In the context reported, the products manufactured by us enjoyed a very good appreciation from all categories of consumers.

For the future, the company where the research was performed aims to further diversify its range of chicken meat products, so as to cover the ever increasing requirements of consumers. At the same time, will expand the range of natural flavors for the products manufactured so as to meet the diversified requirements of consumers in this regard.

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RESEARCH RELATED TO THE REFINEMENENT OF THE POULTRY AS PRODUCTS WITH PROTECTIVE MEMBRANE

Lilica COCLEA[1], Angela GAVRILAŞ[2], D. SIMEANU[2], Cristina SIMEANU[2]
[1] SC Tabco-Campofrio SA Tulcea, [2]USAMV Iaşi

Abstract
The present work has the purpose of testing the quality of the raw chicken meat and that of one membrane product manufactured in the framework of SC "Tabco-Campofrio" SA – Tulcea. The analysis carried out has been in the range of organoleptic, physical, chemical and microbiology tests. Following these tests we have noted that the raw materials utilized were of good quality, thus leading to final products well valued by consumers, especially since all links in the manufacturing technology have been followed. For the analyzed product high protein content has been detected; at the same time, fat content was low. Microbiologically, the studied product did not endanger the consumer's health. In the reported context, the product manufactured by the above mentioned company has enjoyed a good appreciation of consumers from all categories. For the future, the company where the research was carried out plans to diversify more the chicken meat product range, so as to cover the growing consumer requirements. In the same time the natural flavors range will be extended for the manufactured products, so as to satisfy consumers requirements in this respect as well.

Key words: Vienna sausage, products, organoleptic, physical, chemical.

In the supply population with food, with high biological value, a particularly important role rests to the animal origin products, and among these, meat and meat derivatives are prevalent. Thus, poultry occupies a special place, whereas it features characteristics that makes it not only very nutritious, but dietary as well.

Concerns for superior refinement of poultry as products are a growing concern induced by a more open market for such products.

The area in which research has been carried out, North Dobrogea respectively, the problem addressed is very topical, since here is recorded a lesser consumption of chicken meat and products, due various reasons like:

- lack of tradition in poultry consumption
- limited range of chicken meat products in the grocery stores and high price of the existing ones, not meant to stimulate public interest in their consumption
- lack of meat poultry farms, ensuring a supply of processing units with rhythmic material and to determine the lowest production cost for finished products

To those shown we believe that our research results will lead to an increase in chicken meat processing in the area and will provide incentives for business to invest in poultry.

MATERIALS AND METHODS

This work has analyzed the product "Chicken Vienna sausage" in regards with organoleptic, physical, chemical and microbiological characteristics, which allowed us to make a proper assessment of the product's quality, in conformity with the quality of food required by current Romanian legislation as well as by EU regulations.

Organoleptic characteristics were related to: product appearance, color, it's consistency and smell, respecting the provisions of the STAS 7031/83.

pH value

The acidity is assessed *objectively* (by electrometric measurement, using a pH-meter or a iono-meter).

Easily hydrolysable Nitrogen

Nitrogen from amino groups is released by hydrolysis with a weak base and together with the free ammonia, is driven by steam distillation in an acidic solution quantitatively and qualitatively known. Excess acid is determined by titration with an equivalent alkaline solution.

Hydrolysable Nitrogen derives both from amino groups and free ammonia. Free ammonia is only a part of the easily hydrolysable nitrogen, so it should not be confound.

Hydrogen sulfide

Hydrogen sulfide in the sample to be analyzed forms in reaction with the lead acetate the lead sulfide (dark-brown compound).

Water test was done by the oven drying method. Principle of the method consists in drying the sample to be analyzed in the oven at a temperature of +105 ^{o}C.

Minerals test

To determine the ash, calcinations method in the electrical furnace was used. Principle of the method consists in calcinations of the meat sample at +550 ^{o}C in an oven.

Protein test was carried on using Kjeldhal method.

The principle of the method: combinations of organic nitrogen, by heating with concentrated sulfuric acid, in the presence of a catalyst, are converted into ammonium sulfate. By adding a strong base (NaOH 33%), ammonia is released, and through distillation, captured into a fixed amount of sulfuric acid with known normality. The excess acid was titrated with an alkaline solution of same normality, and the difference was the total amount of N fixed. Chemical reactions that occur are:

$$N\ (protein) + H_2SO_4 \rightarrow (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2\ NaOH = H_2SO_4 + 2\ NH_4OH$$

$$2\ NH_4OH + H_2SO_4 = (NH_4)_2SO_4 + H_2O$$

Fat test was carried out using Soxhlet method. Principle of the method consists in fat extraction with organic solvent.

Microbiology

N.T.G.M.A. test is based on the inclusion of an amount of meat to be tested in a suitable nutrient environment, poured in Petri plates. Following incubation, each group of germs or each microorganism will develop a colony.

For this determination the following culture environments are used: yeast extract agar – glucose – gelatin (Frazier); agar tryptone – yeast extract – glucose (Plate count agar)

Principle of the method. Presence of Salmonella is detected by seeding the sample in an pre-enriching non-selective liquid environment, incubation at 37 ^{o}C, then seeding in two enriching selective liquid environments using cultures from the pre-enriching environment on selective solid media, which after incubation at 37 ^{o}C are checked for the presence of Salmonella suspected colonies and confirmed by identification test.

The main experimental data were statistically processed, calculating: arithmetic average, variance, average standard deviation, variation coefficient, and the homogeneity of the experimental variances were tested by *Fisher test* (*Sandu Gh., 1995*).

RESULTS AND DISCUSSIONS

Characteristics of the sort "Chicken Vienna sausage"

Organoleptic characteristics

For the fabrication of the product "Chicken Vienna sausage" skinless chicken breast and pork lard are used. The raw material is used chilled, but it comes both from fresh and frozen meat.

Chicken Vienna sausages have the appearance of cylindrical bars with a diameter of 18 mm and a length of 13 cm, coated with a transparent membrane, edible. Following heat treatment the product color becomes brown. According internal fabrication rules, taste and smell are pleasant, specific to products boiled and smoked, enhanced by the flavors and spices used in the recipe. Cross cut layout is that of a fine paste of meat, well connected, compact and uniform, without showing air gaps or agglomerations of fat. In analyzing the product there were not noticed deviations from the internal fabrication norms of this product.

Physical characteristics

pH value

pH testing was carried out on a 60 days period, which is the shelf life of the product. Values obtained are shown in *table 1*, where we notice the decrease of the pH value from 6.57, value obtained on the fresh product, to 6.38 on the product with 30 days consumed of the shelf life, for at it's conclusion, 60 days after, pH value to reach 6.22. Calculated coefficients of variation have been low, bellow 10%.

Table 1. **pH value dynamics in the studied product: "Chicken Vienna sausage" (**n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$	**V %**
Fresh product	6,57±0,25	5,34
Product at half of the shelf life (after 30 days storage)	6,38±0,28	4,63
Product at the end of shelf life (after 60 days storage)	6,22±0,26	5,42

Easily hydrolysable nitrogen

The results acquired regarding the easily hydrolysable nitrogen are presented in the *table 2*, these ranging between 19.33 to 24.61 mg NH_3/100 g product. The values obtained are bellow the maximum allowed by law level of 30 mg NH_3/100 g product (*Ord. 560/2007*). Low values of the calculated variance coefficient indicate a good homogeneity between samples.

Table 2. **Easily hydrolysable nitrogen contents dynamics in the product "Chicken Vienna sausage" (**n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$ (mg NH3/100 g)	**V %**
Fresh product	19,33±1,05	6,34
Product at half of the shelf life (after 30 days storage)	22,31±0,83	5,21
Product at the end of shelf life (after 60 days storage)	24,61±0,72	5,39

Hydrogen sulfide

The presence of the hydrogen sulfide was not detected after laboratory analysis.

Chemical characteristics

Water content

An important parameter is analyzing meat products is the water content.

In *table 3* are presented the values obtained by laboratory analysis of the water content of the product "Chicken Vienna sausage". One may note that over time the water content

decreases slightly, due the product is packaged by vacuum, the only loss being those given by juice expression after pasteurization. The 51.76% water content, value recorded for the fresh product, ends at 51.68% at the end of the shelf life, the maximum 68% water content, as it is legislated, not being exceeded. Coefficients of variance had low values, bellow 10%, demonstrating the homogeneity of the studied characteristics.

Protein content

The boiled and smoked meat products have a minimal protein content value of 11% set by the legislation.

In laboratory investigations it was determined that our product has a much higher protein content, and that is 15.31% for the fresh product, 15.26% for the product at half of the shelf life, after 30 days of storage, and 15.19% for the product in the expiration threshold, as seen in *table 4*, exceeding the amount required by law. The variance coefficients calculated for this parameter were bellow 10%.

Table 3. **Water content of the sort "Chicken Vienna sausage" (**n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$ (%)	**V %**
Fresh product	51,76±1,25	6,34
Product at half of the shelf life (after 30 days storage)	51,70±1,16	4,58
Product at the end of shelf life (after 60 days storage)	51,68±1,20	5,34

Table 4. **Protein content of the sort "Chicken Vienna sausage"** (n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$ (%)	**V %**
Fresh product	15,31±0,13	6,31
Product at half of the shelf life (after 30 days storage)	15,26±0,11	5,27
Product at the end of shelf life (after 60 days storage)	15,19±0,12	6,19

Fat content

Maximum allowed fat content for the boiled and smoked meat products, without structure, as is the case in this situation, is 26% (*Ord. 560/2007*).

For the Chicken Vienna sausages, subject of our research, we have obtained values slightly bellow the value required, results ranging from 24.53% to 24.6% fat content (*table 5*). The variance coefficients did not exceed 10%, which correlated with a very good homogeneity of the studied characteristic.

Table 5. **Fat content of the sort "Chicken Vienna sausage"** (n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$ (%)	V %
Fresh product	24,53±0,26	6,21
Product at half of the shelf life (after 30 days storage)	24,56±0,30	5,38
Product at the end of shelf life (after 60 days storage)	24,60±0,24	5,49

Salt content

Salt content values obtained were bellow the maximum 3% allowed by legislation, these oscillating between 1.03-1.045% (*table 6*). Calculated variance coefficients were low, which reflects the homogeneity of the studied characteristic.

Table 6. **Salt content of the sort "Chicken Vienna sausage"** (n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$ (%)	V %
Fresh product	1,03±0,09	5,34
Product at half of the shelf life (after 30 days storage)	1,04±0,08	6,27
Product at the end of shelf life (after 60 days storage)	1,045±0,10	5,43

Nitrites content

Nitrites content tests indicated values between 2.73-2.74 mg NO_2/100 g product, data which may be found in *table 7*. Compared with outstanding norms, these results fall within normal values for the analyzed parameter. Viewed in terms of variance coefficients values, the results are homogenous.

Table 7. **Nitrites content of the sort „Chicken Vienna sausage"** (n=25)

Shelf life	$\bar{x} \pm s_{\bar{x}}$ (%)	V %
Fresh product	2,74±0,11	5,29
Product at half of the shelf life(after 30 days storage)	2,73±0,09	5,81
Product at the end of shelf life(after 60 days storage)	2,73±0,10	6,02

Microbiological characteristics of the sort "Chicken Vienna sausage"

Laboratory analysis revealed that the product is fit for consumption, no contamination with Listeria or Salmonella being indicated (*table 8*).

Table 8. **Microbiological characteristics of the sort „Chicken Vienna sausage"** (n=25)

Shelf life	Salm./25g	List.m./25g
	Met. SR EN ISO 6579:2003	Met. SR ISO 11290-1:2000
Fresh product	abs.	abs.
Product at half of the shelf life (after 30 days storage)	abs.	abs.
Product at the end of shelf life (after 60 days storage)	abs.	abs.

CONCLUSIONS

From our findings clearly emerges that in our research was used a high quality raw material, which led to obtain finished goods valued by consumers, especially since all fabrication technological steps have been followed. All finished products analyzed had high protein content, while the fat content was low. Microbiologically, the studied products did not endanger the health of consumers. In the context reported, the products manufactured by us enjoyed a very good appreciation from all categories of consumers.

For the future, the company where the research was performed aims to further diversify its range of chicken meat products, so as to cover the ever increasing requirements of consumers. At the same time, will expand the range of natural flavors for the products manufactured so as to meet the diversified requirements of consumers in this regard.

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IN VITRO ACTIVITY OF SOME NATURAL ESSENTIAL OILS AGAINST THE YEAST- LIKE ALGA PROTOTHECA

Bouari (Cuc) Cosmina, Gh. Răpuntean, C. Cătoi, N. Fiţ, G.Nadăş, P. Bolfă, M. Taulescu, A.Gal, R. Moussa, A. Nagy, F. Tăbăran

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca
Faculty of Veterinary Medicine
Mănăştur street, no 3-5
cosminacuc@yahoo.com

Abstract

The goal of this study was to determine the in vitro susceptibility of Prototheca isolates to conventional antifungal agents and to essential oils, provide an alternative to classical therapy with natural products, a strong tendency in the last years.

Twelve Prototheca zopfii isolates from mastitic milk samples and one P. wickerhamii referent strain (RE-4608014 ATCC 16529), from American Type Collectin, were submitted to antifungal susceptibility testing by classical diffusimetric method.

For both Prototheca strains tested, the highest efficiency were registered for: fir, savory and coconut essential oils. The tested isolates were shown to be resistant to rattle, marigold and eucalyptus essential oils.

Difficulties in treating protothecosis in humans and animals with conventional drugs and the potent in vitro activity of essentials oils demonstrated here raise the interest in further investigations on the therapeutic use of these non-conventional natural products.

Keywords: *Prototheca, therapy, essential oils*

INTRODUCTION

The genus *Prototheca* (*Trebouxiophyceae*, *Chlorophyta*) includes unicellular achlorophylous yeast-like microalgae that reproduce by formation of a variable number of sporangiospores within a sporangium (5). The genus Prototheca was established by Kru¨ger in 1894, and five species: *Prototheca wickerhamii*, *Prototheca zopfii*, *Prototheca stagnora*, *Prototheca ulmea* and *Prototheca blaschkeae*—have been recognized (1,7). In addition, three distinct biotypes of *P. zopfii* have been defined on the basis of phenotypic characteristics, and recently by molecular characterization (6,7).

Prototheca species are ubiquitous and can be isolated from various environmental sources, such as slime flux of trees, grass, fresh and salt water, and wastewater. A pathogenic potential has been indicated for *P. wickerhamii* and *P. zopfii*. Although the former is the predominant cause of human infections, the latter causes infections in animals, particularly in cows and dogs (2,3).

P. zopfii has been identified as inducing a therapy-resistant inflammation of the mammary gland in dairy cows leading to severe losses in an infected herd (1). These algae do not respond to routine mastitis therapy and the only control measure to date has been the elimination of the infected animals (3).

Recently, the potential antimicrobial effects of essential plant oils have attracted serious attention within the scientific community. Several reports have documented the efficiency of essential oils extracted from various plant species, such as *Melaleuca alternifolia* (tea tree) and, more recently, from *Citrus bergamia* (bergamot) (4,8).
The aim of this study was to investigate the *in vitro* susceptibility of *Prototheca* isolates to conventional antifungal agents and to essential oils.

MATERIALS AND METHODS

Twelve *P. zopfii* strains isolated fom mastitic milk samples and one *P. wickerhamii* referent strain were used for the study. The isolates were identified on the basis of microscopic, cultural (growth in glucose broth and on gelose mediums) and biochemical features (assimilation of glucose, galactose, glycerol, sucrose and trehalose). *In vitro* susceptibility testing was performed by classical diffusimetric method. Eight natural extracts: coconut , polioel 3, eucalyptus (*Eucalyptus globules*), rattle (*Calendula officinalis*), marigold (*Hypericum perforatum*), fir (*Abies alba*), aloe (*Aloe barbadensis*) and savory (*Satureja hortensis)* comparative with positive and negative controls (Lincocin forte and distillated water) were tested upon both *Prototheca* species. Two concentration of each extract (0,1 and 0,2%) in distillate water were used.

The vegetal extracts used in the study were plants extracts found in Romania flora and other countries with subtropical climate. Were purchased commercially as recommended standardized products to combat various diseases becaused of the bactericidal and antimycotic potential. These products have the advantage that the bactericidal effect have a low environmental impact, the only approved by European Union for use in humans and animals.

For *in vitro* testing of these products were used: Petri dishs with glucose agar medium, wells cut dies, automatic pippets, sterile tips, *Prototheca* culture in glucose broth medium or suspension in saline solution performed from colonies developed on agar surfaces. Warking technique were classical diffusimetric method on solide glucose medium.

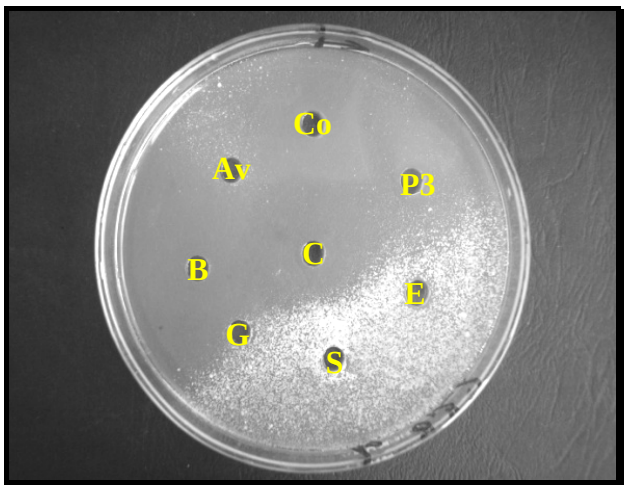
RESULTS AND DISCUSSION

The researches performed in the last period, regarding protothecosis combating developed function of knowledge level and of technological possibilities. Pharmacological protocol for therapy of this infection has not been developed yet either in human or in veterinary medicine, treatement remains a controversial problem. Therefore the establish therapy, is a long-term, and the results even effective innitialy, while proving the contrary by relapses occurring. Basic treatement in this disease remained some drugs which still fairly wide applicability.
Almost exclusively drugs therapy utilization (antibiotics and chemiotherapics) has led to the antibioresistance phenomenon. Very important as finding alternatives to the use of these products have proven to be harmful to animals and humans by residues accumulation in animals products and in the environment.

Tests carried out in the direction of establishing the algaecide effect of essential oils showed different aspects according to the plant from which it was obtained, extract concentration and also to the species of *Prototheca* tested.

For both *Prototheca* tested species the best results were registered at extract concentration of 0.2, the most efficient proved to be: fir, savory and coconut essentials oils.

Both *Prototheca* species have shown a reduce sensitivity to aloe vera extract and a total resistance to rattle, marigold, and eucalyptus essential oils (fig 1 and 2).



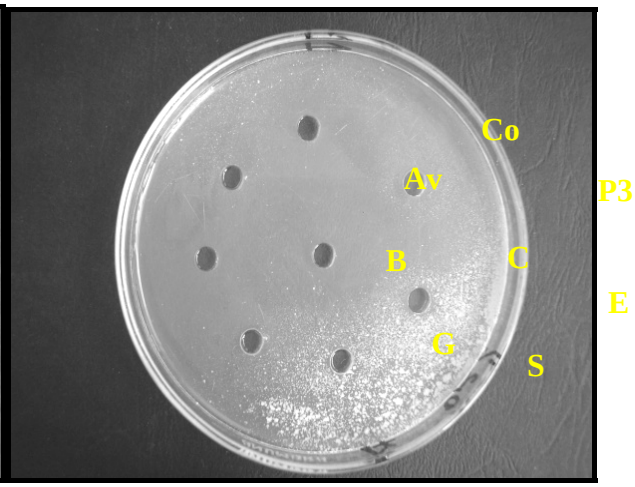


Fig.1.*P. zopfii* (10 strain) – vegetal extracts efficacity: Co – coconut, P3 – Polioel 3, E - eucalyptus, S – rattle, G – marigold, B – fir, Av – aloe, C – savory.

Fig.2.*P. wickerhamii* – vegetal extracts efficacity: Co – coconut, P3 – Polioel 3, E - eucalyptus, S – rattle, G – marigold, B – fir, Av – aloe, C – savory.

Table 1 summarizes the in vitro susceptibility profile of the *Prototheca* isolates to the plants extracts in two different concentration tested, compareing with positive and negative controls.

Table 1

Vegetal extracts efficaciry comparative with positiv and negative controls

Testated strains		Vegetal extracts and concentrations testated																		
		Aloe		Fir		Rattle		Marigold		Coconut		Savory		Eucalyptus		Polioel 3		+ctrl		- ctrl
		0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	0,2%	0,1%	-
P. z	1	15	8	27	10	0	0	0	0	20	14	20	17	0	0	24	22	10	8	0
	2	0	0	26	15	0	0	0	0	22	10	25	22	0	0	18	16	8	0	0
	3	18	11	24	18	0	0	0	0	22	18	20	18	0	0	20	18	18	16	0
	4	18	9	27	12	0	0	0	0	23	12	20	20	0	0	22	20	0	0	0
	5	10	10	25	10	0	0	0	0	20	0	22	22	0	0	0	0	0	0	0
	6	15	9	25	10	0	0	0	0	21	14	23	19	0	0	24	22	12	10	0
	7	18	10	27	13	0	0	0	0	24	0	21	20	0	0	10	0	20	17	0
	8	14	11	30	15	0	0	0	0	22	18	19	22	0	0	18	16	16	14	0
	9	18	10	27	18	0	0	0	0	20	12	24	17	0	0	15	0	16	14	0
	10	0	0	30	12	0	0	0	0	22	0	25	18	0	0	0	0	20	17	0
	11	15	11	27	18	0	0	0	0	20	14	25	22	0	0	0	0	10	8	0
	12	18	11	30	18	0	0	0	0	25	18	24	21	0	0	20	18	24	20	0
Average of inhibition area diameter (mm)		13,25	8,33	27,08	14,08	0	0	0	0	21,75	10,8	22,33	19,83	0	0	14,25	11	12,8	10,3	0
P. w		15	10	30	18	0	0	0	0	20	10	21	17	0	0	17	15	10	7	0

In the figure bellow can be observed a graphic representation of the average of inhibition areas, depending of the concentration used, obtained for each plant extract tested compared with positive control for both *Prototheca* species (fig. 3, 4).

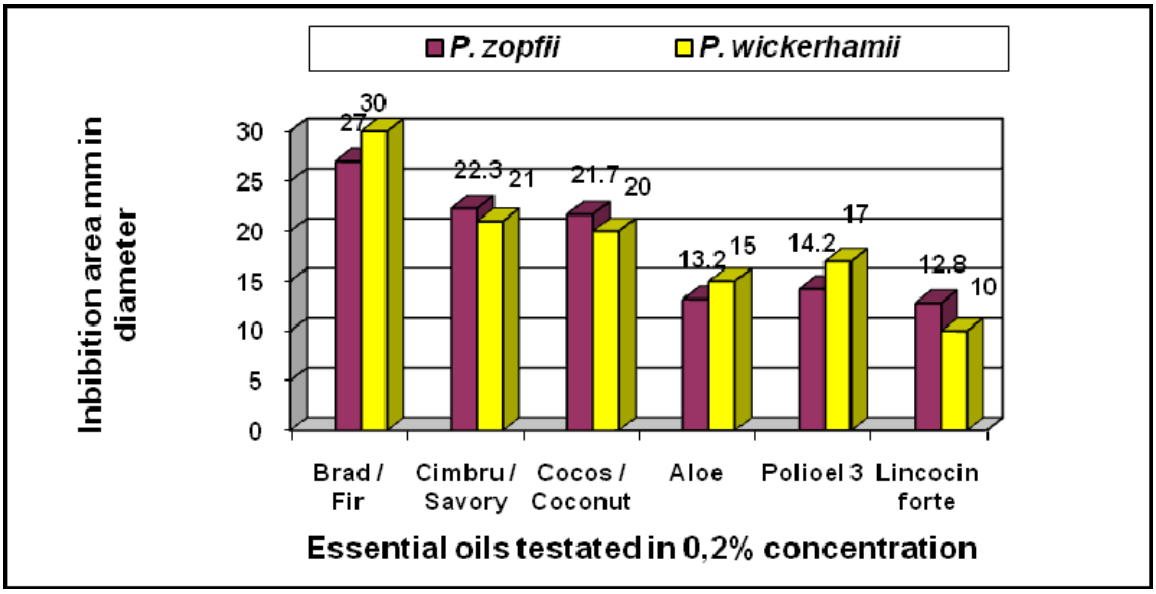
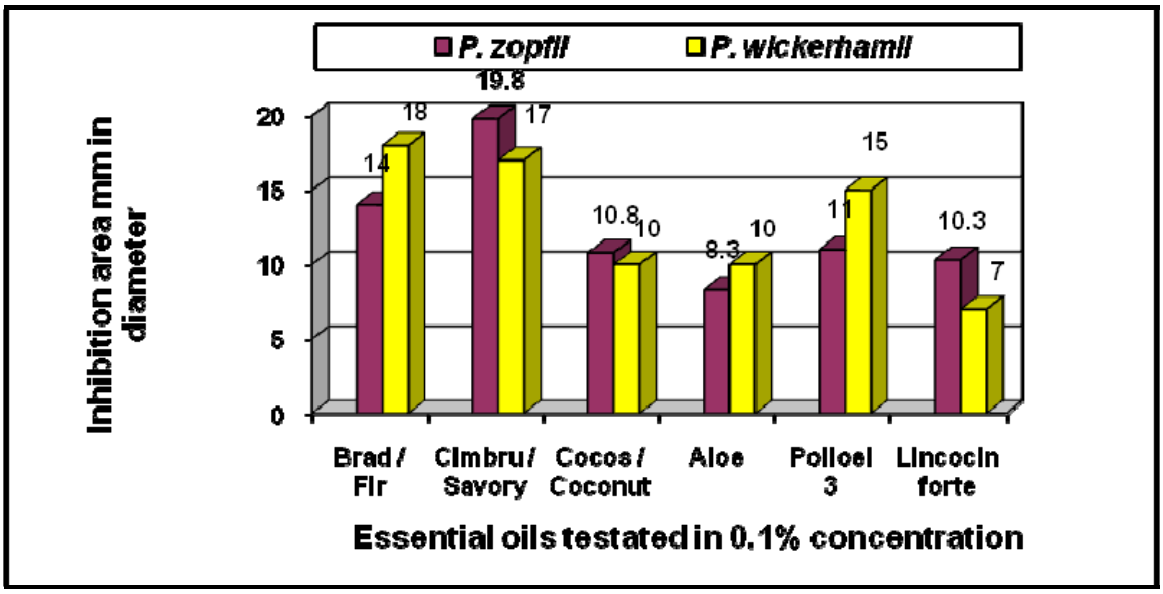


Fig.3, 4. Representation of average efficacity in terms of essential oil concentration, comparative with positive control, on both *Prototheca* tested species.



Fir essential oils in both concentration tested proved a highest efficiency for *P. wickerhamii*, with a value of average of inhibition area of 30 mm, versus 27 mm for *P. zopfii.* For both concentration tested, *Prototheca* strains showed a higher efficacy compared to conventional drug used as positive control (Lincocin).

Savory essential oil as 0,2 and 0,1% concentration was more efficient for *P. zopfii* than for *P. wickerhamii*. For both species this extract showed a higher efficiency compared to positive control.

The tested isolates were shown to be resistant to rattle, marigold and eucalyptus essential oils.

Ather study were realised in order to select a new antifungal compound, of vegetal origin. Was evaluated *in vitro* the efficiency of the following extracts: *Camelia sinensis*, *Cupressus sempervirens*, *Pistacia lentiscus* and *Glicine soja* seeds extracts, upon some strains of *Candida albicans*, *Criptococcus neoformans* and *Prototheca wickerhamii*. Among those *Camelia sinensis* (green tea) extracts, at MIC of 300-4800 µg/ml extract solution, which corresponds to a concentration of 130-2010 µg/ml total poliphenols prouved efficacy upon *P. wickerhamii* tested strains. Tests performed in which were used only pure polyphenols present in the extract of *C. sinensis* showed that only epicatechin 3-O galate (ECG) and epigalocatechin 3-O galate (EGCG), prouved anti-*Prototheca* activity (10). As an alternative to usage conventional antifungals, an other study reported the efficacy of essentials oils of *Maleleuca alternifolia* (tea tree) and that of *Citrus bergamota* (bergamout) (4,8,9). Plant action is based on these chemical composition, on principals active components and on synergic activity of all components. Important is that plants, throught there content in bioactive substances acts directly on pathogen microorganism and on some physiological functions, or indirectly in the biological processes that modified favorable, having positive influence on whole body health. Plants act as the antiseptic, antioxidante, anti-inflammatory, hepatocytes stimulating, antimitotics, immunomodulatory, endocrinoregulation and anthelmintic.

In conclusion, despite the *in vitro* activity of several antifungal drugs, difficulties in treating animals with these conventional drugs and the potent in vitro activity of essential oils demonstrated here raise the interest in further investigations on the therapeutic use of these non-conventional natural products.

CONCLUSIONS

► The most efficient non-conventional products, for both *Prototheca* species ware: fir, savory and coconut essential oil in 0,2% concentration. Those natural products in 0,1 as weel as in 0,2% concentration shown a higher efficiency than conventional antifungal drugs.

► On the other hend rattle, marigold and eucalypus essential oils showed no effect on any *Prototheca* strain tested.

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ORGANOLEPTIC RESEARCH OF SALAMI DURING THE VALIDITY PERIOD (FOOD DETERIORATION)

F. M. FOICA [1], M. CARP-CĂRARE [2], Dana-Simona DRUGOCIU[3]
[1] A.I.A. College, Țara Bârsei Prejmer – Braşov,
[2] Faculty of Veterinary Medicine, Iasi
[3] Faculty of Veterinary Medicine, Bucharest
dr_mikeflavius@yahoo.com

ABSTRACT. Before assessing the nutritional potential, that is, checking if the product is nourishing or not, the consumer makes decisions based on its psychosensorial effect, which brought us to the present research. Thus, we isolated several meat products covered in membrane, which were studied during the whole validity period, the results being different from one product to another and from one sort to another, with changes that would either shorten the validity period or extend it for certain products.

Key words: sensory analysis, salami, validity.

The sensory analysis of food products is, basically, as old as mankind, but progress was made no sooner than the latest three decades, by the intensification of the scientific research aiming at objectivizing it. This special interest for the sensory analysis of food raises from the progress made in physics, chemistry, microbiology, biochemistry, histology, technology, merceology, as well as in other domains of the technique and science.

The systematic research made during the last decades in the field of sensorics resulted in a rich resource regarding the way of applying the sensorial analysis in the control and quality assessment of the food products. The interest in the issues raised by the sensorics is currently very high, which is not at all by chance, as the sensorial properties of food products always draw the attention of the consumers, who react sensitively and also promptly to all the basic changes which affect certain products, and especially those changes related to sensory characteristics.

If, for most of the other industrial products, quality is characterised by a well-defined property or group of physical or chemical properties, in the case of food products quality is determined by three different essential criteria: innocuity, nutritional value and sensory quality. Sometimes, food quality is assessed by means of elements such as packaging and labeling, which are important for the product's protection and aspect.

MATERIALS AND METHODS

We isolated three batches of products from a company which produces sausages in the county of Brasov, a company which occupies an average market segment; the isolation was made as follows:

BATCH I – fresh salami: pork "parizer" (thick rosy sausage made of boiled minced meat), cremwurst, Polish sausages made of chicken breast

BATCH II – boiled and smoked: rustic salami, summer salami, baked salami

BATCH III – air-dried: "Săcele" salami, "Chorizo" salami, "Baciu" salami

These products were subject to examinations and sensory assessment based on scores, and we also made a statistic study regarding the consumers' opinion about sausages in different stages of the validity period, that is, at the beginning, middle, and at the end of the validity period.

The sensory properties will be examined in the following order: the condition of the transport package, outer aspect and shape of the product, the aspect in cross-section, smell, taste and consistency.

Dimensions are checked immediately after checking the outer aspect and product shape. In order to check the cross-section aspect, the pieces of meat products are cut perpendicular to the longitudinal axis, either in slices or in pieces, using either a thin-bladed and very well sharpened knife or a meat cutting machine (the machine is not used for aspic, black pudding, smoked bacon and sausages).

The order of the examination is the following: we will start with the less spiced sorts (cremwurst, Italian salami, etc.) and we will continue gradually with the more spiced ones ("Agnita" sausages, sausages with cumin, etc.).

Members of the commission are not allowed to exchange opinions, as this might influence the result of the sensory evaluation.

At the sensory evaluation we could notice:

Outer aspect: whole pieces showing a clean surface, without any impurities or spots of mould – except for the raw salami or sausages with special noble mould on the surface). The membrane has to be neat and without any wrinkles for fresh semi-smoked meat products, it must have wrinkles in the case of semi-smoked air-dried salami (classic summer salami covered in natural membrane), it must adhere to the composition, and under the membrane there should not be any air holes, melted fat, juice, larvae or insect galleries.

Cross-section aspect: compact, well-bound composition, with pieces of bacon of equal size and equally spread throughout the whole composition (for meat products with a mosaic aspect). The composition must not have air holes, melted fat blobs, pockets with juice or albumin precipitate.

For fresh salami, the composition is juicy, but without letting out liquids when pressed moderately.

For semi-smoked salami, the composition is compact, well-bound, firm and elastic.

For products which last longer, the composition is relatively hard, firm and homogeneous.

Colour: on the exterior, the colour is specific according to the product and to the technological process used, as well as to the type of membrane (natural, synthetic or semi-synthetic – the last two being able to be coloured and imprinted). On the cross-section, the colour must be homogeneous, specific to the sort, without areas showing a modified colour. The pieces of bacon are either white or pink, without having a grey, green or yellow hue. The semi-smoked products and products which last longer have a reddish, homogeneous colour on the cross-section, without any darker hue on the margins or any other changes in the colour towards the middle.

Smell and taste: characteristic to the sort, pleasant, moderately salted or spiced, without no changed or improper smell or taste.

The sensory quality of meat products is generally assessed on a 1 – 10 scale, the first five steps of the scale representing products showing positive qualities. **(Banu et al., 2007).**

The products labelled "exceptional" and "very good" (grades 9 and 8) are included in the "superior" category; the products labelled "good", "better than average" or "average" (grades 7, 6 and 5) are included in the 1st category. **(Banu et al., 2007).**

The area refering to the "unsatisfactory" grade represents less than 20% of the whole (Table no. 2).

The sensory analysis is completed with the physico-chemical analysis for the integrity assessment: water, fat, proteins, sodium chloride, nitrates/nitrites, polyphosphates, including starch, vegetal proteins or collagen proteins.

These evaluations are necessary in order to check if the product observes the standards imposed by the company or branch regarding the recipes, technological process or final product parameters.

RESULTS

Sensory characteristics of the PORK "PARIZER"

Validity period: 20 days (stored at 0-4°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: Table no. 1

Table no. 1 – Valability period score

Organoleptic properties	12.08.2009	22.08.2009	01.09.2009	11.09.2009
Outer aspect	8	7	7	7
Colour through the section	6	6	6	5
Flavour	7	6	6	5
Taste	6	6	5	4
Consistency	5	7	6	6
Juiciness	7	7	7	7
Overall assessment of quality	5	5	5	4
Total	44	44	42	38
Score	6.28	6.28	6	5.42

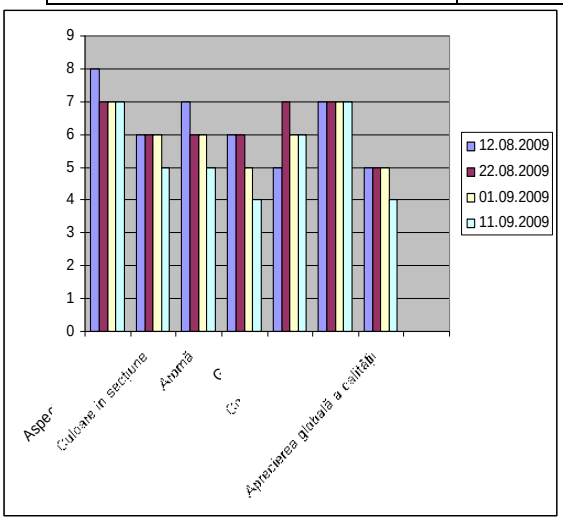

Fig. 1 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Fig. 2. Pork "parizer" – cross-section during different stages of the validity period

Sensory characteristics of the POLISH SAUSAGES MADE OF CHICKEN BREAST

Validity period: 10 days (stored at 0-4°C and relative humidity of air between 75 – 80%) given by the producer. As a result of the score it obtained, we noticed the changes presented in fig. 3.

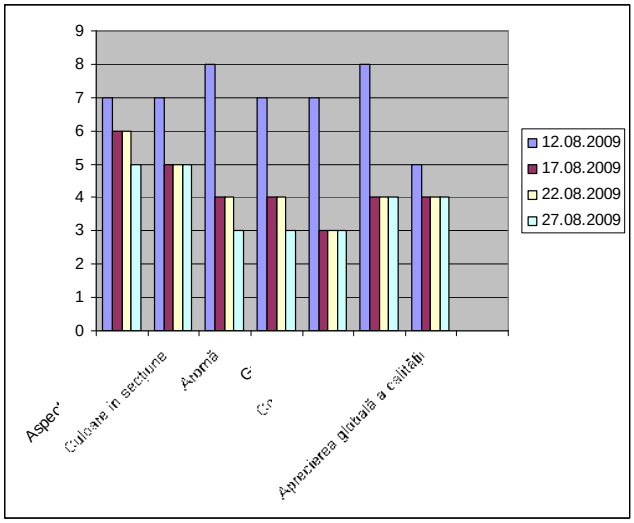

Fig. 3 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Fig. 4. - Polish sausages – outer aspect.

Sensory characteristics of CREMWURST

Validity period: 10 days (stored at 0-4°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 5

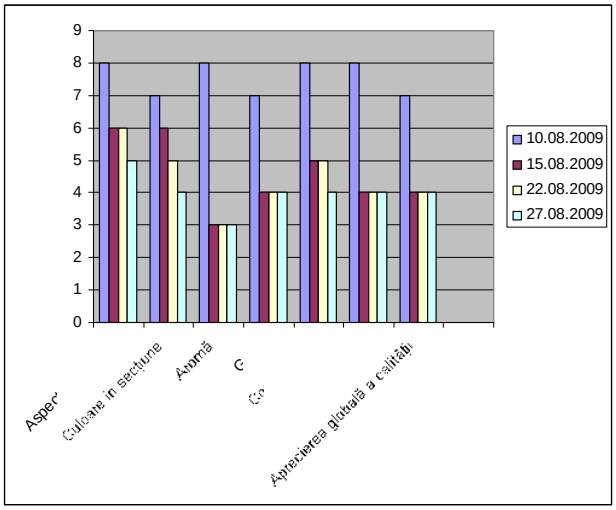

Fig. 5 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Fig. 6. - Cremwurst – outer aspect.

Sensory characteristics of the SUMMER SALAMI

Validity period: 15 days (stored at 10-12°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 7.

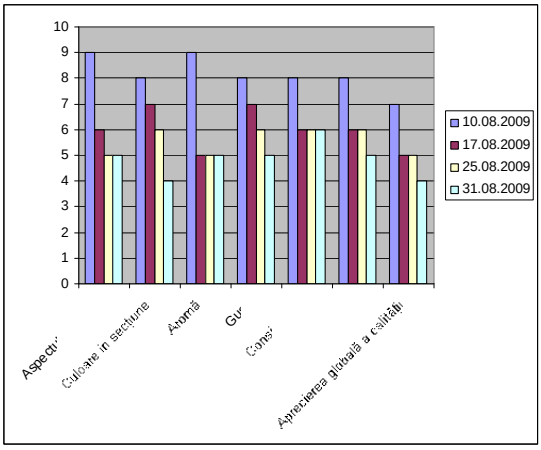

Fig. 7 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Fig. 8. Summer salami – outer aspect.

Sensory characteristics of the RUSTIC SALAMI

Validity period: 15 days (stored at 10-12°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 9.

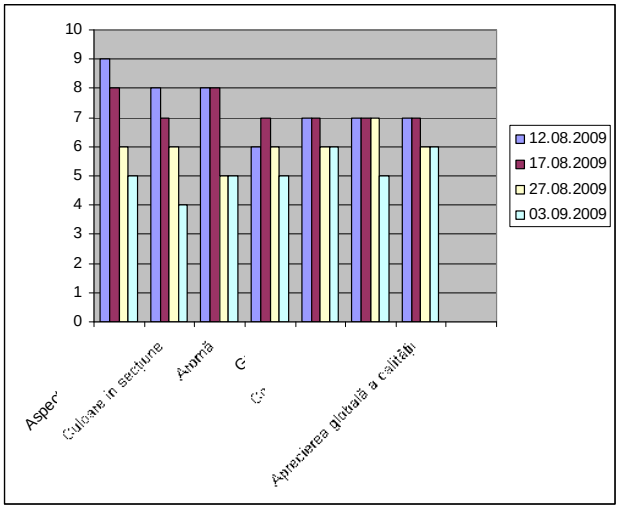

Fig. 9 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Fig. 10 - Rustic salami – outer aspect.

Sensory characteristics of the BAKED SALAMI

Validity period: 15 days (stored at 10-12°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 11.

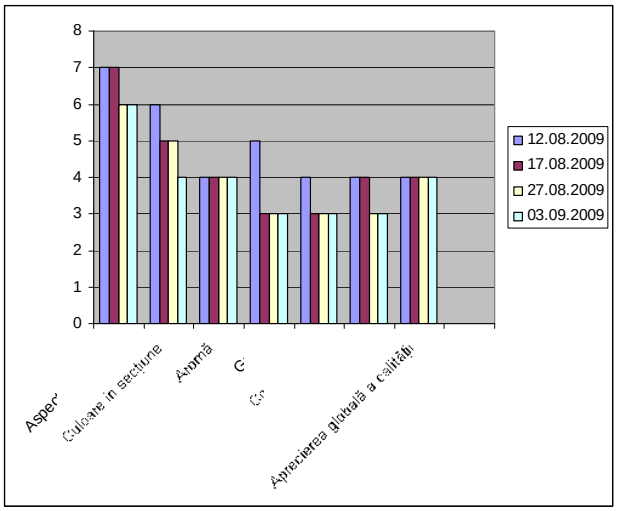

Fig. 11 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Sensory characteristics of the "BACIU" SALAMI

Validity period: 45 days (stored at 10-12°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 12.

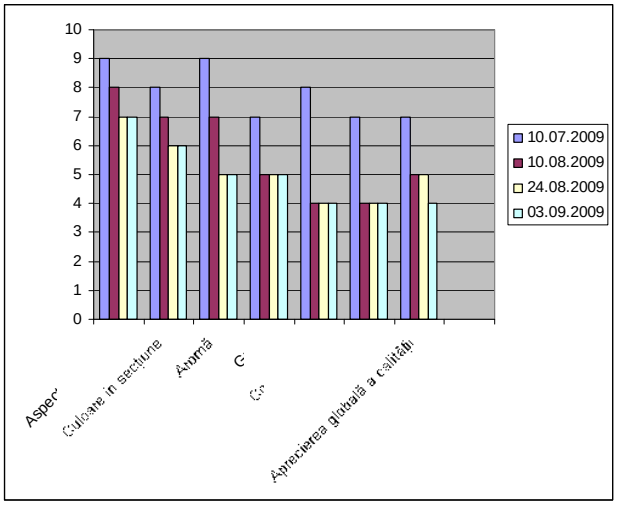

Fig. 12 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Sensory characteristics of the "CHORIZO" SALAMI

Validity period: 45 days (stored at 10-12°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 13.

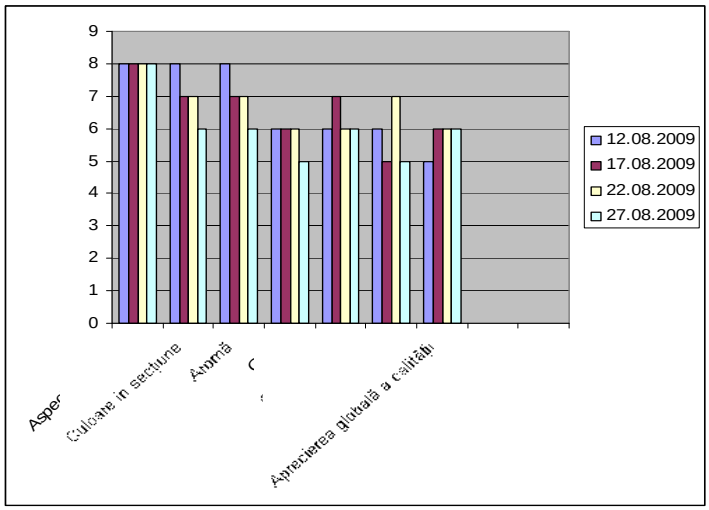

Fig. 13 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

Sensory characteristics of the SĂCELE SALAMI

Validity period: 45 days (stored at 10-12°C and relative humidity of air between 75 – 80%) given by the producer; it obtained the following score: fig. 14.

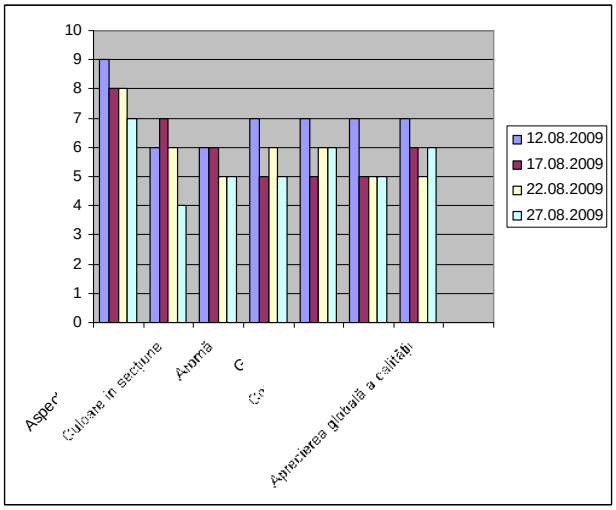

Fig. 14 - Graphic representation of the organoleptic changes undergone during different stages of the validity period.

CONCLUSIONS

1. The best score at the category of "fresh" meat was obtained by the "parizer", in whose case the validity period can be extended at least 5 days.
2. The cremwurst and Polish sausages showed visible modifications and, therefore, had a lower score before the end of the validity period, which results in a 4-day decrease of the validity period (40%).
3. The baked salami was the one which best kept its organoleptic properties within the smoked products, whereas the lowest score was obtained by rustic and summer salami.
4. Raw salami best preserved thei sensory characteristics, with a score that would allow the extension of the validity period with approximately 20–25%.
5. The results of the sensory analysis of the products' quality characteristics must be correlated to the results of the physico-chemical and microbiological analysis, in order to objectivize the quality control as a whole.

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RESEARCH CONCERNING DIARY COWS' WELFARE IN A FARM NEAR BUCHAREST

F. Furnaris[1], Elena Mitranescu[1], L. Tudor[1], Violeta Simion[2], C. Togan[3], B. Tasbac[1], S. Bustani[1]

1) University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 105 Spl. Independentei Street, Bucharest, Romania; ffurnaris@gmail.com

2) Spiru Haret University Bucharest, Faculty of Veterinary Medicine

3) National Sanitary Veterinary and Food Safety Agency

Abstract

A good animal welfare could not be conceived without continue monitoring, based on practical, numerical tools. The present study aims to assess the welfare of diary cows from Holstein-Frisian breed in a farm near Bucharest during May-June 2008 period.

As in Romania there an official numeric system for the assessment of animal welfare does not exist yet, permitting comparisons between different situations and farms, we used the ANI 35L 2000 version, the Austrian official system. This system had in view both animal based parameters (behavioral) and engineering based parameters (housing conditions, paddock quality, microclimate). There are assessed five areas of influence: locomotion, social interaction, flooring, light and air, and stockmanship. Points are awarded within each area of influence for several parameters using five assessment sheets. The researches were done based on the direct measurements, health records or using specific equipment as the Drager Miniwarn electronic gas meter (CO_2 and NH_3 analysis) or the Hill catathermometer (draught intensity). The sum of all points for the five areas of influence gives the overall ANI-score. The obtained final score was 19 points, which indicates a poor welfare. In order to improve cows' welfare in the farm up to an acceptable level measures must be taken referring to the critical points of housing condition.

Key words: diary cows, welfare, ANI 35L, gait score, hygiene

Ensuring a high level of animal welfare is not just a way to increase the production but also a moral duty of man regarding the animals.

Defined by Broom as "the state of individual as regards its attempts to cope with its environment", the animal welfare is an issue more and more approached by general public and world associations and organizations, mainly veterinary, but also in other field of activities.

After joining the European Union, in Romania the consumers of animal origin products became more interested in raising welfare level of farm animals, resulting in an increased number of laws concerning this matter. However, in our country there is not used yet a national practical system in order to assess the level of animal welfare at farm level, a system permitting comparisons between different situations and farms or to rate farms or shelters based on livestock welfare. Therefore, in our researches, we used an Austrian integrative numeric system of animal welfare assessment, respectively the Animal Needs Index (ANI) 35L. This system could be applied in our country, because it covers all infield rearing systems possible: with or without tether system, and all the shelter types: open or closed, with or without paddock and with different types of feeding, watering, lighting facilities.

MATERIAL AND METHODS

The researches aimed to assess diary cows' welfare based on ANI 35 L systems in a farm near Bucharest during May – June 2008. The system is important by its integrative character, gathering animal based parameters (behavioral) and engineering based factors (housing conditions, paddock quality, microclimate etc).

According ANI system, the welfare level was assessed on the basis of five areas of influence: locomotion, social interaction, flooring, light and air, stockmanship – each area of influence containing several factors which are awarded by comparison with reference values, using the 5 system sheets. The sum of all points awarded in the five areas of influence gives the overall ANI-score, which indicates the welfare level of the diary cows.

The assessment was done in one of the four sheds of the farm, in which was housed a number of 168 animals from Holstein-Frisian breed (235 Animal Weight Units = 168 animals x 1.4 multiplier), with an average weight of 540 kg, an average body length of 1.95 m and an average height at withers of 1,55 m. The shed have 72.65 m length and 15.5 m width. Concerning space internal structure, it is organized in 4 rows of stalls with vertical tether system. It is used the mixed lighting system, through the 96 windows of 1.2 m/0.85 m, respectively 55 m^2 shed surface and 38 light bulbs of 100W. The ventilation is natural, made by 20 air outlet openings of 30/25 cm and 4 air inlet openings of 50/60 cm.

The researches were conducted using the following methods: metric measurements for the calculation of available floor area, light index or other parameters; processing animal health records; specific equipment (Drager Miniwarn gas-meter for assessing the CO_2 and NH_3 level in the air, Hill catathermometer for measuring draught velocity). In addition, in order to improve the system's methodology (to make more it objective), we used for assessing the cleanliness of animals a method proposed by N.B. Cook (2002) and for conditions of hooves the gait scoring modified by N.B. Cook (2005).

RESULTS AND DISCUSSIONS

The results are presented in Table no.1.

Table 1

The ANI overall score calculation

Area of influence	**Factors**							Score /area of influence
	a	b	c	d	e	f	g	
I. Loco-motion	Space allowance (m^2/AWU)	Lying down, lying and rising	Stall size and boundaries	Movement of the tether	Outdoor yards access (days/year)	Pasture access (days/year)		**2,5**
Points awarded			0	0	2,5			
II. Social interaction	Space allowance (m^2/AWU)	Herd structure	The management of the youth	Access to outdoor yards (days/year)	Access to pastures (days/year)			**2,5**
Points awarded	0	0		2,5				

III. Flooring	Softness in the lying area	Cleanliness in the lying area	Degree of slipperiness in lying area	The quality of the activity area and passage ways	Outdoor yards type and quality	Pasture type and quality		**5**
Points awarded	2,5	0	1	0	1,5			
IV. Light and air	Daylight	Air quality	Draughts in lying area	Noise	Access to outdoor areas			**5,5**
					Days/year	Average hours/day		
Points awarded	0,5	0,5	- 0,5	1	2	2		
V. Stock-manship	cleanliness of pens/feeding /drinking areas	technical condition of equipment	condition of integument	cleanliness of animals	conditions of hooves	technopathies	animal health	**3,5**
Points awarded	0,5	-0,5	1	0,5	0,5	0,5	1	
OVERALL ANI SCORE								**19**

Within the first area of influence – locomotion – there are approached 3 factors: stall size and boundaries, movement of the tether and outdoor yards access (days/year). There could not be approached neither the factors referring strictly to loose housing system: *the space allowance (m^2/AWU)* and *lying down and rising*, nor the *pasture access (days/year)* because the cows have not access to pastures. Stall size and boundaries factor is scored with 0 points – stalls being rated as restrictive, because are short (1.8 x 1.2 m) and have high and rigid walls. Movement of the tether factor is scored with 0 points – the back and forth movement allowance being smaller than 0.4 m and the lateral movement smaller than 0.3 m. Outdoor yards access (days/year) factor is scored with 2.5 points – the animals accessing the paddocks 230-240 days/year and the paddock providing 12.37 m2/cow. Thus, the score for the first area of influence is the sum for the 3 factors above, respectively 2.5 points.

Within the second area of influence – social interaction – there are approached 3 factors: s*pace allowance (m^2/AWU)*, *herd structure* and *access to outdoor yards (days/year)*. Space allowance factor is scored with 0 points, being under the value of 6 m^2, respectively 3 m^2 (1.8 m x 1.2 m stall surface added with manger surface of 1.2 m x 0.7 m). For the herd structure the score is 0 points, cows being reared in tether system and the changes of stall allocation are not frequent. Outdoor yards access (days/year) factor is scored with 2.5 points, similar with the scoring within the first area of influence: locomotion. The score for the second area of influence is 2.5 points.

Within the third area of influence – flooring – there are approached 5 factors: *softness in the lying area*, *cleanliness in the lying area*, *degree of slipperiness in lying area*, *the quality of the activity area and passage ways* and *outdoor yards type and quality*. The first factor is scored with 2.5 points, in the assessed house being used as litter a layer of straw thicker than 60 mm. For the cleanliness in the lying area the score is 0 point (dirty floors) and for the degree of slipperiness 1 point. For establishing the quality of the activity area and passage ways there was observed the floor of passage ways to the outside areas. Because the floor of these area are from concrete, a rigid material, with a low thermal insulation factor and which could generates sliding (the rate of lameness was high in the assessed farm: 20-22%), the

score is 0 points. Outdoor yards type and quality factor is scored with 1.5 points. The score for the third area of influence is 5 points.

Within the forth area of influence – light and air – there are approached: *daylight*, *air quality*, *draughts in lying area*, *noise*, *access to outdoor area in days/year* and *access to outdoor areas in hours/day*. For the first factor the score is 0.5 points because the shed is closed and the percentage of window area relative to floor area is 10% (light index 1/10). Air quality is sufficient and it is scored with 0.5 points: the CO_2 concentration being lower than 0.2% and the average concentration of NH_3 is 14 ppm. The draughts in lying area factor is scored – 0.5 points, being registered seldom exceeding of the admitted limits (average speed measured with Hill catathermometer is 1.05 m/s). For the factor noise the score is 1 point, the shed being natural ventilated. The access to outdoor areas in days/year is scored with 2 points (230 – 240 days) and the access to outdoors area in hours/days with 2 points (a mean value of 8.5 hours daily). The score for the forth area of influence is 5.5 points.

Within the fifth area of influence – stockmanship – there are approached 7 factors: *cleanliness of pens/feeding /drinking areas*, *technical condition of equipment*, *condition of integument*, *cleanliness of animals*, *conditions of hooves*, *technopaties* and *animal health*. The first factor gets 0.5 points, the hygiene being medium. For the technical condition of equipment, the score is – 0.5 points due to droppings collecting system malfunction. The condition of integument is good, being observed minor skin lesions in less than 10% of the stock, so the score is 0.5 points. The cleanliness of the animals was assessed using the method proposed by N.B. Cook in 2002 (figure 1), the score being 0.5 points (good).

Conditions of hooves was assessed based on gait score (figure 2) – receiving 0.5 points (medium condition). The tehnopaties incidence is lower than 15% and the lesions are minor – so the ANI 35L factor is scored with 0.5 points.

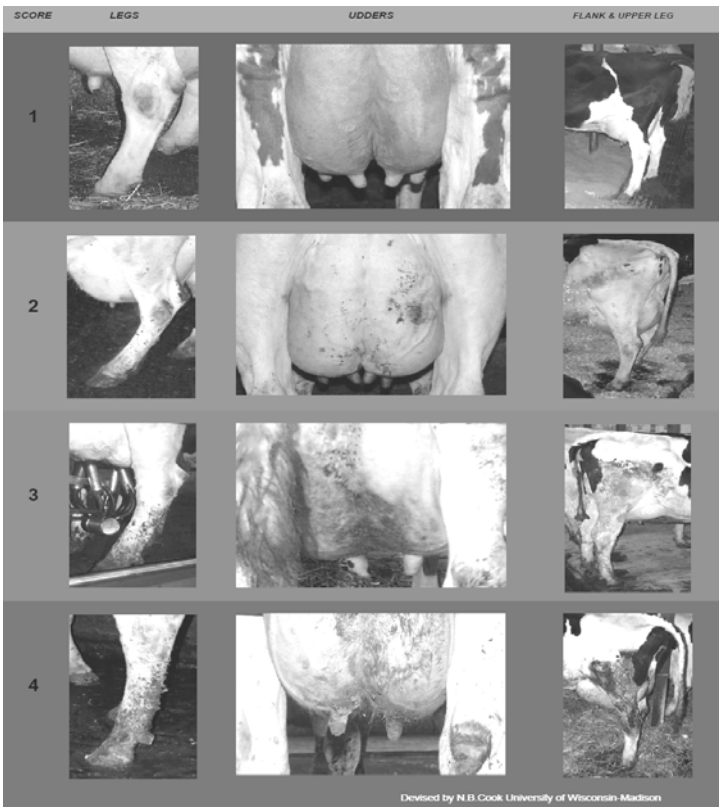

Figure 1 – Body hygiene scoring in cows (N.B. Cook, 2002)

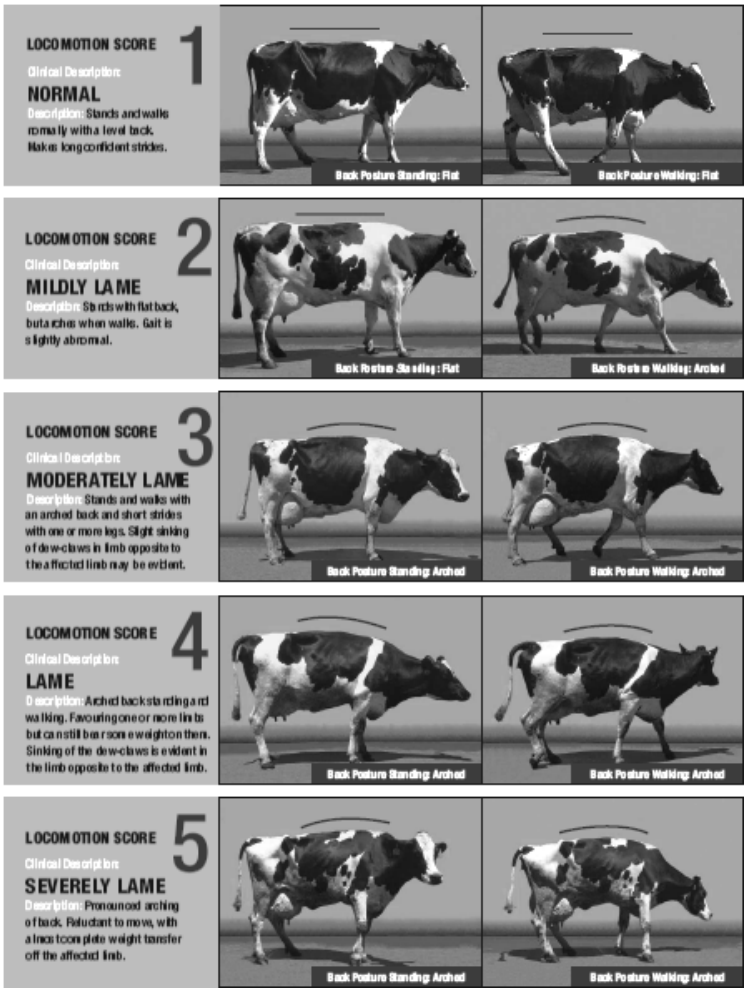

Figure 2 – Gait scoring, adapted by N.B. Cook (2005)

The animal health is qualified as good so it receives 1 point, based on the low mortality (0.8% - respectively 5 cows from a total of 623 cows) and good reproductive indicators (table 2).

Table 2

Reproductive indicators January-June 2008

	Cows	Heifers	Total
Total number of cows	480	143	623
Gestant cows after first insemination	198	85	274
Obtained calves	120	81	201
Total of gestation loss	32	19	51

The overall ANI score is of 19 points, showing that the diary cows' welfare in the studied shed is poor (overall score between 16 and 20).

CONCLUSIONS

1. The diary cows' welfare in the assessed farm is poor, the final ANI 35L score being 19 points.
2. The critical points of the housing conditions are the following: the spatial restriction, the use of tether system, the lack of pasture access, the increased value of draughts in the resting area, the poor floor hygiene, the equipment malfunction and the slipperiness of the activity area and passage ways.
3. In order to get the cows' welfare to an acceptable level need to be taken immediate actions regarding the critical points above.

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RESEARCHES REGARDING AIR QUALITY IN NEAMT COUNTY

F. Furnaris[1], Elena Mitranescu[1], Catalina Lala[2], L. Tudor[1], Magdalena Goncearov[1], Mariana Țerbea[3], L. I. Ilie[1]

[1] University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 105 Spl. Independentei Street, Bucharest, Romania; ffurnaris@gmail.com

[2)] Roche Romania. SRL

[3] National Sanitary Veterinary and Food Safety Agency

Abstract

In an area of the old province of Moldavia, special by its parks and reservations, a study on air quality was made. There were assessed gaseous pollutants, heavy metals by spectroscopy, sedimenting air particulates and noise intensity. The assessments were made for gaseous pollutants by using the colorimeter method, for heavy metals by atomic absorption spectroscopy, for sedimenting air particulates by the sedimentation method, and for noise intensity by sonometer.

Following the study, air quality in Neamt area ranged within the admitted limits in terms of concentration of gaseous pollutants, sedimenting particulates quantity and concentration of heavy metals, but noise level was exceeded by 17% in schools, nurseries, kindergartens and by 100% in parks and railway areas.

Key words: air, gaseous pollutants, particulates, noise, heavy metals

INTRODUCTION

In our country environmental protection is legislated, being a matter of national interest. Accumulation of pollutants in the environment will break the ecological balance, degrading the primordial factors of life: air, water, soil, through which plants and animals are affected, and, directly or indirectly, man too.

Nowadays, the concept of environmental damage is no longer confined to germs and potentially harmful climatic factors, but damage occurs also by thermal and sound pollution, vibrations, photonic radiations etc, generated by elements whose absence is inconceivable in some stage of industrial and economic development.

The area had in view in the above study (Neamt) is special by its rich vegetation, national parks (Ceahlau and Hasmas - Cheile Bicazului, Vanatori national park), 20 nature reserves, 6 natural monuments and 2 avifaunal special protection areas, being very transited by both foreign and Romanian tourists.

MATERIALS AND METHODS

In order to establish air quality in Neamt there were determined main gaseous pollutants (NO_2, SO_2, and NH_3), heavy metals (Pb, Cd, Zn, Mn, Cr, Cu, Ni, Co), sedimenting air particulates and noise intensity.

Gaseous pollutants were established in five control points on a variable number of samples, from 183 to 204, depending on the sampling point.

For the assessment of heavy metals in the air in Neamt area, samples were collected from eight points and sedimenting air particulates were measured in five control points.

For noise intensity determinations were made in markets, retails, outdoor restaurants, schools, nurseries, kindergartens, playgrounds for children, parks, recreation areas and railways.

Gaseous air pollutants were assessed by classical colorimetric methods and heavy metals by atomic absorption spectroscopy method.

Sedimending air particulates were assessed by sedimentation method, and for the noise intensity level there was used BK 2250 sonometer.

Results interpretation was done according to regulations in force.

RESULTS AND DISCUSSIONS

Average concentrations of gaseous air pollutants in the area are shown in Table 1.

Table 1

Average annual values of gaseous air pollutants – Piatra Neamt city

Sampling point	Station type	The pollutant type	No. of samples	Average annual concentration (µg/m^3)	Maximum admitted limit according to Order 592/2002 and STAS 12574/87 (µg/m^3)
Wheather station	Background station	NO_2	202	17,1521	100
		SO_2	202	6,8338	250
		NH_3	202	27,7798	100
Dumbrava Rosie Fuel station	Traffic station	NO_2	203	20,9723	100
		SO_2	203	5,1647	250
Savinesti Center crossroad	Industrial station	NO_2	204	10,9611	100
		SO_2	204	5,8833	250
		NH_3	204	42,1671	100
Savinesti–Socin	Industrial station	NO_2	183	7,6502	100
		SO_2	183	4,7192	250
		NH_3	183	40,9707	100
Turturesti	Industrial station	NO_2	201	16,4097	100
		SO_2	201	4,9660	250

Analyzing the data from the table it is noticed that there were no exceedings of the admitted limits recorded for any gaseous pollutant in any point of the five checkpoints.

In Table 2 are shown the annual average values of heavy metals in air from Neamt area.

There were assessed the concentrations of lead, cadmium, zinc, manganese, chromium, copper, nickel and cobalt in 8 control points from Neamt county, respectively: Piatra Neamt, Savinesti, Roznov, Turturesti, Roman, Bicaz, Tasca and Viisoara.

Analyzing the values in the table, it can be noticed that there was not registered average values over the admitted limit in any sampling point for any assessed element.

Doing a comparative analysis between sampling points and the assessed elements concentrations finds that, in terms of concentration of Pb, the highest annual average values were recorded in Savinesti, cadmium in Roman, zinc in Tasca, manganese in Tasca, chrome in Tasca, copper in Savinesti, nickel and cobalt in Tasca.

Sedimenting air particulates average values determined in five points (Piatra Neamt, Bicaz, Tasca, Targu-Neamt, Roman) are presented in Table 3.

Table 2

Average annual values of air heavy metals– Piatra Neamt city

Area	Average annual concentrations (ppm)							
	Lead	Cadmium	Zinc	Manganese	Chrome	Copper	Nickel	Cobalt
PIATRA NEAMŢ	76.636	6.991	980.558	441.915	16.580	77.315	19.945	15.161
SĂVINEŞTI	138.300	2.817	887.067	622.900	21.310	193.067	29.645	20.855
ROZNOV	84.245	1.575	701.750	416.200	38.660	104.035	28.420	17.310
TURTURESTI	99.133	0.356	769.633	326.500	46.770	109.240	48.500	15.450
ROMAN	93.511	4.100	1250.903	536.147	25.146	141.813	45.484	24.855
BICAZ	80.028	0.754	1159.434	500.788	39.250	111.214	33.873	12.782
TAŞCA	104.122	0.798	1673.729	759.790	78.847	122.905	67.481	41.916
VIIŞOARA	74.730	1.835	847.900	576.600	32.125	85.223	39.030	16.510

Table 3

Average annual values of sedimanting air particulates– Piatra Neamt city

City	Sampling point	No. of sample	Average values g/m^2/30 days
Piatra Neamţ	19 Noiembrie Street	34	6
	Solidaritatii Street		6
	Ciocarliei Street		7
Bicaz	Mărceni	36	8
	Dodeni		7
	House		11
Taşca	House family 1	36	7
	House family 2		12
	House family 3		13
Târgu-Neamţ	Mărăşeşti Street	27	8
	Mihai Eminescu		10
	Aleea Castanilor		13
Roman	Hospital	48	16
	Health center 6		15
	Republicii Boulevard		7
	School no. 8		7
Maximum admitted limit			***17***

Analyzing the obtained results for the concentration of sedimenting air particulates it can be noticed that in all sampling points, values were below the maximum admitted limit of 17g/m^2/30 days.

Values close to the maximum admitted limit were recorded in the hospitals and health centers from Roman sampling point.

Noise intensity was determined in open spaces, schools, nurseries, kindergartens and railways areas. The averages values are shown in Table 4.

Table 4

Average annual values of noise intensity - Neamt area

Sampling point	***No. of assessments***		***Measured values (db)***		***% Exceeding***	
	At the functional areas limit	***Interior***	***At the functional areas limit***	***Interior***	***At the functional areas limit***	***Interior***
Markets, retails, outdoor restaurants	5	3	63,6	67,5	0	0
Schools, nurseries, kindergartens, playgrounds for children	6	4	75,3	68,2	17	25
Parks, recreation areas	20	8	78,8	64,4	100	25
Railways	48		79,1		100	

Analyzing data from the table there are found to be critical points where the intensity noise exceeded admitted limits by 17% in schools, kindergartens, playgrounds for children and by 100% in parks, recreation and leisure area, as well as in railway area.

CONCLUSIONS

1. Air quality in terms of the gaseous pollutants, the amounts of heavy metals and sedimenting air particulates range within the admitted limits.

2. Noise intensity is exceeded in parks and railway areas by 100%, and in schools, nurseries, kindergartens by 17%.

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RESEARCH ON AVIAN COLIBACILLOSIS CONDITIONS IN INTENSIVE REARING

Diana Galațanu[1], T. Perianu[2], Oana Tănase[2]
[1]Morandi Com. Lipovăț Vaslui, [2] FMV Iaşi
diana.galatanu@gmail.com

Colibacilozele birds are contagious infectious disease that causes economic losses in affected flocks. They are produced by E. coli, are frequently encountered and superimposed infections with other bacteria and/or other viruses.
Disease was studied in a flock of 22000 chickens breed Issa Brown. The percentage of morbidity was CREC and the mortality reached 6.3%. Following isolation of 51 strains of E. coli and performing antibiogram has been established for five days treatment with floron, resulting in low mortality rate.
Losses were not significant chicks reached maturity at the age of productive performance.

Key words: colibacillosis, E.coli, intensive rearing, sistem, poultry.

Avian colibacillosis is a serious problem for the poultry industry is one of the main causes of morbidity, and mortality and birds. Pathogenic strains of Escherichia coli in poultry may cause aerosaculită, omfalitis, peritonitis, salpingitis, synovitis, sepsis, cellulitis, necrotic dermatitis and granulomatous syndrome in poultry. Considering the frequent presence of disease in flocks of birds kept in intensive and consequences of this disease (unevenness in fact, decrease the percentage of laying low body weight, seizure at slaughter) were conducted epidemiological surveys, clinical and morphological of colibacillosis birds under intensive growth.

MATERIAL AND METHODS

Epidemiological investigations were conducted on a sample of 22,000 chickens breed Issa Brown in a poultry farm in Vaslui during May-August 2005. Poultry litter are kept permanently on the ground sunflower husks and shavings, and the density of 15 birds / sq.

The first signs of illness occurred at the age of 24 days following vaccination against pseudopestei. Morbidity rate increase (up to 50%) and mortality rate reached 6.3% at 48 days after that, return to the clinical normal. (Table 1)

Diagnosis of avian colibacillosis was confirmed by laboratory tests and by clinical examination and necropsy. There were a total of 450 samples taken to establish a diagnosis and treatment optim.S have isolated a number of 51 bacterial strains identified as E. coli from the liver, heart, air sacs, lung. Were performed sowing the usual media-broth agar medium but also specific for the diagnosis of E. coli (Levin). All APEC strains studied fermented lactose on culture media, the biochemical character is considered constant at APEC strains. All lactozo-positive strains were subjected to further testing by the minimum set TSI mu Congo red. Colibacillosis diagnosis is confirmed by morphological and biochemical characters.

Necropsy was performed on a total of 650 birds being observant air bags lesions fibrinous- 76.9% -89.2% fibrinous lesions of pericarditis, hepatitis hemoragico-necrotic - 15.3%, fibrinous salpingitis -23% -53.8% fibrin-purulent peritonitis.

Table 1. Mortality in the flock of chickens

Birds age	No dead birds/day	No total birds/day	Percentage mortality
25 days	10	10	0,04 %
26 days	15	25	0,11 %
27 days	30	55	0,2 %
28 days	40	95	0,43 %
29 days	50	145	0,65 %
34 days	70	215	0,97 %
35 days	75	290	1,31%
38 days	80	370	1,68%
40 days	100	470	2,1 %
41 days	110	580	2, 63 %
42 days	200	780	3,54 %
43 days	280	1060	4,8 %
44 days	150	1210	5,5 %
45 days	100	1310	5,9 %
46 days	50	1360	6,1 %
47 days	25	1385	6,2 %
48 days	10	1395	6,3 %

650 birds from the necropsy lesions were found air sack fibrinous - 76.9% -89.2% fibrinous lesions pericarditis, hepatitis, necrotic hemoragico - 15.3% -23% salpingitis fibrinous, purulent peritonitis fibrin -53.8%. (Table 2)

Table 2. Type and frequency of pathological changes

Birds age	No dead birds/day	Air sack fibrinous	Pericarditis	Hepatitis	Salpingitis	Peritonitis
25-34 days	290	68,9 %	86,2%	15,5 %	-	8,6%
35-40 days	845	93,4 %	94,6%	23,6%	35,5 %	76,9%
41-48 days	335	28,8 %	59,7%	-	-	14,9%

Movement of poultry material, globally, to disseminate APEC strains and hence the release of multiple resistance to antibiotics, which requires the monitoring of this view of APEC strains isolated from birds reared in intensive system, the reference laboratories , which in many countries is already in place.

Following the performance tulpinide antibiogram for the 51 E. coli isolates, there was increasing resistance to most strains of antibiotic susceptibility and restriction, present in particular to newer antibiotics. (Table 3). The best results were obtained in florfenicol (90% sensitivity).

Other 36 strains of E. coli tested were found to be sensitive in more than 50%, the following antibiotics: flumequin (61.11%), enrofloxacin (58.3%) and norfloxacin (52.77%).

Regarding antibiotic resistance, the 51 strains tested in the first set proved to be resistant to more than 50% the following antibiotics: doxicilină (92.57%), tetracycline (85.13%), neomycin (77.69%), ciprofloxacin and erythromycin (60.34%), spectinomycin (57.85%).

Table 3. Behavior results from antibiotic strains of E. coli

Antibiotics	Sensibile %	Moderately susceptible %	Rezistent %
Neomicina	*10*	*12,31*	*77,69*
Enrofloxacin	*58,3*	*23,7*	*8*
Apramicina	*68,2*	*27,2*	*4,6*
Streptomicina	*7,5*	*23,8*	*68,7*
Colistin	*75,4*	*23,4*	*1,2*
Furazolidon	*62,4*	*26,8*	*10,8*
Tetraciclina	*6,7*	*8,17*	*85,13*
Amoxicilina	*51,4*	*13,7*	*34,9*
Spectinomicina	*20*	*22,15*	*57,85*
Lincomicina	*-*	*-*	*100*
Gentamicina	*40,2*	*28,3*	*31,5*
Sulfametoxazol+ Trimetroprim	*11,5*	*34,6*	*53,9*
Ampicilina	*2,7*	*21,6*	*75,7*
Fluorfenicol	*90*	*6,4*	*3,6*
Lincospectin	*89,5*	*-*	*10,5*

Episode triggered by avian colibacillosis in poultry farm because of the microclimate-supraalomerare deficiencies, excessive dust, stress, vaccination, mortality losses varying by them. (Fig. 1).

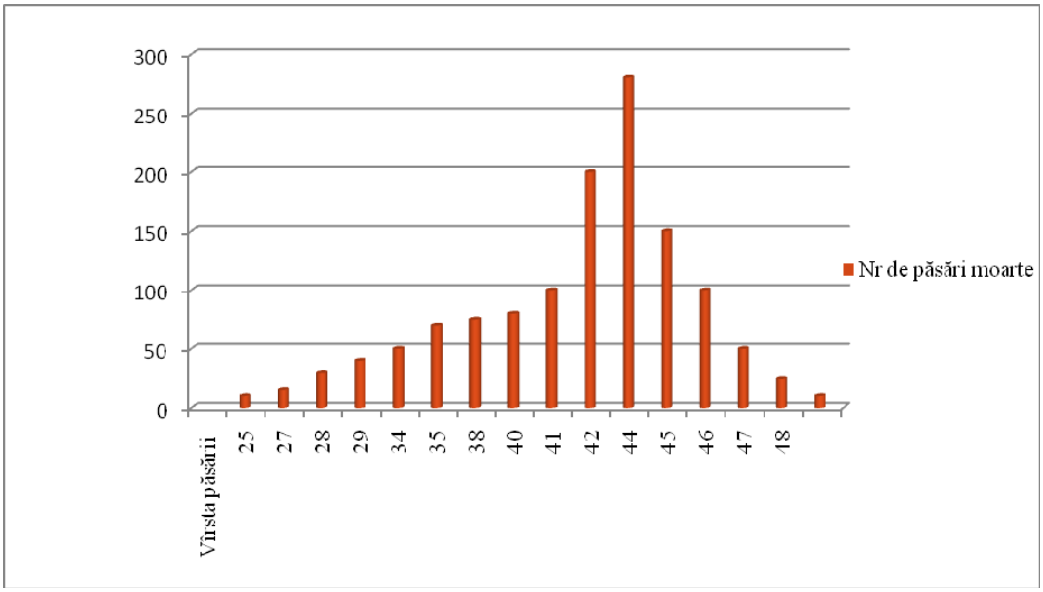

Fig.1 Mortality in a flock of chickens

Disease occurs in birds affected by sneezing, serous ocular and nasal catarrh, cough, shortness breath and sinus infraorbitale deformation. Birds at necropsy air bags appear fine deposits of fibrin that later turns into fibrin deposits caseous the appearance of ouă. Pulmonii greaves are hiperemiaţi and increase in volume, tongue lesions of pneumonia. Subsequently, fibrin color of air bags closed envelope. Serofibrinoase serous pericarditis occur and

subsequently followed by liver and serous inflammation serous abdominal tututror then, all bodies are covered by fibrin. (Fig. 2, Fig. 3).

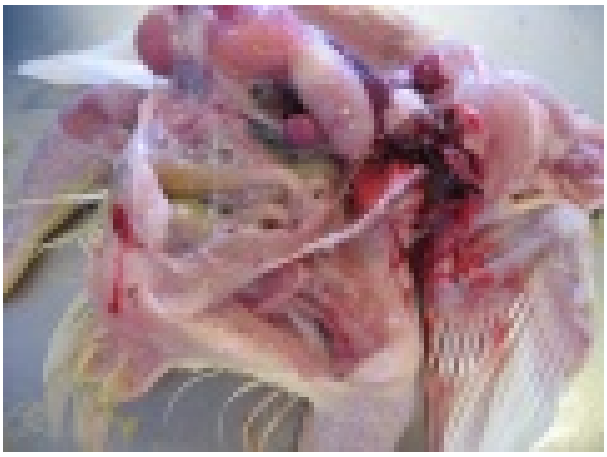

Fig. 2 Foamy exudate in the air bags birds infected with E. coli

Fig. 3 Exudate cheese in the air bags birds infected with E. coli

As the evolution of the disease, liver or dark green, increased consistency and pectoral muscles are congested increase in volume, brittle and outbreaks of hepatitis hemoragico-necrotic. It finds and fibrin purulent peritonitis, salpingitis fibrinous. (Figure 4, Figure 5) have normal bowel inflammation blue, often with the serous intestinal bleeding points. Bleeding is found also in the pericardium, endocardium and in other organs.

Fig. 4 Liver hypertrophy in chickens

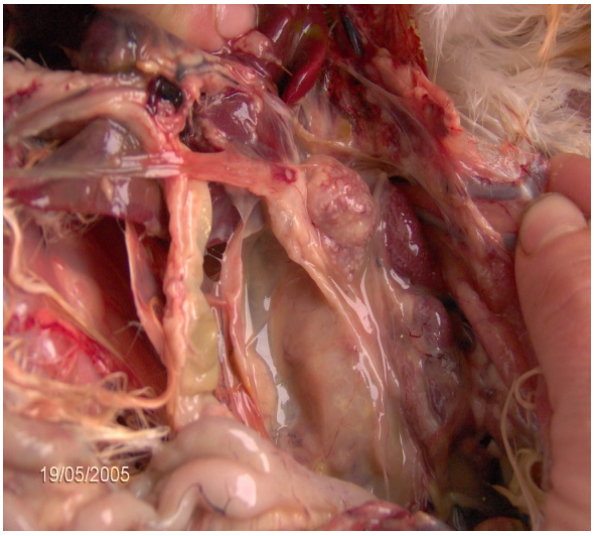

Fig. 5 Aerosaculită and salpingitis in chickens aged 40 days 40 days old

A high frequency of aerosaculită lesions, pericarditis, hepatitis, salpingitis and peritonitis were found at the age of 35-40 days. During e evolution of the disease were thinning birds Hall transfer to other halls and drug treatment was applied following completion of antibiograms for five days with floron - 200ml / 100litri water in drinking water since the age of 40 days. After 2-3 days of administration of the mortality rate began to fall. (Table 4).

Table 4. Mortality rates after treatment with antibiotics

Birds age	No. ad birds	Percentage mortality
41 days	110	0,5 %
42 days	200	0,9 %
43 days	280	1,27%
44 days	150	0,53 %
45 days	100	0,45 %
46 days	50	0,22 %
47 days	25	0,11 %
48 days	10	0,04%

CONCLUSIONS

Research avian colibacillosis in a group of chickens reared in intensive system led to the following conclusions:

1. Illness were found in a consignment of 2,200 chickens reared in intensive vaccination because of stress and inappropriate conditions microlimat.
2. Colibacillosis occurred after vaccination because of stress and high density of birds in the hall.
3. The disease started at the age of 25 days, the morbidity rate is high (50%), but mortality reached 6 procentil 3%.
4. Colibacillosis diagnosis was established by laboratory tests (by cultivation on agar, broth) Morphological and biochemical examinations, clinical and necropsy.
5. There were 51 strains of E. coli isolation and antibiogram was observed after performing strains increased sensitivity to flurofenicol.
6. Losses were so significant and have affected poultry flocks, chicks reaching productive performance in terms of egg production when he reached adulthood.

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RESEARCH CONCERNING THE INFLUENCE OF THERMAL TREATMENT ON THE QUALITY OF FLORAL HONEY PRODUCED IN ROMANIA

ANCA MARIA GALIŞ, I. ȚOGOE,
L. TUDOR, I.L. ILIE[1], MITRĂNESCU Elena
Faculty of Veterinary Medicine Bucharest

Abstract
Romanian honey is processed in different ways in order to extend its shelf life and prevent crystallization, thus the producers heating it at temperatures with values below 100°C.
It is believed and recent studies have partially demonstrated that the thermal treatment of honeydew and floral honey determines a transformation of the hydroxymethylfurfural content (abbreviated HMF in the research field). The formation of this compound is highly influenced by the water content, total acidity, pH value and mineral substances content.
The honey samples were subjected to heating at different temperatures, of 50, 75, 100°C for a period of time between 15 and 60 minutes, afterwards being analyzed for the HMF content by using HPLC.
As it was already proved in other previous studies, a significant increase concerning HMF content in honey samples was not observed even when heating the samples at 100°C. The reason for HMF content severe increase is probably due to another factor, probably related to production or storage step.

Keywords: floral honey, thermal treatment, quality

INTRODUCTION

Since the earliest times, honey has been used as food and medical product, as it is a natural substance produced by honeybees from the nectar of flowers or exudates of trees or plants (2, 9). Honey is considered a very valuable product and it has frequently been considered a subject for adulteration by different manufacture practices worldwide (1). The chemical composition of honeydew honey is different from the floral type, concerning pH, mineral substances content and sugar profiles (3). In spite of the fact that most scientists consider that raw honey is the most suitable variant of honey for the consumers to eat, in time, the heating of honey has become a practical method to prevent the post-bottling crystallization, thus nowadays during the processing, honey is usually submitted to thermal treatment (4, 10). The main degradative compound of heated honey is considered to be hydroxymethylfurfural (HMF), the process of HMF formation comprising hexose dehydration especially at a pH value of 5 or even lower, or through Maillard reaction (5, 6). HMF is an indicator of heat stress to food products during processing, its toxicological nature being still uncertain. In high concentrations, HMF is cytotoxic, causing irritation to eyes, upper respiratory tract, skin and mucous membranes (7, 8, 11). Honey composition is very important in significance considering HMF formation. In this investigation, we determined the most common properties of floral honey such as: water content, total acidity, mineral elements and HMF, examining meanwhile the influence that the thermal treatment has on the HMF content of honey, revealing its final quality.

MATERIALS AND METHODS

The samples were collected in number of 10, from different producers all over the southern area of our country, and the vegetation of the selected areas is mainly composed of mixture of various plants, like linden, acacia, sunflower and several forest trees. The samples were introduced in glass jars and stored at +4°C until analysis.

The total acidity, mineral substances content and water content were investigated according to International Standards.

HMF content was analyzed using HPLC method. From each sample, 1 g was selected and introduced in 9 ml of distilled water. This mixture was centrifuged at 10,000 rpm for 15 minutes, in order to remove the fine debris that might be present in the sample. The obtained supernatant was filtered using a membrane of 0,5 mm and 25 ml of filtrate substance was introduced in HPLC column, where the mobile phase comprised a mixture of methanol and water. The HMF quantity was determined by using an external calibration curve, and the detector for HPLC was set at 280 nm.

The thermal treatment comprised different temperature values, from 50 to 100°C, (50, 75 and 100°C) for a 15, 30, 45 and 60 minutes, cooled and analyzed afterwards. The samples were introduced in thermal treatment tubes and hermetically sealed, being in duplicate. There have been included a total number of 240 tubes, due to the number of 10 types of honey samples, and for each temperature, we have included a duplicate of samples for each period of time. After heating, the samples were cooled at 4°C.

RESULTS AND DISCUSSION

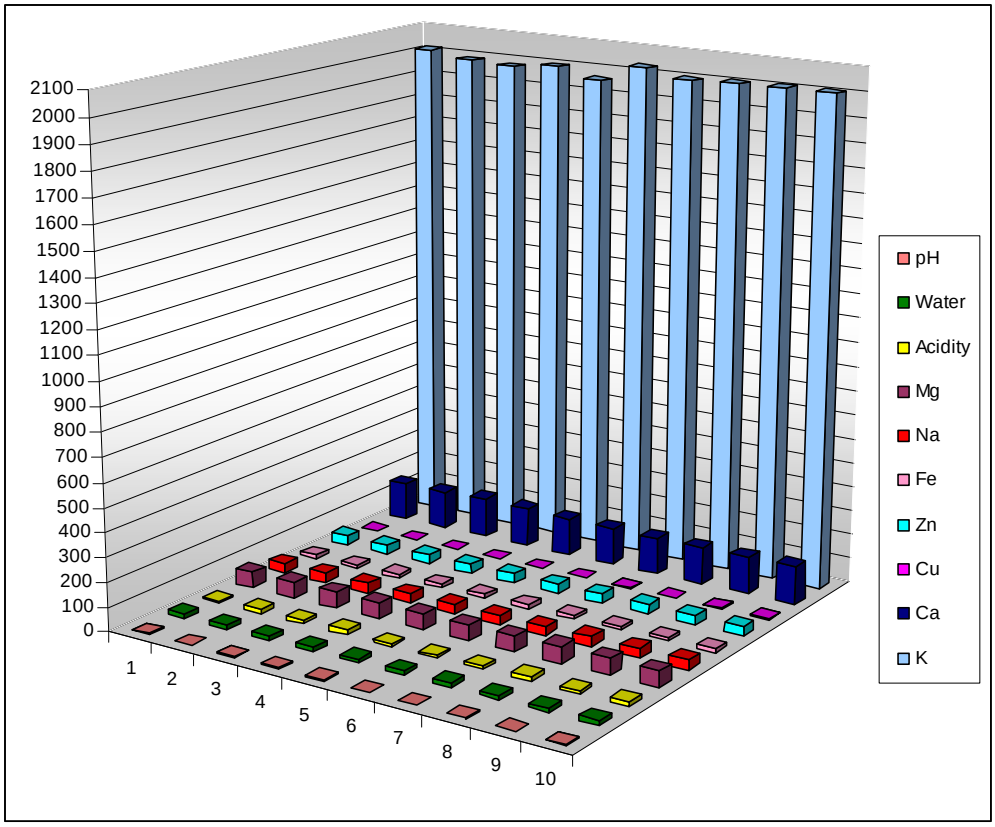
The chemical characteristics of the honey samples included in the study are comprised in Table 1 and Fig.1.

Table 1
The chemical characteristics of honey samples

Sample	pH	Water	Acidity	Mineral content						
				K	Ca	Mg	Na	Fe	Zn	Cu
No.	-	(%)	(mmol/ kg^{-1})	(mg/kg^{-1})						
1.	4,2	19,01	8,23	2012,45	157,59	65,61	40,32	15,54	39,80	2,02
2.	3,7	19,16	16,44	1986,78	155,48	66,01	41,55	15,01	38,62	1,98
3.	3,6	16,87	17,36	1980,23	165,12	67,23	40,98	16,17	38,94	1,64
4.	4,1	16,52	21,49	1997,65	160,23	67,51	39,47	15,89	37,62	2,05
5.	4,0	17,98	10,84	1959,45	154,15	66,32	38,65	15,81	39,05	2,03
6.	4,4	16,45	8,50	2029,60	153,64	65,98	38,12	15,67	39,12	2,01
7.	3,5	16,13	13,38	1997,92	150,23	64,85	37,25	15,64	39,56	2,10
8.	3,8	17,81	19,05	2004,59	151,87	68,56	39,91	15,73	38,67	2,09
9.	3,4	17,35	11,79	2007,69	152,84	66,54	39,76	16,04	38,42	2,05
10.	3,5	19,41	15,84	2006,74	156,65	65,40	38,67	15,59	39,09	2,06

Taking into account the first parameter, the pH, sample no. 6 presented the highest value, of 4.4, meanwhile the lowest one was registered to sample no.9. Considering the water content, in percentage values, sample no.10 presented the highest value, of 19.41, and the lowest was characteristic to sample no.7, with a value of 16.13. Sample no.4 had the highest value of total acidity, 21.49 mmol/kg^{-1}, and sample no.1, the lowest, 8.23 mmol/kg^{-1}. The potassium content, was correlated with a 2029.6 mg/kg^{-1} value for sample no.6, and with 1980.23 mg/kg^{-1} for sample no.3. Meanwhile, the calcium quantity was of 165.12 mg/kg^{-1} for sample no.3 and of 150.23 mg/kg^{-1} for sample no.7. The magnesium content was 65.4 mg/kg^{-1}(the lowest) for sample no. 10 and of 68.56 mg/kg^{-1} for sample no.8. The highest value registered for sodium content was for sample no.2, with 41.55 mg/kg^{-1} and the lowest for sample no.7, of 37.25 mg/kg^{-1}. The minimum value for iron quantity was 15.01 mg/kg^{-1} for sample no.2 and the maximum was registered for sample no.3, of 16.17 mg/kg^{-1}. The zinc was quantified at 37.62 mg/kg^{-1} in sample no.4 and 39.8 mg/kg^{-1} for sample no.1, while for sample no.3 the copper content was 1.64 mg/kg^{-1} and 2.1 mg/kg^{-1} for sample no. 7. In other words, sample no. 1 presented the highest value for zinc content, and the lowest for total acidity; sample no.2 had the highest content of sodium and the lowest considering iron; sample no.3 had the highest values for calcium and iron content and the lowest for potassium and copper; sample no.4 presented the highest values of for total acidity and the lowest for zinc content; sample no.6 had the highest value for pH and for the potassium content; sample no.7 had the highest content of copper and zinc and the lowest value for calcium, sodium and water content; sample no. 8 registered the highest value of magnesium content, while sample no.9 the lowest pH and sample no.10 presented the highest content of water and the lowest in magnesium.

Fig. 1 – The chemical characteristics of honey samples – graphic and values

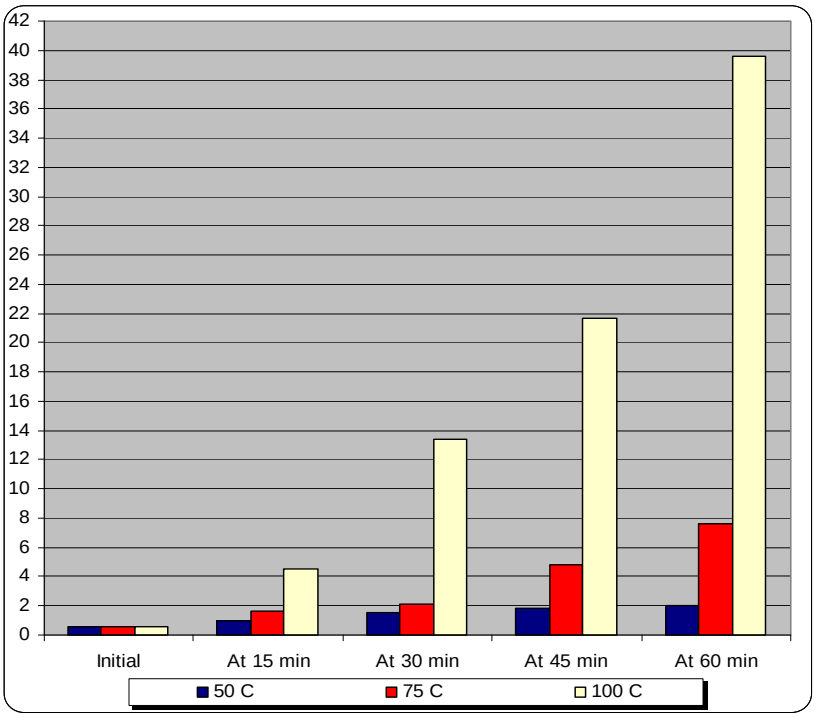


The HMF content in samples is included in table 2 and fig. 2.

Table 2
The formation of HMF in different types of honey samples at different temperatures (mg/kg^{-1}) (n = 10)

Time (min)	Heating temperatures		
	50 (^{o}C)	**75** (^{o}C)	**100** (^{o}C)
Initial	0,54	0,54	0,54
15	1,01	1,67	4,52
30	1,57	2,11	13,41
45	1,82	4,83	21,67
60	2,04	7,64	39,61

Fig. 2 – The formation of HMF in different types of honey samples at different temperatures (mg/kg^{-1}) (n = 10) – graphic and values



In what concerns the HMF content, the highest value can be observed at samples heated for 60 minutes, these increasing at the same time with temperature value. After investigating and obtaining the data for HMF content, the main results are that at a temperature of 50°C, no matter the time used for heating, the HMF content is stable, within limits of 0.54 initially to 2.04 after thermal processing for 60 minutes. At a temperature value of 100°C HMF content is already 4.52 mg/kg^{-1} after 15 minutes, 13.41 mg/kg^{-1} after 30 minutes, 21.67 mg/kg^{-1} after 45 minutes and 39.41 mg/kg^{-1} after one hour.

CONCLUSIONS

Due to the fact that honey is subjected to a thermal treatment in order to prevent post-bottling crystallization, the hydroxymethylfurfural content increases, due to the heating process at temperatures within the range of 50 to 100°C. The data show that HMF content is directly related to the temperature value used for thermal processing of honey and less with the processing time, because honey heated at 50°C for 60 minutes has an HMF content of 2.04 mg/kg^{-1}, in comparison to 39,61 mg/kg^{-1} for honey heated at 100°C for the same period of time.

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RESEARCH CONCERNING THE MOISTURE CONTENT AND VISCOSITY OF ROMANIAN HONEY STORED AT DIFFERENT TEMPERATURES

ANCA MARIA GALIŞ, I. ȚOGOE,
L. TUDOR, I.L. ILIE, MITRĂNESCU Elena
* Faculty of Veterinary Medicine Bucharest

Abstract
The moisture content is the quantity of water contained in a material. In order to see how the moisture content of honey and the storage temperature affect the final quality of honey, we measured the viscosity of 5 types of floral honey, specific to the southern part of Romania. We started by measuring the initial water content, and afterwards we increased this parameter to 20 %, 25 %, 30 % and 35 %. Each type of processed honey was stored at different temperatures: 20 ^{o}C, 25°C, 30 ^{o}C, 35 ^{o}C and 40°C. The results showed variable values concerning this parameter, with ranges from 0,581 to 20,165 Pa-s, and the conclusion is that viscosity is definitely influenced by moisture content, temperature and even its botanical origin.

Keywords: floral honey, moisture content, temperature, viscosity.

Honey with a very high quality presents a low water content. If the water content is greater than 19%, honey will ferment and lose its freshness, because raw honey (unpasteurized) contains yeast strains, that produce fermentation, this producing a high acidity, deteriorating the honey.(1, 3, 7) Besides that, honey has the property of being very hygroscopic, being able to easily absorb moisture from the air.

Viscosity is considered one of the most important characteristics of honey, being able to affect the final quality of this product (2,6). The importance of this parameter is high especially in the sector of honey production, starting from the extraction stage and ending with the packing one (8, 9).

The optimum temperature for honey storage is 10-16°C. Some researchers consider that the ideal temperature for crystallization is 14°C, so their recommendation is to store honey at 20-23°C (4, 5). Meanwhile, at low temperatures crystallization is slowed down. Nevertheless, honey quality decreases with increasing the temperature, the HMF content increasing as well, while the enzyme activity decreases (10).

The main objective of this study was to investigate the effect of moisture content and storage temperature, related strictly to viscosity, on the final quality of honey destined for human consumption.

MATERIALS AND METHODS

Five raw types of honey were studied, namely linden, forest, acacia, sunflower and polifloral honey. The samples were obtained directly from the producers, and not from the market. The weight of each original honey sample was approximately 1000 g.

The water content of the samples was measured with a digital 2522 HHR-2N refractometer and afterwards, the refractive values were converted to moisture contents.

Each sample's viscosity was measured initially at the original moisture content, and after this step, an amount of distiller water was added to each sample, in order to increase the water content value to 20 %, 25 %, 30 % and 35 %. The viscosity was measured with a Schott rotational viscometer, which is equipped with an incorporated LCD that shows the viscosity in mPas. In order to analyze the honey at the maximum precision, the samples of 50 ml each were heated at 45°C for 2 h, in an incubator, to remove entirely the air bubbles and the crystals that were already formed. After 24 hours, viscosity was measured at 20 ^{o}C, 25°C, 30 ^{o}C, 35 ^{o}C and 40°C, for each of these storage temperature values, a number of 10 samples was used, in duplicate.

RESULTS AND DISCUSSION

The initial moisture content of honey samples is included in table 1. From the obtained data, acacia honey seems to have the highest value concerning moisture content – 18,7 % – while polifloral honey had the minimum from all five types of samples, only 16,2 %.

Table 1
The initial moisture content (average value) of the samples (at the initial amount of sample taken directly from the producer)

Sample	Moisture (%)
Linden honey	16,7
Forest honey	15,6
Acacia honey	18,7
Sunflower honey	18,1
Polifloral honey	16,2

Concerning the viscosity data (showed in table 2 and revealed in figure 1), we analyzed it by applying different predefined moisture content values of 20 %, 25 %, 30 % and 35 %, much above the normal moisture content values that the honey shows. Correlated with the moisture content values, we analyzed the viscosity as a function of the storage temperature, by applying 5 different values of this parameter: 20 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35 ^{o}C, and 40 ^{o}C.

The results showed that the values of viscosity (measured in Pascal-seconds – Pa s) were comprised in ranges from 25,654 Pa s measured for linden honey, with a moisture content of 20 % and stored at a temperature value of 20°C, to 0,431 Pa s, for polifloral honey, with a moisture content of 35 % and stored at 40°C. These results show that the viscosity decreases during the increase of temperature and moisture content, so its fluidity is raising.

Table 2

The values for viscosity (Pa s) for the different types of analyzed honey, as a function of moisture content at different temperatures

	Moisture (%)	20 °C	25 °C	30 °C	35 °C	40 °C
Linden	20	25,654	20,628	14,627	8,147	4,632
	25	16,549	11,498	8,961	6,321	2,617
	30	10,625	8,624	6,375	2,746	1,587
	35	5,458	3,726	1,834	1,003	0,627
Forest	20	24,857	19,362	13,384	7,612	2,034
	25	18,521	12,327	6,519	3,631	1,981
	30	10,627	8,629	3,337	2,006	1,527
	35	5,321	4,218	2,994	1,089	0,942
Acacia	20	21,628	18,569	15,627	7,615	4,216
	25	17,684	14,623	10,289	5,912	3,378
	30	12,458	8,746	6,210	4,252	2,080
	35	8,667	5,941	3,319	2,113	1,001
Sunflower	20	18,510	15,812	10,917	6,819	3,397
	25	15,519	12,157	7,142	4,618	2,178
	30	11,258	8,449	4,617	2,176	1,127
	35	7,027	3,227	1,394	0,687	0,887
Polifloral	20	18,006	14,176	10,914	6,701	3,803
	25	14,611	12,113	7,517	3,515	1,567
	30	8,887	6,941	4,283	1,275	1,492
	35	5,127	3,934	1,307	0,560	0,431

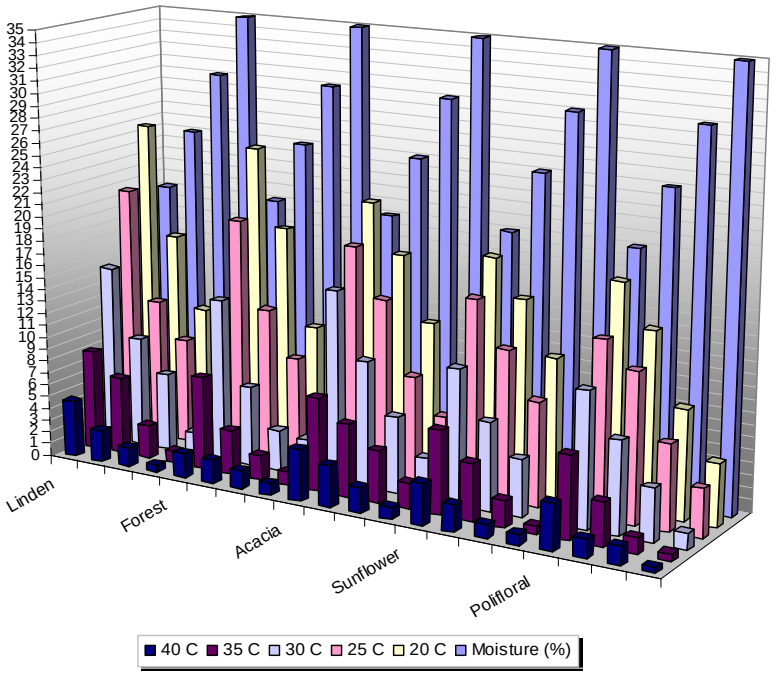

Fig. 1 – Viscosity values of different types of honey when moisture content was artificially modified and different temperature values were applied

CONCLUSIONS

1. The moisture content of honey is influenced directly by its botanical origin. The highest moisture content of non-processed honey was observed for Acacia honey (18,7 %) and the lowest one was obtained for polifloral honey.
2. The viscosity of honey varies with temperature and moisture content, and indirectly by its botanical origin. The viscosity of linden honey was definitely higher than the viscosity shown for the rest of the honey types. The lowest values for viscosity were obtained for polifloral honey, no matter the storage temperatures applied.
3. Even though polifloral honey had a specific moisture content of 16,2 %, its values for this parameter were increased artificially and its viscosity data were also the lowest from all 5 species investigated in this study. On the other side, even though its original moisture content was only 16,7 %, linden honey presented the highest viscosity values of all.

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AUDIT-SYSTEMATIC AND INDEPENDENT EXAMINATION OF EFFECTIVE IMPLEMENTATION OF SANITATION PROGRAMS

MAGDA GONCIAROV[1], R. POPA[2], L. TUDOR[1], DOINA LUPU[2], ELENA MITRĂNESCU[1]

1)U.S.A.M.V.,Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei, District 5, Bucharest, 050097, magdagonciarov@yahoo.com

2)Sanitary Veterinary Direction and for the Food Safety,

Summary

The audit is the systematic and independent examination to determine whether the activities and results comply with planned programs are implemented effectively and are suitable for meeting targets.

The audit is a comprehensive assessment of a food processor .The audit is conducted by one or more auditors , who are competent to perform audits .One of the auditors shall be appointed as chief auditor of the audit team leader .If necessary , make the team with expert technical knowledge of the area of activity audited .

Key words:food safety audit, inspection, HACCP, risk

The audit plan includes areas/activities audited, the date scheduled audit functions of the system to be audited, the applicable reference documents (audit criteria).

During the audit findings will be communicated to the audit during the audit in order to avoid further misunderstandings.

The findings should be written, must coincide with the requirements. Inspections are unannounced, audit is announced. The audit is directed towards continuous improvement.(2)

Audit work of good hygiene practices shall verify that food business operators apply procedures continuously and properly concerning at least :

- checks the information food chain;
- design and maintenance of facilities and equipment;
- personal hygiene;
- training on procedures and hygiene;
- pest-control:
- water quality
- temperature-control;
- checks on entry and exit of food / and the undertaking and any accompanying documents.

The nature and intensity of auditing tasks for each unit are based on estimated risk. In this respect, the competent authority shall evaluate periodically:

- the risks to public health and , where appropriate , animal health ;
- if slaughterhouses , animal welfare issues ;
- type of processing performed and results ;
- previous record of the food business operator in respect of legislation on food . (1)

European Commission experts may carry HACCP system in order to verify food business operators apply these procedures permanently and properly. They determine in

particular whether procedures provide guarantees to the possible extent, that products are of animal origin:

- comply with microbiological criteria laid down in Community legislation;
- are in accordance with Community legislation on residues, contaminants and prohibited substances;
- no physical risk such as foreign bodies. (4)

The implementation of audit tasks competent authority shall in particular:

- to ensure that all stages of production , on personnel and its activities in the unit complies with relevant requirements of Community law .To complete control , the competent authority may conduct proficiency checks to ensure that, in terms of skills , staff meet the parameters specified ;
- to verify all relevant records of food business operators ;
- to take samples for laboratory analysis , if necessary ;
- to justify the items considered and the audit findings.

European Commission, general and specific audits in all Member States , mainly aimed at checking if official controls are carried out according to control plans (The National Framework for Inspection and Control) and whether the food legislation regarding feed is respected .

If the checks are carried out in Romania ,The National Sanitary Veterinary and Food Safety experts to ensure all necessary assistance to fulfill their duties. The European Commission will inform the National Sanitary Veterinary and Food Safety results of the checks.

If a seriously risk to animal health is identified during an audit or an inspection of the European Commission, The National Sanitary Veterinary and Food Safety Authority must immediately take all necessary measures to protect animal health. If these measures are not taken or if they are deemed insufficient, the European Commission, in accordance with comunitary procedure, adopt measures necessary to protect animal health and inform the National Sanitary Veterinary and Food Authority on this. (3)

Documentation required for audit farm classification requires consideration of existing documents in the unit:

- veterinary authorization issued (original);
- control-notes, evaluation sheets following previous inspections;
- recommendations ,sanctions or other measures officially arranged;
- official results of sampling;
- commercial/economic documentation (contracts/invoices/lists suppliers/distribution lists ,etc…).

Documents shall specify the amount and results of activities ,including data useful new risk assessment and new planning. For all controls, including controls ad hoc or emergency, there must be papers on : the type of control , control results and the measures / improvement.

In addition to general requirements concerning audits for good practice in terms of hygiene, the official veterinarian must ensure that the food business operator times its own procedures for each collection, transportation, storage, handling, processing and use or disposal of animal by-products animal origin, including specific risk materials are the responsibility of food business operator. Besides the general requirements of the HACCP-based audits the official veterinarian must ensure that operators provide assurances that procedures where possible, the meat:

- doesn’t present pathologically abnormalities or deterioration;

- does not contain specified risk material, unless this is provided by laws, and were produced in accordance with Community legislation in the TSE.(5)

If it meets the duties of the inspection, the official veterinarian must take into account the results of audits performed .It is appropriate, if necessary ,he shall identify the inspection duties accordingly.

The official veterinarian must monitor and analyze relevant information from the registers holding of origin of animals for slaughter and to take into account the results of this check and justified grounds of this analysis when performing ante-mortem and post mortem. If it meets the duties of the inspection , the official veterinarian must take into account official certificates accompanying the animals and any statements of veterinarians engaged in the primacy production controls , including the official veterinarian. If the food business operators involved in the food chain take additional measures to reassure on food safety , by independent third parties or by other means and, where such measures are detailed enough and the animals included in these systems can be identified with certainty, the official veterinarian may consider all this in the duties of inspection and verification procedures based on HACCP system.

Steps for conducting the audit are :

- opening session;
- audit of the departments;
- documented findings and discuss of them with audited;
- meeting of auditors;
- closing meeting;
- drafting the audit report.

Audit team leader has the final decision on how to conduct the audit and the formulation of the findings, prepare audit report, coordinate the work of auditors;

Auditors:

- make/create audit objectively, in accordance with established audit plan and moral principles;
- review with the audit to obtain concrete evidence, so that the objectives and sufficient to assess the functionality of the HACCP system.

It is necessary to establish a program of audits, and frequency o f audits should take into account:

- size of the organization audited and technological features;
- the number of items or groups of products obtained;
- risk group of products;
- number of non-compliance of the previous audit.

The auditors found that the procedures, work instructions and other documents are known, available, understood and applied by the staff of the organization.

Requirements for auditors:

- to have a spirit of observation;
- to adapt to different situations and personal being audited;
- to be strong, balanced, tenacious, diplomatic, with moderation;
- to have critical thinking;
- to be familiar with the audit criteria;
- know-assessment audit process;
- be familiar with HACCP principles and activities;
- to assess whether the steps are properly implemented HACCP system;
- how to assess whether monitoring of the CCP is appropriate;

- to identify and make non-conformity;
- to make some recommendations;
- to make comment on the findings. (6)

CONCLUSIONS

1. The audit is a comprehensive assessment of a food processor. The audit is conducted by one or more auditors, who are competent to perform audits. One of the auditors shall be appointed as chief auditor of the audit team leader. If necessary, the team is completed with an expert in technical knowledge of the area of activity audited.

2. The audit represents systematic and independent examination to determine whether the activities and results comply with planned programs and if these programs are implemented effectively and are suitable for meeting targets.

3. European Commission, general and specific audits in all Member States, mainly aimed for checking if official controls are carried out according to control plans (egg The National Framework for Inspection and Control) and whether the legislation on products food is respected.

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OFFICIAL CONTROL-CONTROL METHOD FOR CHECKING COMPLIANCE WITH FOOD LAW

MAGDA GONCIAROV[1], R. POPA[2], L. TUDOR[1], DOINA LUPU[2], ELENA MITRĂNESCU[1]

1)U.S.A.M.V.,Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei, District 5, Bucharest, 050097, magdagonciarov@yahoo.com

2)Sanitary Veterinary Direction and for the Food Safety,

The official controls should be carried out without prior warning, except audit work where is required prior notice to the operator for animal food domain or the food sector. Also, official ad-hoc controls may be made.

The official controls are carried out in any of the stages of production, processing and distribution of animal food or feed and animal products.

Official controls have to be performed using appropriate control methods and techniques such as: monitoring, supervision, checking, auditing, inspection, sampling and analysis.

Key words:food, control, audit, risk, monitoring

The official control represents any kind of control made by the competent authority or the European Commission and EU member states, to verify compliance with food law.

Must ensure that official controls are carried out regularly, based on a risk analysis and an appropriate frequency, given by:

1) Identified risks associated with animals, animal food, feed, business activity in animal feed, food business enterprises, use of feed or animal food or with any process, material, substance, activity or operation that can influence animal food or feed security, health or animal welfare;

2) Previous records of animal food or feed operators, on compliance with legislation in the feed or food law or the rules of health and animal welfare;

The reliability of the already done self checks;

Any information that may indicate non-compliance. (5)

Official controls should be carried out without a prior warning, except audit activities, where the prior notice of the food or feed operator is necessary. Also, official checks may be made ad hoc.

Official controls are carried out in any of the stages of production, processing and distribution of animal food or feed and animal products.

These should include checks on business activity in the feed and food business, regarding the use of feed and animal food, food and feed storage or any process, substance, activity or operation including transport feed or food and live animals. Competent authorities for performing official controls should meet a number of operational criteria in order to ensure their impartiality and effectiveness. They should have enough staff, with adequate qualifying and experience and to possess adequate facilities and equipment to properly perform their duties.

Competent authorities should ensure that, in cases in which the official controls requires the participation of different control units, they apply and effectively implement appropriate coordination procedures. Competent authorities should also ensure that, in cases in which the competence of performing official controls has been delegated from a central level to a regional level, between the central and the regional or local level exists an efficient and effective coordination. (1)

Laboratories participating in the analysis of official samples should operate in accordance to certain internationally approved procedures or rules based on performance criteria and to use analysis

methods that were considered valid, as far as this was possible. These laboratories should provided especially with equipments that allow the correct determination of the standards, like the maximum levels of residue set by legislation. Also, the national and community reference laboratories should contribute to achieving a high level of quality and consistency of analytical results. This objective can be achieved through activities such as application of validated analytical methods, the availability of reference materials, organizing comparative testing and forming laboratory staff. There should be proper procedures for cooperation of the competent authorities of a member state and of different member states, especially where official controls reveal that food issues concern more than one member state. In order to facilitate this cooperation, member states should assign one or more liaison bodies with the purpose of coordinating sending and receiving requests for assistance.

Official controls on products of animal origin should include all matters that are important in the protection of public health and, where appropriate, animal health. These controls should be based on the most recent relevant information and therefore should be possible to adapt them in light of new relevant information available. Official controls on meat production are necessary in order to verify that food business operators and inspections, including checks of controls even operators.

Member states shall ensure that food business operators provide all necessary assistance to ensure effective implementation of official controls by the competent authority. The competent authority shall carry out controls to ensure that food business operators comply with the requirements of Regulation (EC) no. 852/2004, Regulation (EC) no. 853/2004 and Regulation (EC) no. 1774/2002. (4)

The competent authority in Romania as a destination, can control the authorities responsible to its food compliance with food law through discriminatory controls. For reasons strictly necessary for the organization of official checks, the competent authority may require operators who have their goods that were supplied form the European Union member states to report the arrival of such goods.

If during a check destination or during storage or transport in Romania, the competent authority finds non-compliance, it may take steps which may include return of European Union member state of origin.

The competent authorities must ensure:

- the effectiveness and appropriateness of official controls on food in all stages of production, processing and distribution;
- that those carrying out official controls are out of any conflict of interest;
- that they own or have access to adequate laboratory capacity for testing and staff suitability qualified and experienced enough so that official controls and control responsibilities to be carried out efficiently and effectively;
- that they have facilities and equipment maintained in proper condition to ensure that staff can perform official controls efficiently and effectively;
- that they have legal powers to carry out official controls and to take appropriate action;
- that they have contingency complained place and ready to implement such complaints the situation of need;
- that food business operators are obliged to undergo any inspection carried out and assist staff in carrying out its authority.

The competent authorities must Ensure that all their staff performing official controls:

a) receive, for competence, appropriate training allowing him to perform competently and to carry out official duties consistently.

b) inform the purpose and days to follow, if necessary, additional training regularly;

c) provide skills for multidisciplinary cooperation. (3)

The official checks shall be performed using appropriate control methods and techniques, such as: monitoring, supervision, checking, auditing, inspection, sampling and

analysis. Monitoring is achieving planned sequence of observations or measures in order to obtain a general assessment of the level of compliance with food law;

Supervision is a careful examination of one or more businesses with food business operators to work food or their activities. Monitoring means observing the detail of various aspects of products and units which fall within the competence of ANSVSA - DSVSA, following the incidence of potentially having impact on food safety.

Verification is control, by examination and consideration of evidence, in terms of meeting the requirements specified. Verification is a method of testing by examining objective evidence and analysis on legislative requirements.

The audit is the systematic and independent examination to determine whether the activities and results comply with planned programs and these programs are implemented effectively and are suitable for meeting targets.

Inspection is the examination of any aspect of feed, food, health and welfare, to verify that such matters are in accordance with the requirements of legislation on feed and food law and health and welfare rules animals.

The inspection is based on the element of surprise. If it is considered that, by explaining why the completion of the inspection can distort the result, will not communicate a particular view. Sampling for analysis is sampling of feed or food or other substances representative of the production, processing and distribution of feed or food or animal health (including environmental) in order to verified by analysis of compliance with legislation in the feed or food law or animal health rules;

Sampling methods and analysis used in official controls must comply with EU rules into national law, or:

a) if such rules do not exist, with internationally agreed rules and protocols, as those that the European Committee for Standardization (CEN) has accepted or those agreed by national legislation or

b) where those referred to above, other methods for their purpose or developed in accordance with scientific protocols.

Official controls on food should include, the following activities:

1. examining control systems that feed business operators and those with pet food business have implemented and results achieved;
2. inspection:

- primary-production facilities, business activity in feed and food business enterprises, including their surroundings, facilities, offices, equipment, facilities and equipment, vehicles, and feed and food;
- raw materials, ingredients, processing auxiliary agents and other products used in the preparation and production of feed and food;

semi-products;

- materials and articles intended to come into contact with food;
- products and processes for cleaning and maintenance and pesticides;
- labeling, presentation and advertising;

3. controls on hygiene in business activity in feed and food business enterprises;
4. evaluating Procedures for Good Manufacturing Practice (GMP), good hygiene practices (GHP), good agricultural practice and HACCP (Hazard Analysis and Critical Control Point), taking into account the use of guides established in accordance with national legislation transposing EU legislation specifies;
5. examining written materials or other records that may be relevant to assess compliance with legislation in the feed and food law;

6. interviews with animal feed business operators, with the activity in food and their staff;
7. reading values recorded by measuring instruments business activity in the feed or food business enterprises;
8. controls' own instruments made by the competent authority to verify the measurements made by operators of animal feed business and those with food business. (4)

Multiannual national control plan should contain general information on the structure and organization of food control systems, in particular on:

- strategic objectives of the plan and how to control priorities and allocating resources reflect these objectives;
- risk-classification of those activities;
- designation of competent authorities and their functions at national, regional and local levels and the resources available to these authorities;
- management and general organization of official controls at national, regional and local level, including official controls in individual companies;
- control systems applying to different sectors and coordination between various services of the competent authorities responsible for official controls in these sectors;
- where appropriate, delegated tasks to other control bodies;
- methods to ensure compliance with operational criteria;
- staff training for performing official controls;
- documented procedures;
- organization and operation of contingency Plans for emergency animal disease or in case of food poisoning due to contamination of food and other situations of public health risk;
- organization cooperation and mutual assistance.

CONCLUSIONS

1. Official control means any form of control that made him competent authority or the European Commission and EU Member States, to verify compliance with food law.
2. Responsible authorities for official controls should meet a number of operational criteria to ensure their impartiality and effectiveness.
3. Official controls must be carried out without prior warning, except audit work that is required prior notice to the operator for animal feed business activity or the food. Also, official checks may be made ad hoc. Official
4. Controls must be made in any of the stages of production, processing and distribution of feed or food and animal products.
5. Official controls must be made using appropriate control methods and techniques, such as: monitoring, supervision, checking, auditing, inspection, sampling and analysis.

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ANTIMICROBIAL SENSITIVITY OF *E. COLI* STRAINS ISOLATED FROM PIG SEPTICEMIC COLIBACILOSIS

V. HERMAN, Corina PASCU, Luminița COSTINAR, B. FAUR, Ioana VĂDUVA, Anca SURPAT, Sorina IRIMIE
Faculty of Veterinary Medicine Timişoara
Str. Calea Aradului No.119, 300645, Timisoara, Romania
E-mail: cabesti@yahoo.com

Abstract
In the paper are presented the results concerning antimicrobial sensitivity for 23 strains of E. coli type 1 isolated from pigs with septicaemic lesions.
In vitro testing by diffusimetric method for sensitivity to 12 antimicrobials (ceftiofur, colistin, amoxicillin, amoxicillin-clavulanic acid, enrofloxacin, gentamycin, penicillin, oxytetracycline, lincospectin, tiamulin + tetracycline, erythromycin, doxycicline) showed less susceptible strains and many strains resistant to tested antimicrobials.
Key words: *E.coli*, antimicrobial sensitivity.

Diseases caused by *E. coli* produce important economic losses in pigs. To institute appropriate therapy is necessary to isolate bacteria that can cause the disease and to know the behavior to therapeutic substances, for choosing the most effective (2, 4).

Antibiogram by using diffusimetric method is quick and easy to realize. Not always have the highest accuracy, but provide a rapid response to the recommended antibiotic therapy (1, 3, 4, 5).

This paper presents *E. coli* strains sensitivity to different antibiotics.

MATERIALS AND METHODS

Between 2008 and 2009 were examined piglets that provided from fattening farms from the west area of the country, which at the necropsy were reported septicaemic lesions.

Culturing was made from parenchymal organs, using the usual media. Samples were incubated 24 hours at 37°C. Identification of *E. coli* was based on cultural characters on Levin media, after passage of isolates from primary culture on the usual media.

For testing susceptibility of *E. coli* strains were used discs with antimicrobial substances produced by different companies. The discs are placed in tubes of 50 microcomprimates, each tube is specified name and quantity of the antimicrobial substance: micrograms (µg) or IU (2, 4).

Following the known principles, using diffusimetric method, was carried out antibiogram. From the liquid medium that contained pure culture, after 24 hours, using Pasteur pipette was taken a quantity of 1 to 1.2 ml, which was deposited on the Mueller-Hinton agar surface. The plate was introduced in thermostat for 15 minutes, then was put the microcomprimates on plate, and the result was read and interpreted after 24 hours of incubation at 37°C.

Studied *E. coli* strains were tested against the following antimicrobials: ceftiofur, colistin, amoxicillin, amoxycillin/clavulanic acid, enrofloxacin, gentamycin, penicillin, oxytetracycline, lyncospectin, tiamuline+tetracycline, erythromycin, doxycicline.

Results reading and interpretation consisted in inhibition zone size induced by antimicrobials, area where microbial colonies are lacking. Diameter of inhibition zone is

directly proportional to the sensitivity of that germ. Interpretation of the results was done using tables for the interpretation of the bacteria sensitivity, as specified by the supplier firms.

RESULTS AND DISCUSSIONS

Antimicrobial susceptibility testing results of the 23 strains of *E. coli* isolated are presented in table 1.

As seen from table 1 were less strains of *E. coli* sensitive to tested antibiotics. Susceptibility of *E. coli* strains to some antimicrobials is due to their recent introduction in medicine practice.

Table 1

Antibiogram results

Antimicrobials	**Results**			**No. of tested strains**
	S	**I**	**R**	
ceftiofur	9	5	3	17
colistin	3	10	3	16
amoxicillin	1	-	18	19
amoxicillin- clavulanic acid	7	5	5	17
enrofloxacin	6	6	11	23
gentamycin	5	9	9	23
penicillin	-	-	14	14
oxytetracycline	-	-	5	5
lincospectin	-	1	17	18
tiamuline+tetracycline	-	-	8	8
erythromicyn	-	-	13	13
doxycicline	-	2	10	12

Legend: S-sensitive; I-intermediate; R- resistant

For some antimicrobial that are commonly used in pig farms (oxytetracycline, lincospectin, tiamuline + tetracycline, erythromycin, doxycicline) was observed that strains showed consistently resistance.

It was noted phenomenon of multiple resistance to tested antibiotics for 15 strains of *E. coli* from the 23 isolated strain. In the literature, multiple antibio-resistance is defined as the resistance of the same strain of bacterial to four or more antimicrobials. Antimicrobial resistance is due to genetic mutation mechanisms and recombination, after frequent use of antimicrobials (2, 4, 5).

Multiple resistance of the 15 strains of *E. coli*, are as follows:

- 2 strains to 8 antimicrobials;
- 5 strains to 7 antimicrobials;
- 4 strains to 6 antimicrobials;
- 1 strain to 5 antimicrobials;
- 3 strains to 4 antimicrobials.

CONCLUSIONS

Were isolated 23 strains of *E. coli* type 1 from pigs with septicaemic lesions. In vitro testing by diffusimetric method for susceptibility to 12 antimicrobials (ceftiofur, colistin, amoxicillin, amoxicillin-clavulanic acid, enrofloxacin, gentamycin, penicillin, oxytetracycline, lincospectin, tiamulin + tetracycline, erythromycin, doxycicline) showed less susceptible strains and many strains resistant to tested antimicrobial substances. 15 strains of *E. coli*, were presented multiple resistance of antimicrobial substances.

To establish an effective therapy is recommended to be performed prior antibiogram, considering the different behavior to the studied antimicrobials.

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STUDY CONCERNING THE SEASONAL VARIATION OF CATTLE MILK PH VALUES AND THE OVERALL INFLUENCE ON MILK QUALITY

L.I. ILIE, L. TUDOR, Anca-Maria GALIŞ, Elena MITRĂNESCU, R.F. POPA
Faculty of Veterinary Medicine Bucharest

Abstract
This research had the main objective of evaluating the seasonal variations in cattle milk pH values, on a lot of 10 dairy cows, from a farm near Bucharest, an important provider of milk and milk products for the city. From the total number of cows, we selected a group of 10 cows, from which we collected one sample per week. These samples were analyzed using a multi-parameter device, designed to perform physico-chemical analysis of milk. The data that we obtained were used in statistical formulas, in order to calculate the average values per week and month, the frequency of certain interval values, and the standard deviation. The results revealed an overall pH value of 6,82. The frequency analysis showed that 41 % of the samples had values of pH of 6.8-6.9, 25 % with pH values of 6.8-6.9 and 23 % with 6.9-7.0. We also obtained an overall standard deviation value of 2,765. This last value shows the way that the values differ from the overall average value. The main conclusion is that pH values vary with weather conditions, physical status of the animals, the quality of feed and the shelter and maintenance hygiene. The pH values varied each month, with no tendency of increasing or decreasing for a larger period of time. The month of August presented the highest value, 6,9 while May the lowest 6,77. Considering the fact that fresh milk (immediately after milking) has a pH value situated very close to 7, and that this decreases in time due to processing to 6,7 (before pasteurization) and 6,6 after processing and at refrigeration temperature, the final conclusion is that the milk produced by the selected farm is suited for human consumption in any commercial form.

Keywords: milk pH, seasonal variation, standard deviation, quality.

INTRODUCTION

Milk contains a large number of buffer substances and consequently behaves as a buffer solution. Fresh cows milk has a pH value close to 7, this decreasing during raw milk refrigeration to 6.7-6.5, while for cold processed milk the values are situated between 6,59 and 6.33(1,6,8). Because milk is a buffer solution, considerable acid development may occur before the pH changes. A pH lower than 6.5 indicates that considerable acid development has taken place. This is normally due to bacterial activity. The pH measurements are often used as acceptance tests for milk (4,5).

Measuring milk acidity is an important test used to determine milk quality. Acidity measurements are also used to monitor processes such as cheese-making and yoghurt-making (2,7). The titratable acidity of fresh milk is expressed in terms of percentage lactic acid, because lactic acid is the principal acid produced by fermentation after milk is drawn from the udder and fresh milk contains only traces of lactic acid. However, due to the buffering capacity of the proteins and milk salts, fresh milk normally exhibits an initial acidity of 0.14 to 0.16% when titrated using sodium hydroxide to a phenolphthalein end-point (3, 9).

In this study, we investigated the variation of milk pH values over a ten months period of time, on a group of 10 cows in order to observe the tendency of this parameter, and to evaluate the influence on overall milk quality.

MATERIALS AND METHODS

A number of 10 dairy cows were selected and the milk samples were prelevated once a week, for an period of 10 months, selecting each month the same dates: the 5th, the 12th, the 19th and the 26th. The experimental group comprised 10 cows of almost the same age, with the same diet and bodyweight, and entering together at almost the same time in the lactation period. The milk samples were collected from each cow, one per cow each week, with a total of 4 samples/month, and 40 samples per entire experimental period/cow, with a total of 400 samples analyzed per entire period/lot.

The selection of the experimental dairy cows followed several criteria, like:

- the absence of mastitis or any kind of disease that may interfere with the milk analysis;
- a bodyweight of almost 500-550 kg;
- a milk production of 8000-10000 kg.

The samples were introduced in 50 ml plastic bottles, introduced at refrigeration and analyzed in a maximum period of 2 hours from the collection moment.

The analysis was carried out using EcoMilk Ultrasonic Analyzer, a multi-parameter calibrated device designed for rapid analysis of raw and processed milk. The samples were left for 5-10 minutes at room temperature before proceeding with the analysis, and afterwards, using an automatic pipette with a 5 ml tip, a quantity of 15 ml from each sample was introduced in the analysis tubes.

RESULTS AND DISCUSSIONS

The results showed slight variation, but increasing and decreasing from a month to another. The highest value was registered for the month of August – 6,90 – while the lowest was observed in May. In February, the pH had an average value of 6,83, this decreasing to 6,80 in March, increasing again in April, reaching 6,77 in May. June came with an increase of pH value to 6,81, in July observing again a decrease to 6,78 and in August reaching the highest value, of 6,90. For the next three month, the pH value was maintained in the same range, with 6,83 in September, 6,84 in October and 6,82 in November. All the data are shown in table no.1, fig.1 and fig. 2.

Table 1

The average values of milk pH per month and for each week

Month/Week	1	2	3	4	Monthly average
February	6,78	6,83	6,85	6,87	6,83
March	6,76	6,82	6,81	6,81	6,80
April	6,81	6,83	6,86	6,83	6,83
May	6,78	6,78	6,73	6,80	6,77
June	6,81	6,77	6,85	6,81	6,81
July	6,81	6,8	6,72	6,81	6,78
August	6,84	6,89	6,92	6,95	6,90
September	6,91	6,86	6,73	6,82	6,83
October	6,86	6,81	6,81	6,89	6,84
November	6,89	6,82	6,81	6,78	6,82

Fig. 1 - The average values of milk pH per month

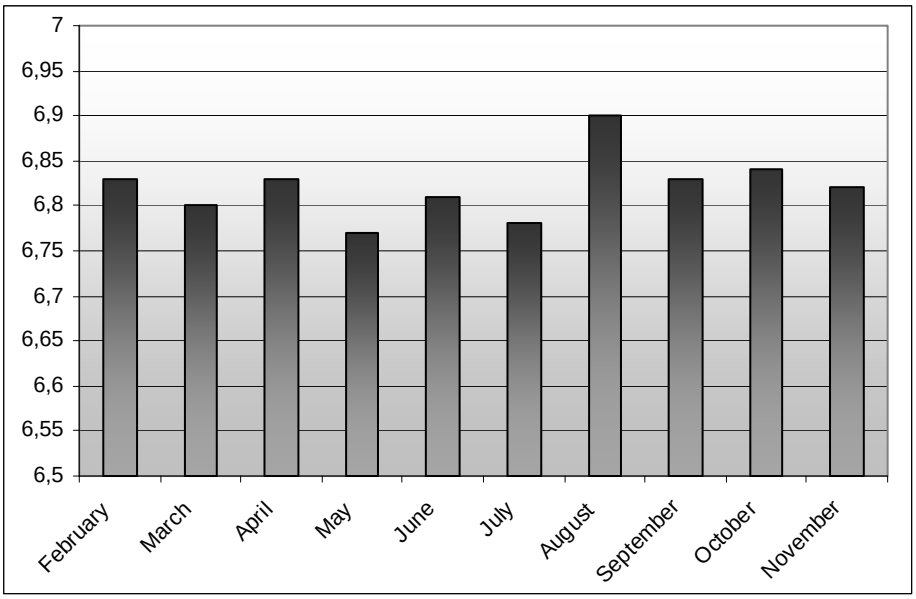
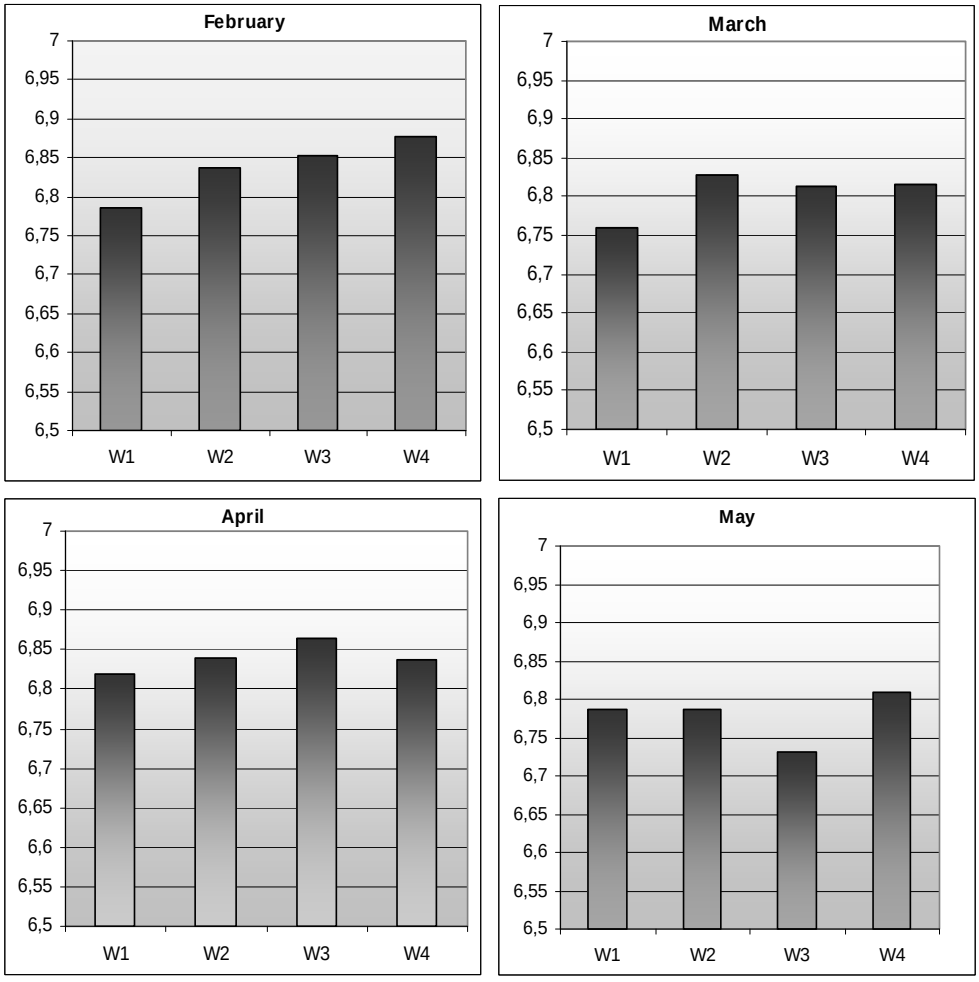
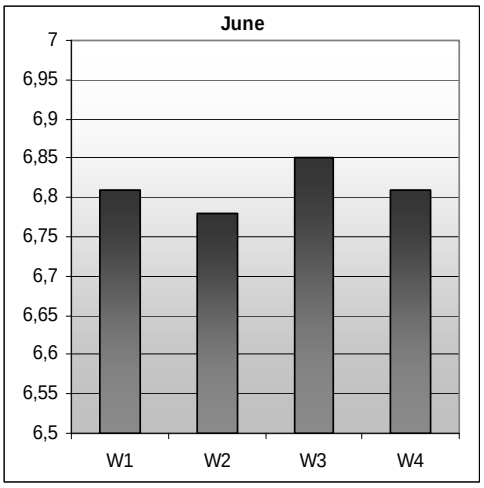
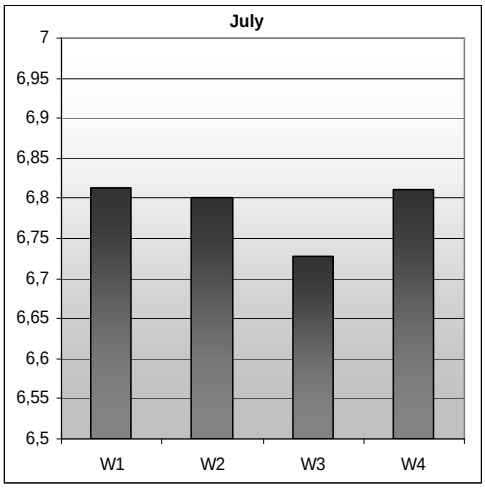


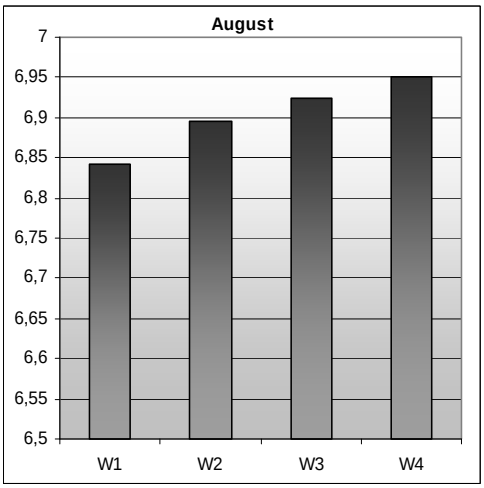
Fig. 2 - The average values of milk density per each month and week

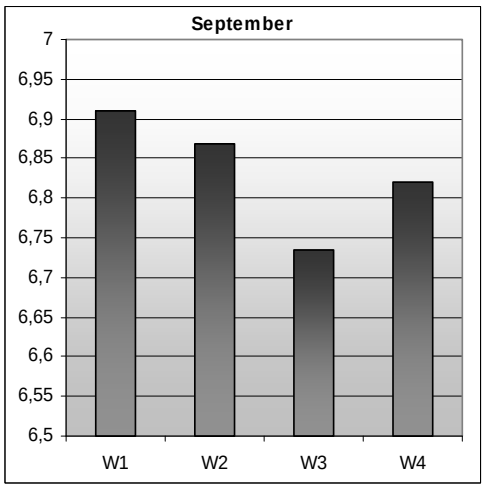


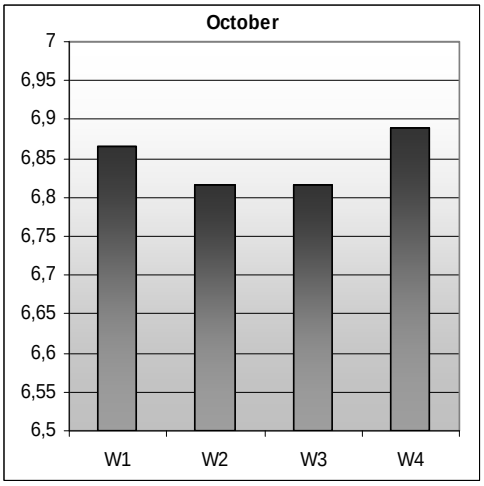


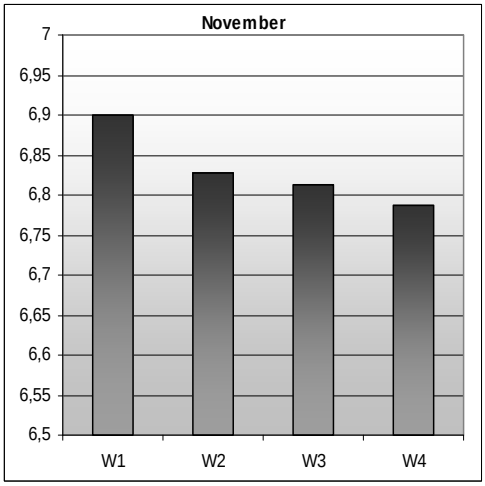


Considering in-month variation, the average values presented an increase tendency from week 1 to week 4 in February and August and a decrease tendency in November, with mixed tendency during the rest of the months. Except for the month of September, when between week 2 and week 3 the difference is higher than in the rest of the cases, the variation presented slight slopes.









From the total number of samples, 164 (41 %) presented values between 6,8 and 6,9, 99 samples (25 %) had values between 6,7 and 6,8, and 90 samples (23 %) had values of 6,9-7,0. From the rest of the milk samples, 40 (10 %) presented values of 6,6-6,7 and only seven (2 %) had values of 6,5-6,6. These data are included in table 2, fig. 3 and fig. 4.

Table 2

The frequency of the milk pH values
(number of samples and percentage for each category)

Month/Value category	6,5-6,6	6,6-6,7	6,7-6,8	6,8-6,9	6,9-7,0
February	0	4	8	17	11
March	3	6	10	15	6
April	0	4	10	14	12
May	3	6	13	17	1
June	1	3	12	21	3
July	0	4	18	10	8
August	0	1	2	15	22
September	0	3	8	23	6
October	0	3	8	17	12
November	0	6	10	15	9
Total	7	40	99	164	90
Percentage	2%	10%	25%	41%	23%

Fig. 3 – The number of samples for each category of frequency, per month of analysis

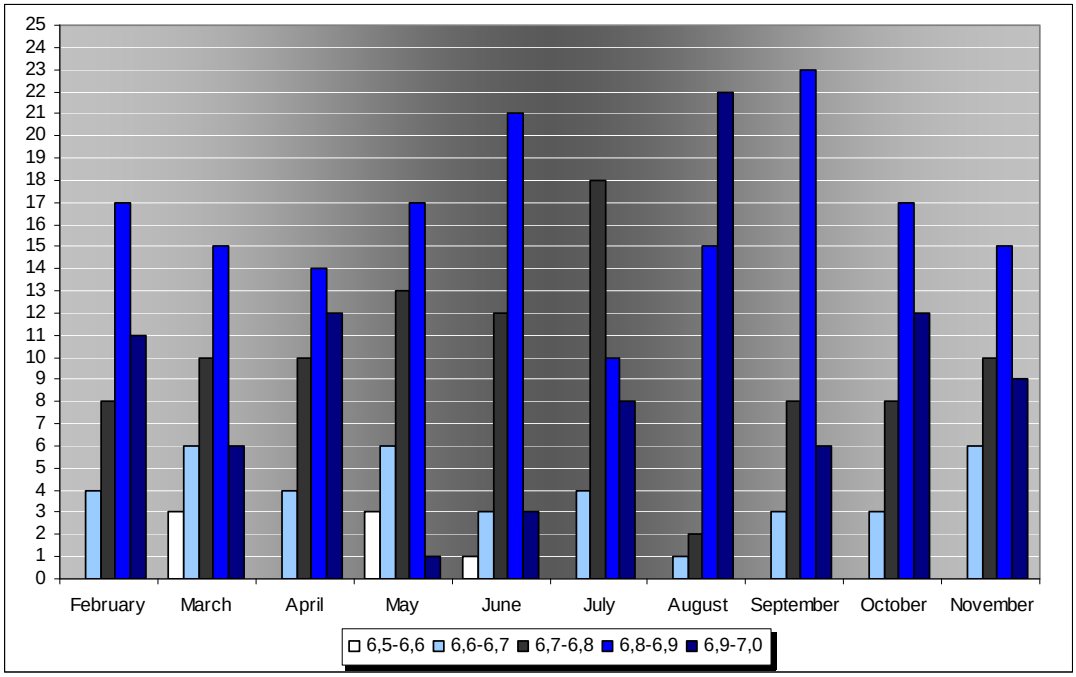
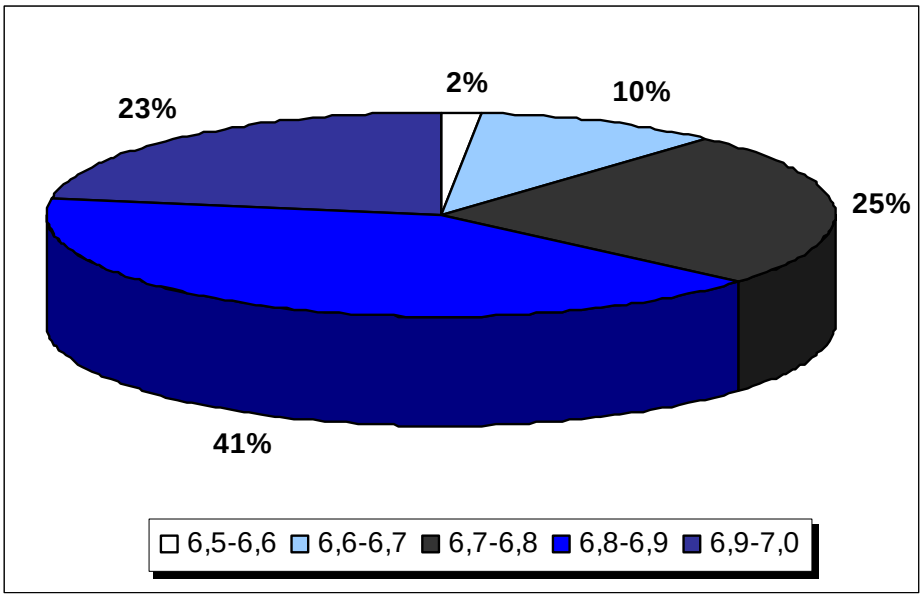


Fig. 4 – The overall pH values percentage for the number of samples included in each category of frequency

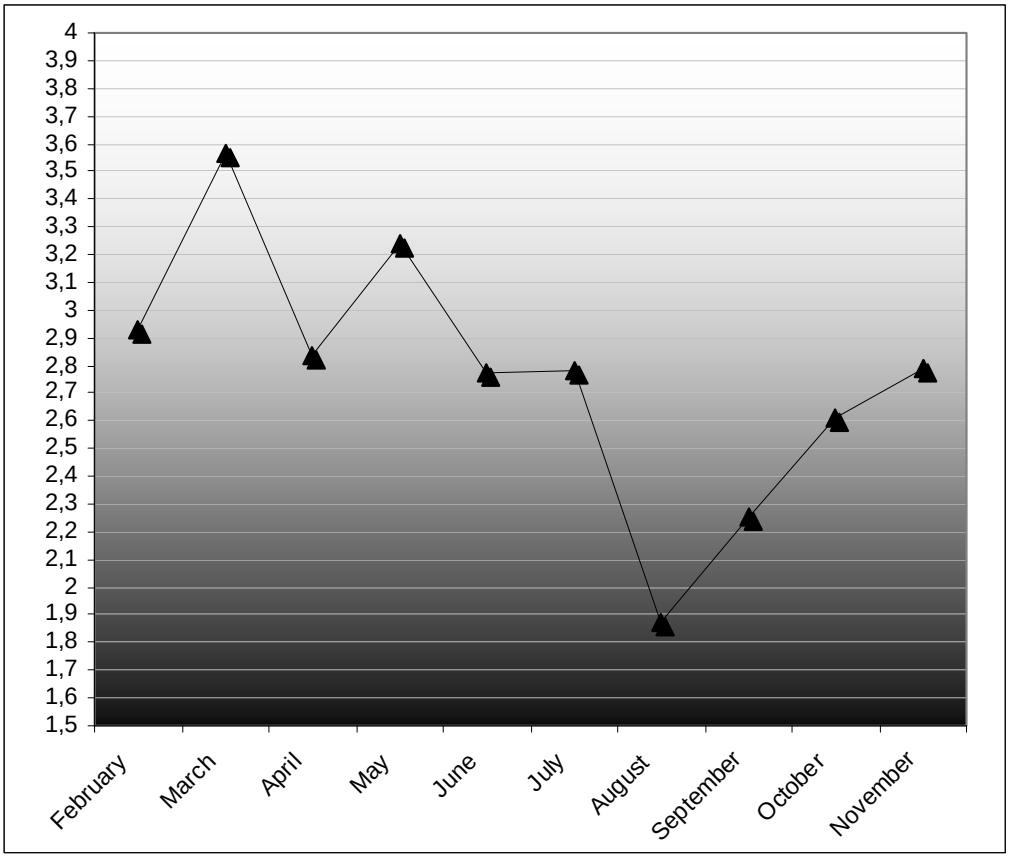


Considering the standard deviation of the values we obtained, this specific statistic indicator presented range values from 0,0202608 to 0,04552472, as shown in table 3. The lowest value of this indicator was observed in August, correlated with the highest monthly pH average value, of 6,90, while the highest value of standard deviation was observed in March. The slightest difference was observed during the months of June and July. From February to June, the tendency was different, from an increase in February to a decrease in May, while between July and August the decrease that we registered was very abrupt. The tendency of increment was observed only in the latest four months, from August to November.

Table 3
The standard deviation values for milk pH values

Month/Statistic indicator	Standard deviation of milk pH values
February	2,932138725
March	3,56432848
April	2,8384745
May	3,23874011
June	2,77415528
July	2,779826883
August	1,873966382
September	2,252429244
October	2,61092656
November	2,78796521
Average per period	2,765295137

Fig. 5 – The standard deviation graphic for dairy cows milk density values for a period of 10 months



CONCLUSIONS

Milk pH is considered a very important physico-chemical trait, among others like added water, superficial tension, viscosity, freezing point, conductivity and density, being an instrument of milk quality evaluation. Fresh milk presents a pH of 7 (immediately after milking), and in time this value tends to decrease to 6,7 (after refrigeration) and even 6,5 after processing and refrigeration storage. For fresh milk, like the one included in the samples of this study, a pH of 6,5 or lower indicates a high bacterial load, with acid development. In comparison to this value, only a few of the samples presented values between 6,5 and 6,6 (7 samples) and 10 % of the samples values between 6,6 and 6,7, this indicating a possible non-detected subclinical mastitis. The lowest value registered overall was 6,51. Of course, through processing and refrigeration storage, the bacterial load reduced to minimum, this milk not being considered in the end a threat to human health.

Overall, the frequency of the samples with optimal pH values (over 6,7) was of 88 % (41 % between 6,8-6,9, 25 % between 6,7-6,8 and 23 % of them with values of 6,9-7,0). From the quality control point of view, these results may be considered appropriate, and the milk may be therefore used after processing in many ways for human consumption.

Also, the standard deviation showed values which can take us to the conclusion that milk pH values do not vary abruptly over time, thus milk composition being influenced by the weather conditions, feed quality, maintenance conditions and shelter hygiene. To sum up, the

pH values cannot be considered as dispersed in a high degree, with normal variations and optimal differences from a period to another.

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RESEARCH CONCERNING THE SEASONAL VARIATION OF CATTLE MILK DENSITY VALUES

L.I. ILIE, L. TUDOR, Anca-Maria GALIŞ, Elena MITRĂNESCU, F. FURNARIS
Faculty of Veterinary Medicine Bucharest

Abstract
In this study, we collected and analyzed a series of milk samples, in order to observe the variation of the density, during a period of ten months. We selected a group of ten cows, from which we collected one sample/week. The milk samples were analyzed using a multi-parameter device, designed to perform physico-chemical analysis of milk. After obtaining the data, the average formulas were used in order to observe the variation of the average values per each week during a month of study and per month for the entire period. The monthly average values had ranges between 1,0280 and 1,0295. The frequency analysis revealed the fact that only 51 % (a number of 201) of the samples were considered "appropriate" based on the milk standard value ranges of 1,029 to 1,031. Also, using the data already obtained for this parameter, we calculated the standard deviation. The results showed that the difference from the average value increases during the month of transition from winter to spring, decreases when the dairy cow organism has adjusted to the new weather conditions, increased over the last months of spring and during the summer and autumn it presented a reduction. Only during the last month, we observed again an augmentation.

Keywords: milk density, seasonal variation, quality, standard deviation.

INTRODUCTION

Among other physico-chemical characteristics of milk, density has proven to be one of the main indicators of milk adulteration during the last century. Milk density is a species trait, being situated as general values between 1,026 and 1,034. Cattle milk density comprises values of 1,029 and 1,031, through adulteration by adding water, this value having a tendency of decreasing to 1 (1, 4, 6).

Milk density is generally used for: converting the volume into mass and vice-versa, estimating the solid content of milk (in some situations) and calculating other physical properties, like viscosity (9, 10). Density values depend on: the temperature at the time of measurement, the temperature history of milk (storage temperature), the composition (especially the fat content) and the inclusion of air (a problem when analyzing viscous milk products) (2, 3, 7, 8).

The main objective of this study was to observe the variation of the density values for a period of 10 months, by analyzing the milk coming directly from the selected dairy unit with a calibrated multi-parameter device, once a week.

MATERIALS AND METHODS

A number of 10 dairy cows were selected and the milk samples were prelevated once a week, for an period of 10 months, selecting each month the same dates: the 5th, the 12th, the 19th and the 26th. The experimental group comprised 10 cows of almost the same age, with the same diet and bodyweight, and entering together at almost the same time in the lactation period. The milk samples were collected from each cow, one per cow each week,

with a total of 4 samples/month, and 40 samples per entire experimental period/cow, with a total of 400 samples analyzed per entire period/lot.

The selection of the experimental dairy cows followed several criteria, like:

- the absence of mastitis or any kind of disease that may interfere with the milk analysis;
- a bodyweight of almost 500-550 kg;
- a milk production of 8000-10000 kg.

The samples were introduced in 50 ml plastic bottles, introduced at refrigeration and analyzed in a maximum period of 2 hours from the collection moment.

The analysis was carried out using EcoMilk Ultrasonic Analyzer, a multi-parameter calibrated device designed for rapid analysis of raw and processed milk. The samples were left for 5-10 minutes at room temperature before proceeding with the analysis, and afterwards, using an automatic pipette with a 5 ml tip, a quantity of 15 ml from each sample was introduced in the analysis tubes.

RESULTS AND DISCUSSIONS

The results showed different variations during the experimental period of time, with lower values during the spring and summer months, and higher ones during autumn. For the month of February, the average value of density was 1,0280, this increasing to 1,0282 and remaining at that point for the next two months. In May, we observed an increase to 1,0284, possibly due to the changing of season, this consisting of higher temperatures, green pasture feeding and a different physical status of the animals. In June, the values increased again, reaching a high value, of 1,0294, while in July we have a lower value, of 1,0290. In August, the value increases to 1,0295. In the autumn months, starting with September, the value is maintained to 1,0295. In October, since the weather changes abruptly and the temperatures are lower, the value decreases to 1,0294. Again, in November there can be observed an increase, to 1,0295, due to the fact that the dairy cows have succeeded in adjusting and preserving the physiological parameters, and maintaining an optimal milk production, with physico-chemical properties that suit the standards. All the data are shown in table no.1, fig.1 and fig. 2.

Table 1

The average values of milk density per month and for each week, for the 10 dairy cows group selected for analysis

Month/Week	1	2	3	4	Monthly average
February	1,0286	1,0287	1,0275	1,0273	**1,028**
March	1,0309	1,027	1,0275	1,0277	**1,0282**
April	1,0279	1,028	1,0284	1,0287	**1,0282**
May	1,0276	1,0283	1,0294	1,0285	**1,0284**
June	1,0293	1,0296	1,0297	1,0293	**1,0294**
July	1,0293	1,029	1,0292	1,0286	**1,029**
August	1,0293	1,0293	1,0298	1,0298	**1,0295**
September	1,0293	1,0299	1,0293	1,0295	**1,0295**
October	1,0291	1,0299	1,0294	1,0295	**1,0294**
November	1,0298	1,0292	1,0295	1,0297	**1,0295**

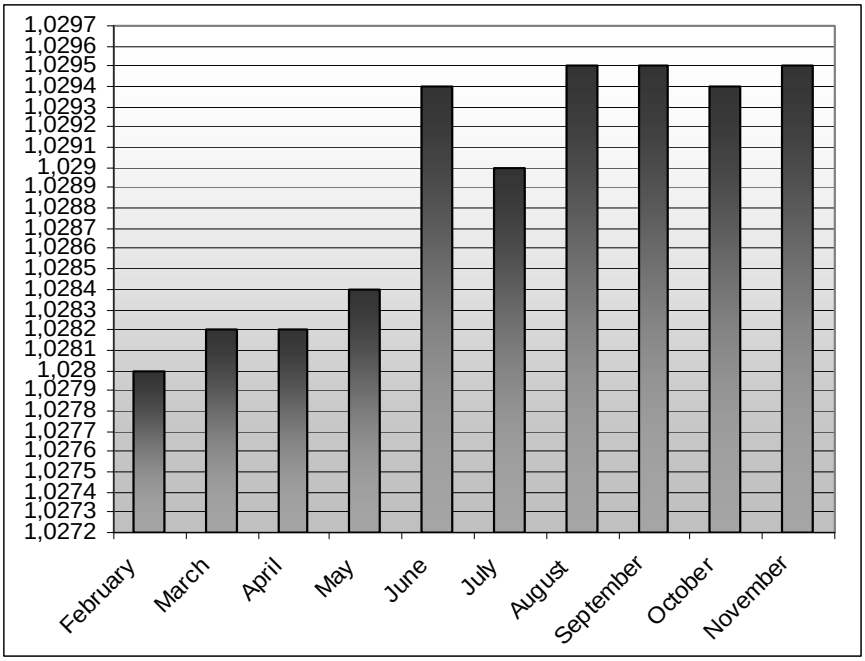
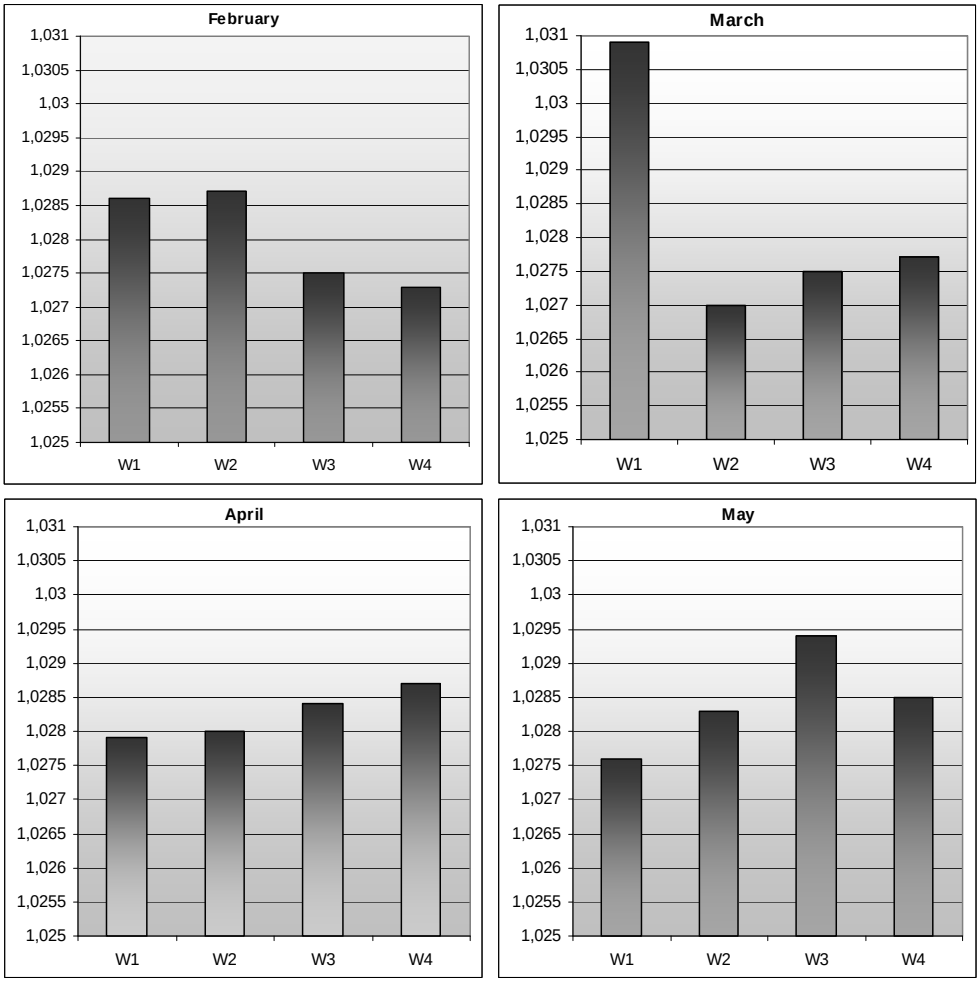


Fig.1 - The average values variation of milk density per month



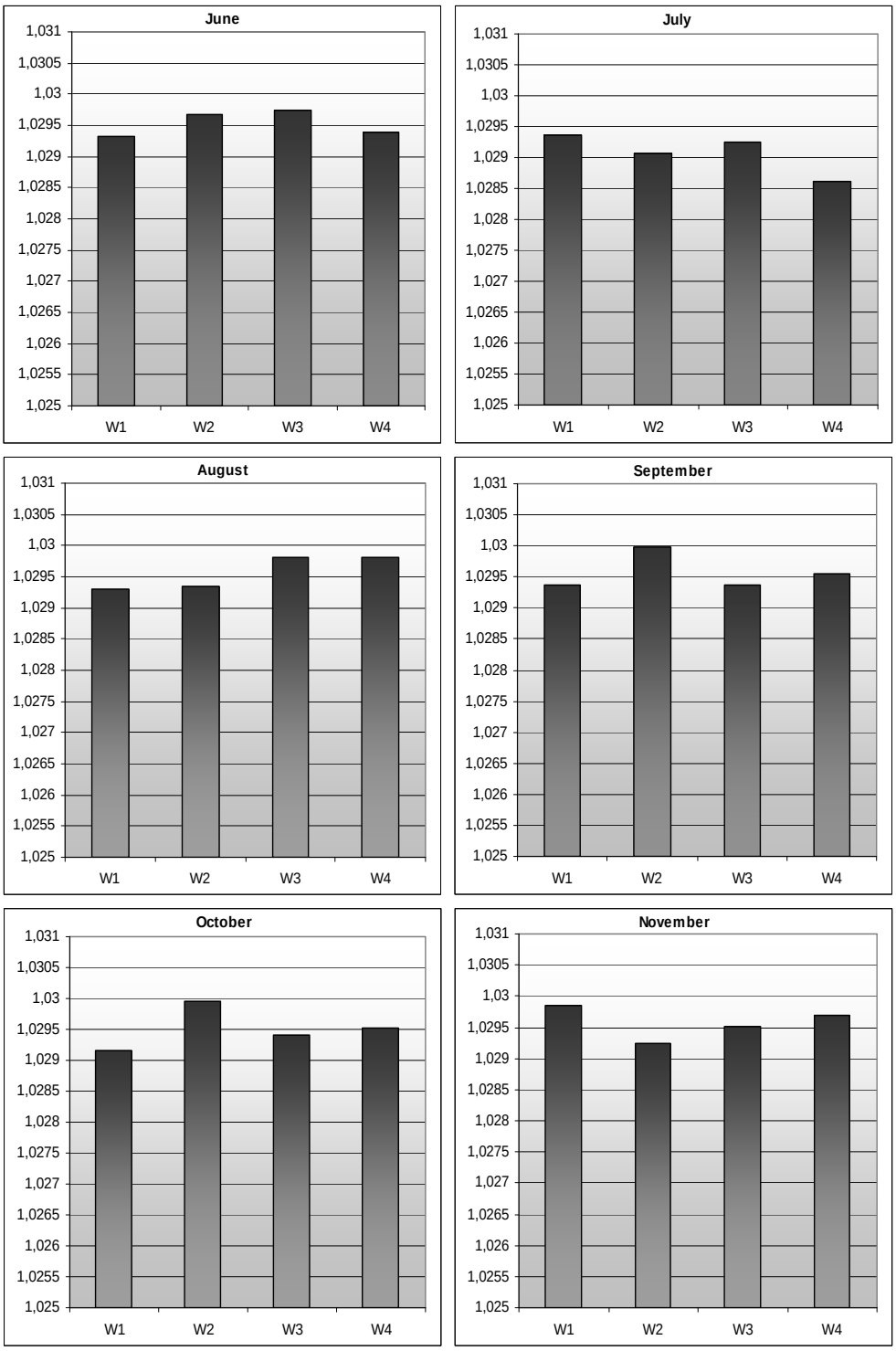


Fig. 2 - The average values of milk density per each month and week, for the 10 dairy cows group selected for analysis

Considering the standard values, in table 2 we included the results for the frequency of the values that we obtained. From the data, we can see that from the total number of 400 samples collected and analyzed during the 10 months experimental period, a number of 27 samples had values between 1,026 and 1,027 (7 %), 57 of them were between 1,027 and 1,028 (14 %), a number of 115 had values between 1,028 and 1,029 (29 %). From 400 samples, only 201 (51%) could be categorized as “framed” in the standard, as the values of density for cow milk are situated between 1,029 and 1,031, therefore, a number of 118 samples had values

between 1,029-1,030 (30 %) and 83 of them presented values of 1,030-1,031 (21 %), as comprised in fig. 3 and 4.

Table 2

The frequency of the milk density values obtained for the 10 dairy cows group selected for analysis (number of samples per each category)

Month/ Value category	1,026-1,027	1,027-1,028	1,028-1,029	1,029-1,030	1,030-1,031
February	11	7	16	2	4
March	9	13	9	2	7
April	1	16	15	6	2
May	5	8	13	11	3
June	1	5	7	11	16
July	0	4	18	10	8
August	0	4	8	14	14
September	0	0	10	20	10
October	0	0	11	20	9
November	0	0	8	22	10
Total	27	57	115	118	83

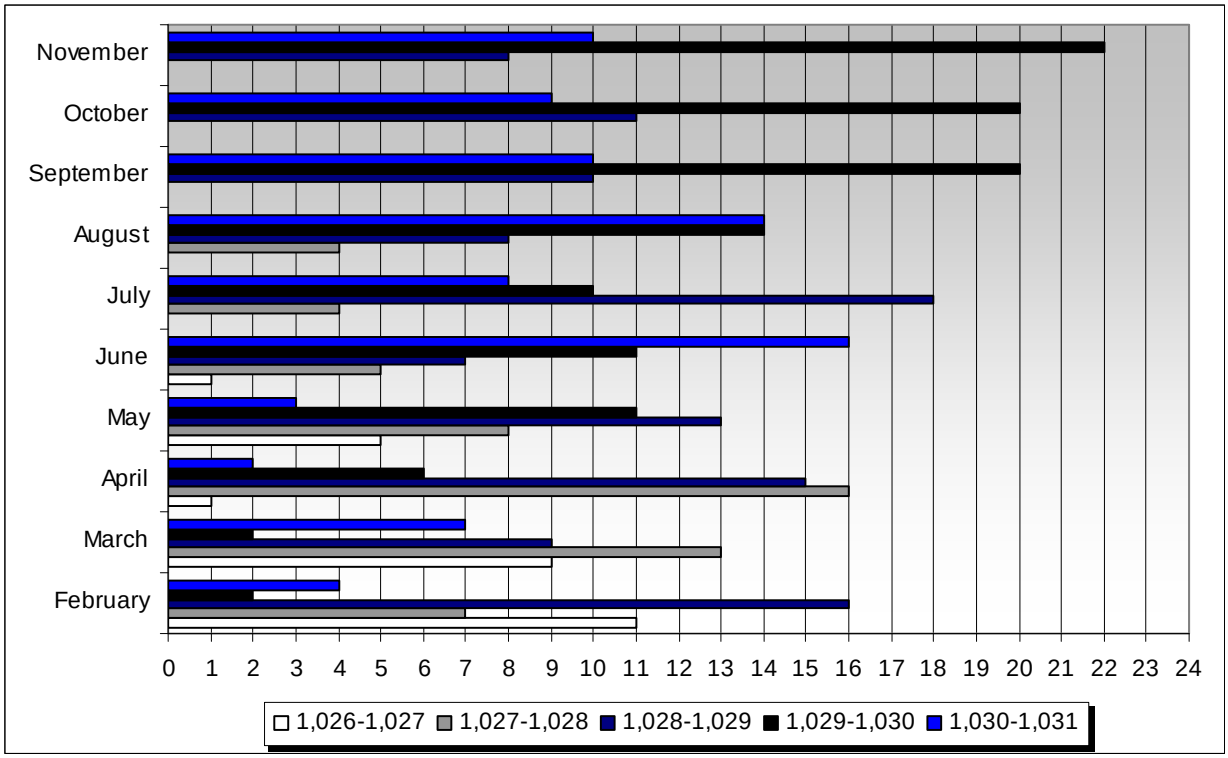


Fig. 3 – The number of samples for each category of frequency, per month of analysis

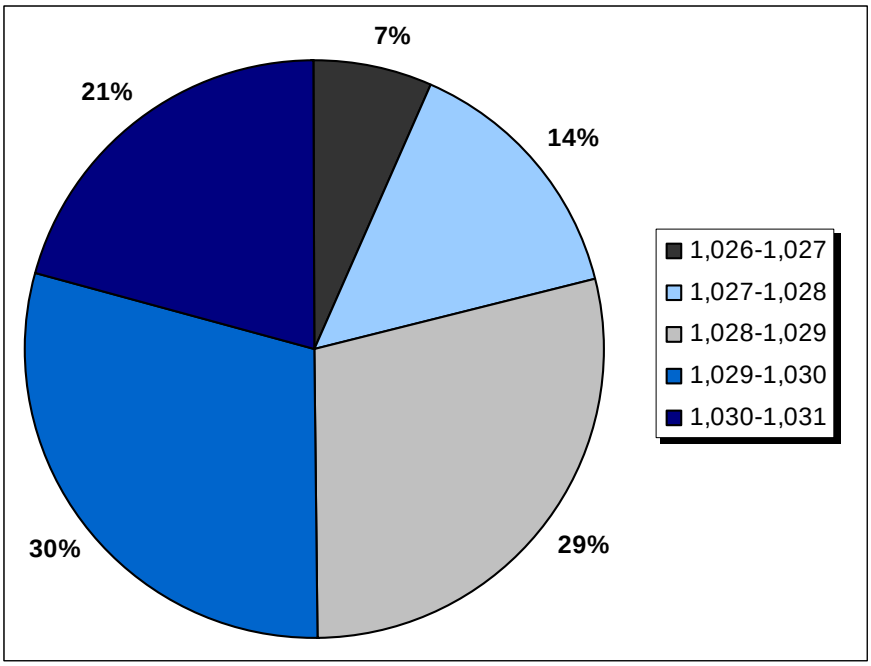


Fig. 4 – The percentage for the number of samples included in each category of frequency

Considering the standard deviation of the values we obtained, this specific statistic indicator presented range values from 0,0202608 to 0,04552472, as shown in table 3. The lowest value of this indicator was registered in October, while the highest one was observed in March. The latter is considered a transition month considering the seasons, from winter to spring, while October may be regarded as a month when the specific weather conditions of autumn may contribute to the body preparation for winter.

Table 3

The standard deviation values for milk density over the 10 months of experimental analysis

Month/ Indicator	Standard deviation
February	0,038577843
March	0,04552472
April	0,02714314
May	0,03405877
June	0,03669809
July	0,030951575
August	0,030430248
September	0,021130547
October	0,0202608
November	0,02304344
Average per period	0,030781917

Considering the differences from a month to another, we observed a major decrease from March to April, possibly due to the spring specific weather conditions and the transformations that occur in the organism. The changes considering the feed may also contribute in a higher or lower degree. Over the next months, from April to June, we registered an augmentation of the standard deviation value, and from June to October, a continuous reduction of this indicator. This is a very good indicator when taking into account milk standards, therefore observing a growth of the quality of milk based on the milk density values and the standard deviation calculated for them. As for the last month of study, an increase was registered, showing us that the diary cows are preparing for winter conditions and also by consuming another type of feed, with specific changes in the composition of milk and properties of milk.

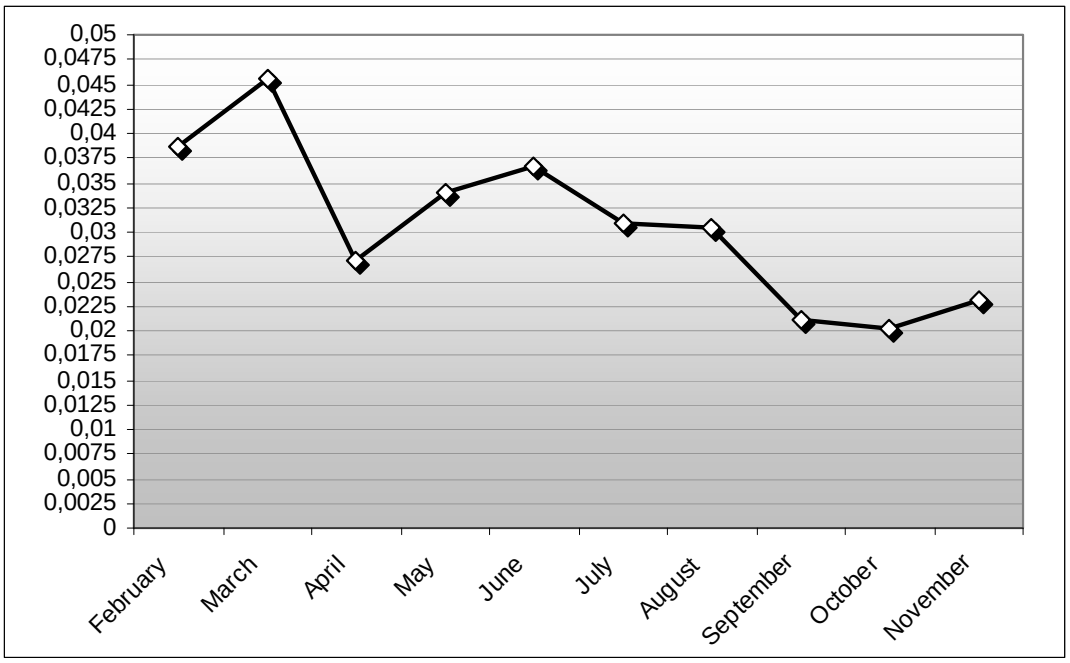


Fig. 5 – The standard deviation graphic for dairy cows milk density values for a period of 10 months

CONCLUSIONS

Milk density represents a valuable parameter used as an indicator when analyzing the quality of milk. In this study, we used it also as an indicator for the milk quality variations registered during several seasons of the year. Monthly average values varied in a range of 1,0280 to 1,0295, showing us a lower overall value of milk density than the standards require, taking into account that the minimum standard value of this parameter is 1,0290. Also, we calculated the value frequency, observing that only 51 % (or 201) of the samples could be considered within the ranges of the milk standards for quality. And by calculating the standard deviation of these values, for a better interpretation of the seasonal variation, we observed that milk samples vary within ranges of 0,0202608 to 0,04552472, the monthly values increasing during the period when dairy cows organism needed an adjusting to the weather conditions and the changes in feed, and decreasing (being closer to the standard value) during the periods of time when the parameters of the dairy cows organism maintained the same pattern.

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A STUDY ON THE BEHAVIOR OF BROWN BEAR (URSUS ARCTOS) FROM ZOOLOGICAL GARDEN TIMISOARA

C. Fl. LĂZĂRESCU[1], Adina BAIAS[1], M. AFRENIE[2]
[1]Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Calea Aradului, 119, 300645 Timisoara, Romania
[2]Zoo Garden Timisoara
e-mali:cristi_lazarescu@yahoo.com

ABSTRACT
The realization of the ethogram in order to estimate the level of well-being is represented by the establishment of the inventory of all the activities that form the behavioral repertoire of the species that is under observation of the behavioral flux. The structure of the behavioral patterns is genetically determined. The captivity affects most the mechanisms that release or that guide the typical actions of the species and to a lesser extent their shape [2, 3]. In order to fully understand and correct the interpretations of the observations it is necessary for the ethologist to understand the normal behavior of the studied animals.

***Key words**: brown bear, behavior, ethogram, zoo garden.*

INTRODUCTION

The aim of this study is the elaboration of an ethogram of the specimens of brown bear (Ursus arctos) that live in captivity at the zoo in Timisoara.

According to the Constitution of the European Association of Zoos and Aquaria (E. A. Z. A.), the zoos are permanent institutions. They are open to the public and they are publicly managed in order to promote nature conservation and in order to ensure the education, information and relaxation of the public, by presenting and conservation the life of wild animals. Ensuring the demands concerning the manifestation of the natural behavior of the animals from the zoos represents a very complex and difficult to attain objective [6].

Assessment of the welfare of animals is very difficult and there are several reasons why we affirm this; firstly, because animal welfare represents the state of the individual concerning his trial to cope with his its environment. From therein, there are different levels of welfare to conspecific individual.

The realization of the ethogram in order to estimate the level of well-being is represented by the establishment of the inventory of all the activities that form the behavioral repertoire of the species that is under observation of the behavioral flux. The structure of the behavioral patterns is genetically determined. The captivity affects most the mechanisms that release or that guide the typical actions of the species and to a lesser extent their shape [2, 4]. In order to fully understand and correct the interpretations of the observations it is necessary for the ethologist to understand the normal behavior of the studied animals.

MATERIALS AND METHODS

In order to draw up the ethogram for the specimens of brown bear (Ursus arctos) that are in captivity at the zoological garden from Timisoara, their behavior has been structured on three different categories: the solitary behavior, the behavior related to their feeding and their social behavior.

Every category implies a series of behavioral patterns. In order to quantify the way in which the animal spends its time it is compulsory for the studied animals to be observed at least six hours [6]. The animals (a male and two females) were under observation for a period of 60 minutes, ten minutes per hours, for six hours. The behavior of the animals was noted, timed and framed in the adequate behavioral pattern. During the observation were noted the atmospheric conditions and the possible unusual situations. The animals were under observation between 7^{40} and 24^{00}. Within the behavioral repertoire at the brown bear the first category is represented by the behavioral activities such as sleeping, rest, performing body hygiene, swimming, exploring the environment, urination, defecation. Another category describes the behavior related to food. This entails the feeding behavior (food consumption), the food-searching behavior and the drinking behavior. The social behavior refers to the action of an individual of coming closer to another at a distance of less than three meters. It also includes the playful behavior and the aggressive behavior. The playful behavior means engaging in an activity of interaction without an aggressive character with other individuals as well as other activities that express a relation of closeness between two or more individuals.

RESULTS AND DISCUSSIONS

Three individuals were under observation: a male (M) and two females (F1, F2). As for the sleeping behavior, 17 periods of sleeping were noted, at different hours, for seven days. In the first day of observation, a single period of sleeping was noted to the F1 female between12^{40}-12^{50}. In the second day two periods of sleep were noticed around 8 o'clock and from 21^{40}. In the third day five periods of sleeping were noticed at: 7^{45}-7^{55}, 14^{30}-14^{40},15^{25}-15^{35}, 22^{15}-22^{25}, 22^{50}-23^{00}. In the fourth day three periods of sleeping were noticed around 8 o'clock and 22 and 23. Between 22 and 23 (at 23 observations were closed) the sleep was uninterrupted. In the fifth day three periods of sleep were noticed between 10^{20}-10^{30}, 23^{00}-23^{10} and 23^{45}-23^{55}. In the last of observation, observations were made between 7^{40} and 18^{40}. Two sleep intervals were noticed between 7^{40}-7^{50} and 9^{50}-10^{00}. As for the rest intervals, we can say thy alternate with the sleeping periods. Most of the rest intervals were noticed between 11^{30} and 15^{30}.

Observations show that animals like to sleep and rest all at the same time. Nine times out of the 17 sleep intervals that were noticed animals were sleeping at the same time but not in the same place. In seven cases two animals were seen sleeping in the same time and in two cases two animals were active. The same thing can be noticed in the rest period cases. During the observations 11 rest intervals were noticed and in seven cases the three animals were resting at the same time. As for terrestrial locomotion, during the observation 42 sequences of the same behavioral pattern were noticed. When animals came at a distance shorter than three meters this fact was registered separately because it entails a certain motivation. From the observations the male manifested this behavior towards both females. Within the 41 sequences, the male showed this behavior in 16 cases as well as the F1 female while F2 female showed this behavior in nine cases.

As for the swimming locomotion and bathing, observations show that animals go in the pool quite often. The pool is used for swimming, bathing and playing. Animals were seen playing in the pool but they were also seen using different objects such as wooden pieces or tires. The playful behavior was also seen outside the pool. Animals were seen together which proves the existence of close relationships among the individuals. Moreover, non aggressive manifestations were noticed during the observation. As for the exploration behavior, it is well represented. It has different forms of expression and different motivations. Animals were noticed when searching for their food: they dug holes and this behavior is quite known to this omnivorous species. The searching food behavior is also well represented during the observations. It is followed by the actual food consumption. The eating intervals were noticed all over the day with a greater intensity between 12^{00}-20^{00}. The male was noticed eating after 22^{00} o'clock.

Table 1

Ethogram model for the brown bear (*Ursus arctos*)

Type of behavior	Behavior	Behavior code	Description of the behavior
I. Solitary behavior	Body hygiene	**Ig**	Animals make the body hygiene (they arrange their fur, they lick their fur)
	Sleep	**S**	The animal adopts specific positions for sleep and does not react to environmental changes
	Rest	**O**	The animal stays still but reacts easily to environmental changes
	Locomote	**L**	The animal moves from one place to another (terrestrial locomotion)
	Swim	**Î**	The animal makes moves in the water
	Defecation	**D**	Adopting the characteristic position and the effectuation of defecation
	Urination	**U**	Adopting the characteristic position and the effectuation of urination
II. Food related behavior	Eat	**H**	The animal eats the food from his habitat
	Search for food	**C.H.**	The animal investigates his habitat in order to find food
	Drink	**A**	The animal drinks water or other liquids found in his environment
III. Social behavior	Play	**J**	The animal interacts with other individuals in activities such as: locomotion, searching, playing with objects, interactions without an agressive character with other individuals from the same species, other activities that express a relation between two or more individuals
	Closeseness	**Ap**	An individual draws closer than 3 meters to another
	Agressive	**Ag**	The animal engages in a physical conflict with another animal from his environment

CONCLUSIONS

- We can conclude on the basis of our observations that all the three individuals of brown bear behaved normally within the behavioral pattern of the species;
- No stereotypical movements and no aggressive manifestations were noticed;
- The behavioral patterns related to the exploration of the environment as well those related to playful behavior prove that the individuals are close to each other;
- In order to establish a time interval for each sequence from the behavioral repertoire of the species longer periods of observation as well as video camera use are necessary.

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RESEARCHES REGARDING IMPLEMENTATION OF A COMPUTERIZED SURVEILLANCE PROGRAM OF MAMMARY GLAND IN A DAIRY COW FARM

Sorana Teodora Matei, I. Groza, L. Bogdan, Simona Ciupe, Anamaria Petrean, Sanda Andrei

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
Faculty of Veterinary Medicine

ABSTRACT: *The purpose of this study was to supervise the activity and health of cow mammary gland, in a unit specialized in milk production, and implementation of a computerized surveillance program of mammary gland. The researche was carried out during 2009-2010 on a total of 489 cows. The activity and health of cow mammary gland was performed using the most advanced management software worldwide for dairy farms, AfiFarm program and Bovi-Vet indicators.Cows diagnosed with subclinical mastitis were treated with 3 therapeutic protocols: Masti Veyxym, Synulox LC and Mastiker E. After monitoring the activity of the mammary gland, were identified a number of 29 cows (5.93%) who noticed an increase in elctrical conductivity, ranged between 10.6 and 13.0 MMHO. Clinical examination showed that only one quarter was affected. Also, we obtained a low milk production, ranged between 2.3 and 9.3 liters of milk. After a single administration of Masti Veyxym a number of 19 (65,52%) cows were cured, 5 (17,24%) cows needed two administrations of Mastiker E and the other 5 (17,24%) cows were recovered after one treatment with Synulox LC. The AfiFarm program allows detection of mastitis diagnosis only when we correlated the cows dynamic activity, milk production, electrical conductivity and milking time.*

Keywords: subclinical mastitis, diagnosis, milk, cow.

INTRODUCTION

Worldwide, mastitis is one of the most important diseases in milk, continuing to be a costly disease facing dairy producers, causing significant economic losses. Subclinical mastitis is difficult to control since it is a disease caused by multiple factors [Oviedo-Boyso Javier, 2006]. Most times, mastitis negatively influences the milk quality having consequences for the dairy industry [Groza, 2006]. Latest trends in the milk production is mainly based on obtaining a higher milk quality and less on getting a major milk quantity. An important role in monitoring mammary gland health and production of higher milk quality is attributed to the veterinary service [Seegers, 2003].

The purpose of this paper was to supervise the activity and health of mammary gland in dairy cows in a unit specialized in milk production in Cluj county, and to implement a computer program for mammary gland health surveillance.

MATERIAL AND METHODS

The research was carried out during 2009 -2010, on a total of 489 cows. Supervision of activity and mammary gland health at the farm level was performed using a specialized program called AfiFarm and Bovi-Vet indicator paper.

AfiFarm program (figure 1) is the most advanced management software worldwide for dairy farms. This program collect and develop necessary information to optimize the productivity of an modern farmer`s farm.The application, developed by Israeli specialists (N° 1 in world milk production), it proves a reliable partner for real farmer, processing and delivering in short time complete data: milk production, herd health, milk electrical conductivity, detection of mastitis, milk separation, etc. The software records and outstanding performance when food its controlled, health monitoring, automatic sorting of cows for treatment or artificial insemination, thus allowing a more efficient management. Also, efficiency increase because of staff drastically reduced to serve a farm. Key components of the module are: daily reports on reproduction and fertility analysis, health monitoring, reports of milking efficiency, monitoring of veterinary checks, including veterinary diagnostic reports, medication and treatments, monitoring procedures for medical treatment, milk production planning in terms of cows number.

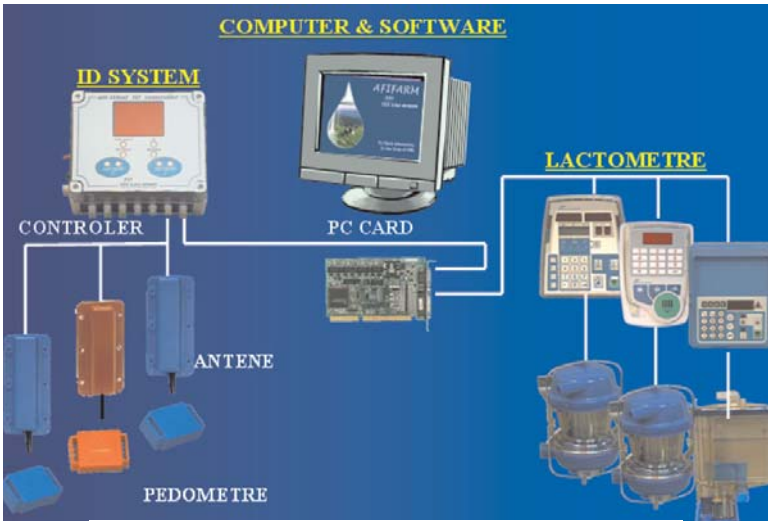


Figure 1: AfiFarm computer program schedule

Bovi-vet paper is an indicator of subclinical mastitis. The method consist in placing a few drops of milk on the special paper then looking for color change which indicates an infected quarter. Due to acidity of milk, the paper will change color from yellow to green or blue-green (figure 2).

Cows treatment was performed with 3 therapeutic protocols: Masti Veyxym – based on proteolytic enzymes and Synulox LC, Mastiker E (antibiotic products).

Figure 2: Bovi-Vet Indicator paper

RESULTS AND DISCUSSIONS

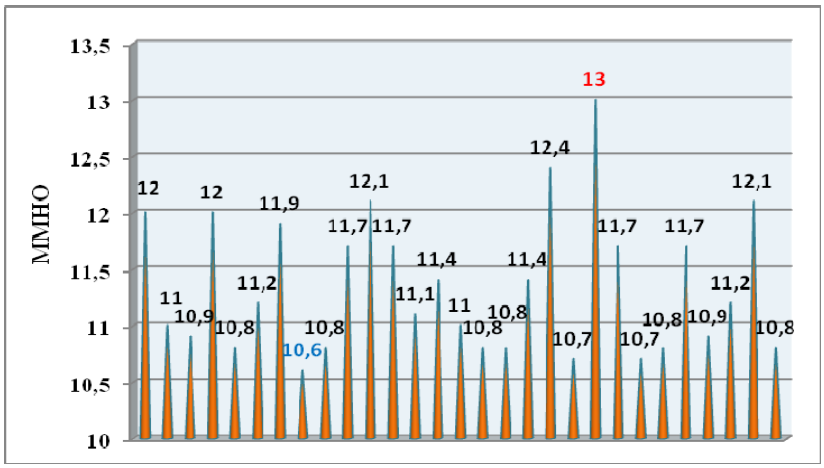
Table 1 – Parameter values obtained using the AfiFarm program

Crt. no.	Registration no.	Milk (liters)	Electrical conductivity (MMHO)	Activity (no. steps/h)
1.	145900	5.2	12.0	47
2.	124452	4.3	11.0	56
3.	125889	8.9	10.9	49
4.	125767	2.5	12.0	51
5.	144132	6.8	10.8	50
6.	238127	6.1	11.2	47
7.	145501	6.0	11.9	48
8.	**125127**	**9.3**	**10.6**	**60**
9.	125134	5.1	10.8	66
10.	125909	4.2	11.7	63
11.	124780	2.9	12.1	49
12.	125477	3.2	11.7	56
13.	145554	2.9	11.1	59
14.	125222	4.0	11.4	62
15.	125434	4.4	11.0	54
16.	125612	5.4	10.8	52
17.	125177	5.6	10.8	61
18.	145111	3.0	11.4	67
19.	145212	4.8	12.4	63
20.	125303	3.1	10.7	59
21.	**145554**	**2.3**	**13.0**	**46**
22.	145617	5.2	11.7	56
23.	145509	3.7	10.7	53
24.	125120	2.8	10.8	63
25.	145066	3.6	11.7	60
26.	136767	4.6	10.9	53
27.	134454	6.0	11.2	55
28.	145556	4.9	12.1	47
29.	145434	6.4	10.8	58

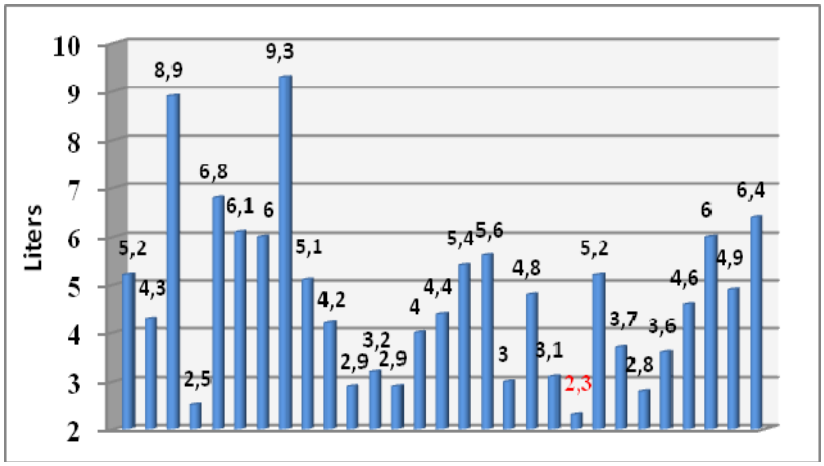
After monitoring the activity of the mammary gland with the AfiFarm computer program were identified a number of 29 cows (5.93%) who noticed an increase in electrical conductivity
(table 1). Clinical examination showed that only one quarter was affected.

Electrical conductivity in the 29 cows diagnosed with subclinical mastitis ranged between 10.6 and 13.0 MMHO (graphic 1), correlated with significantly reduced milk production in subclinical mastitis.

After data provided by AfiFarm program and extensive surveillance of cattle we obtained and low milk production. In the 29 cows that electrical conductivity was increased (>10 MMHO), milk production values framed between 2.3 - 9.3 liters of milk (graphic 2). With this latest software cows were diagnosed with 2-3 days before the occurrence of subclinical mastitis by electrical conductivity changes, being assigned to code 9. Electrical conductivity must always be correlate with milk production, because increase of electrical conductivity, not always occur due to subclinical mastitis. This increase may also depend on high levels of chloride in some feed and advanced lactation.



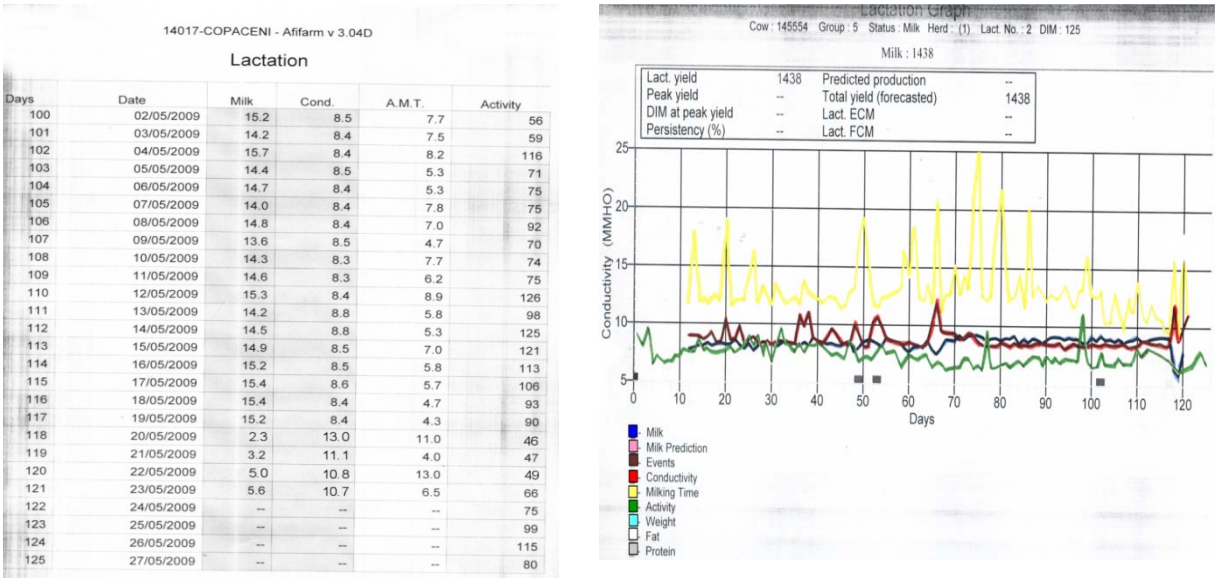
Graphic 1: Graphical representation of electrical conductivity



Graphic 2: Individual milk production in the detection of mastitist

To highlight the evolution of parameters in cows diagnosed with subclinical mastitis, with Afifarm program, we present a inspection summary of one case.

Cow with registration number 145554, in group 5, presented the highest value of parameters obtained. During monitoring, we recorded high values of electrical conductivity on days 38-39 (11 MMHO), day 52 (11 MMHO), day 68 (12,8 MMHO) and the highest value was recorded on day 118 (13.00 MMHO), milk production decreased at 2.3 l, and the number of steps/h at 46 (figure 3).



14017-COPACENI - Afifarm v 3.04D

Lactation

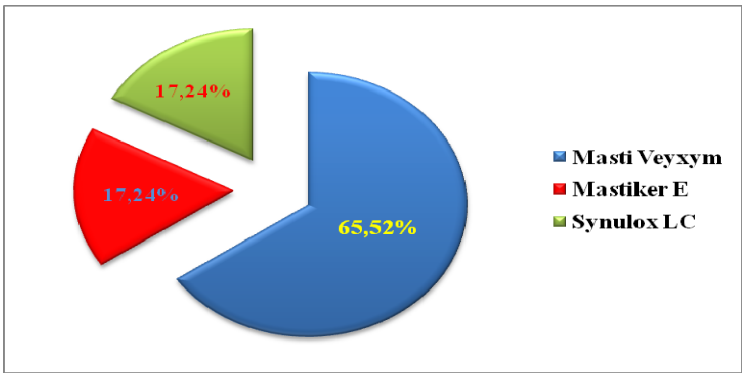
Days	Date	Milk	Cond.	A.M.T.	Activity
100	02/05/2009	15.2	8.5	7.7	56
101	03/05/2009	14.2	8.4	7.5	59
102	04/05/2009	15.7	8.4	8.2	116
103	05/05/2009	14.4	8.5	5.3	71
104	06/05/2009	14.7	8.4	5.3	75
105	07/05/2009	14.0	8.4	7.8	75
106	08/05/2009	14.8	8.4	7.0	92
107	09/05/2009	13.6	8.5	4.7	70
108	10/05/2009	14.3	8.3	7.7	74
109	11/05/2009	14.6	8.3	6.2	75
110	12/05/2009	15.3	8.4	8.9	126
111	13/05/2009	14.2	8.8	5.8	98
112	14/05/2009	14.5	8.8	5.3	125
113	15/05/2009	14.9	8.5	7.0	121
114	16/05/2009	15.2	8.5	5.8	113
115	17/05/2009	15.4	8.6	5.7	106
116	18/05/2009	15.4	8.4	4.7	93
117	19/05/2009	15.2	8.4	4.3	90
118	20/05/2009	2.3	13.0	11.0	46
119	21/05/2009	3.2	11.1	4.0	47
120	22/05/2009	5.0	10.8	13.0	49
121	23/05/2009	5.6	10.7	6.5	66
122	24/05/2009	--	--	--	75
123	25/05/2009	--	--	--	99
124	26/05/2009	--	--	--	115
125	27/05/2009	--	--	--	80

Figure 3: Graphical models obtained by processing parameters using the AfiFarm program

After a single administration with enzyme-based product Masti Veyxym, a total of 19 cows (65,52%) were cured and at the next milking electrical conductivity decreased below 10 MMHO (graphic 3).

The remaining 10 cows that have not healed after the first treatment were transferred to Synulox LC and Mastiker E (antibiotic products). The healing rate was 17,24% after treatment with Synulox LC product after the first administration and 17,24% responded positively after 2 doses of Mastiker E product (graphic 3).

Cows diagnosed with subclinical mastitis and treated with antibiotics were passed on 7 code, thus they are no longer milked by milking machine, but directly to the drum. These codes appeared on the display which is placed in front of the machine milking cow, so the staff will know where to direct the milk.



Graphic 3: Efficiency of subclinical mastitis products used in treatment

CONCLUSIONS

1. Low incidence of subclinical mastitis (5.93%) is possible because the farm implemented a computerized surveillance program of mammary gland - AfiFarm program.
2. Diagnosis of subclinical mastitis showed an increase of electrical conductivity, ranged between 10.6 to 13.0 MMHO and an 15-20% decrease of milk production.
3. Early diagnosis of subclinical mastitis allowed the recovery of 19 cows (65,52%) after the first administration of Masti Veyxym product.
4. Costs treatment of mammary gland diseases were insignificant.
5. Through the most advanced management software worldwide for dairy farms – AFIFARM PROGRAM, the diagnosis of subclinical mastitis can be establish more precisely while a correlation it is done between the cows dynamic activity, milk production electrical conductivity and milking time.

This work was supported by CNCSIS – UEFISCSU, project PN II "IDEI", 1482/2009

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SPECTROPHOTOMETRIC DETERMINATION OF NITRITE AND NITRATE IN MEAT PRODUCTS

Mirela MICLEAN[1], Cecilia ROMAN[1], Laura PARLAPAN[2], Ioan Stefan GROZA[2]
[1]INCDO-INOE 2000, Research Institute for Analytical Instrumentation, Donath St., no 67, 400293 Cluj-Napoca, Romania, icia@icia.ro
[2]University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Mănăstur St. no. 3-5, 400372 Cluj-Napoca, Romania, contact@usamvcluj.ro

Abstract
Nitrate and/or nitrite salts constitute the curing agents of red meat and, in particular, processed or preserved meat products. High dietary intakes of nitrate and nitrite have been implicated in the etiology of human gastric, oesophagus, colorectal and bladder cancer. Also, nitrate and/or nitrite could act as endocrine disruptors, affecting the androgen production in adult vertebrate males.
This paper reports the contents of nitrite and nitrate in processed meat samples using a spectrophotometric method, following aqueous extraction and filtration. Nitrite was determined directly using a Griess–Ilosvay diazo-coupling reaction. Simultaneous nitrite and nitrate determinations were based on nitrate reduction in a micro column containing cadmium. The analysed samples were commercially available minced meat, sausages, Frankfurt and salami. The nitrate and nitrite contents ranged from 0.72 to 68 mg/kg and from 0.90 to 61 mg/kg, respectively. Also, moisture, lipid, protein and salt content were determined for nutritive qualities characterisation.

***Keywords*:** nitrite, nitrate, cured meat, spectrophotometry

Meat and meat products are one of the main components of the human diet. Sodium or potassium nitrate and/or nitrite are usually added to meat as a preservative. The effective substance is nitrite acting as an inhibitor for some microorganisms, also it has strong antioxidant properties, inhibit lipid oxidation, produces the characteristic pink colour of cured meats, and contribute to the flavour of meat products (1). Nitrate may be reduced to nitrite in raw meat products by microorganisms (2). Nitrite is 10 times more toxic than nitrate (3). The red colour is developing in a number of complicated reaction steps until NO-myglobin (Fe2+) is formed (3).

Nitrite could result in formation of carcinogenic n-nitrosamines, by amines present in meat at higher temperatures (4). The underlying mechanism is endogenous nitrosation, which in the case of nitrate must be preceded by reduction to nitrite. Nitrate and nitrite are interconvertible *in vivo*. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary amines and alkyl amides, to generate *N*-nitroso compounds (3).

The consumption of processed or preserved or cured meats (sausages, ham, bacon, hot dogs, etc.), has been related to the incidence of colorectal, gastric, oesophagus and bladder cancer in several epidemiological studies (2, 5). In 2007, a report of World Cancer Research Fund and American Institute of Cancer Research recommended to “avoid processed meats” based on epidemiological studies showing increased risk of colorectal cancer with

increased intake of processed meats (6). Besides, nitrates have higher toxicological potential as they can worsen the physiological low affinity of haemoglobin for oxygen, producing methaemoglobinaemia (7). Also, a high intake of nitrite can pose a human health risk due to the possible allergenic and vasodilator effects (8). Animal studies have shown that nitrate acts as an endocrine disrupter affecting the androgen production in adult males (9).

The regulation regarding the processed food includes a series of tests determining their nutritional composition and how they relate to a healthy diet. One of the most important nutrients is protein and the monitoring of its amount, through the determination of nitrogen, must be accurate to establish the nutritional quality of these meat products. Also, total fat content is probably the most topical of all nutritional components in foods, endangered by obesity and the associated heart disease and diabetes. The salts contained in meat products include common salt (NaCl), nitrite and nitrate, each exerting useful properties to the food (10).

The aim of this study was to investigate the most consumed types of processed meat products: minced meat, sausages, Frankfurt, salami in order to determine the concentrations of nitrite and nitrate, due to the health concern. Also, the content of protein, lipid, moisture and sodium chloride was analysed for quality control purposes. Because of the health implication, the method for quantification of nitrite and nitrate should be fast, sensitive and reliable.

MATERIALS AND METHODS

Sampling and preparation

Thirty commercially available meat products were sampled from local supermarkets situated in Cluj-Napoca, Romania: 5 minced meat, 9 sausages, 7 Frankfurt and 9 salamis.

All meat products were sampled freshly before lipid extraction to prevent any hydrolysis or oxidation before its dispersion in the solvent. The food meat products were cut using a stainless steel knife, and then minced in a porcelain mortar with pestle for homogenization.

Chemical analyses

All the reagents used were of analytical grade, acquired from Merck (Darmstadt, Germany) and double distilled water produced by distiller system (GFL, Germany) was used throughout the experiment. The nitrite stock solution was prepared at 5 g/l level in double distilled water and stored at 4 °C. The stock solution was gradually diluted at the working concentration levels and used for external multilevel calibration.

Salts, moisture, lipid and protein contents were determined according to the official Romanian standardized methods for analysis of meat products.

For the salts (nitrite, nitrate and sodium chloride) content determination, the aqueous extract of meat product was performed by triturating the homogenized meat sample with distilled water and then the sample was filtered through an S&S filter 5892 (Scheicher & Schuell GmbH, Germany) to remove the matrix components present in the meat samples.

For determination, extracted nitrite reacted with Griess–Ilosvay reagent forming a diazonium salt with the sulfanilic acid. This salt was coupled then to alfa-naphtylamine, an aromatic amine to form the coloured azo dye, analyzed spectrophotometrically (Cary UV-Vis spectrophotometer, Varian, USA) at 520 nm wavelength. Nitrate is unreactive in this system. Nitrate was reduced to nitrite with cadmium metal put in a micro column, activated with diluted hydrochloric acid and then determined as described above. Both anions were present

in the same meat product, so extracted nitrite was determined first, then nitrate was reduced to nitrite, which was remeasured, and the original quantity of nitrate was calculated by difference. A single chromogenic reagent (Griess–Ilosvay) containing all the necessary reactants was used in both methods.

Sodium chloride is measured by titration as the chloride ion through its reaction with silver ion added as silver nitrate to extracts of meat products.

Moisture content was determined by dehydration at 105±3 °C until constant weight, in an drying oven (Memmert, Germany) with automated electronic control of temperature and time.

Total lipid content was determined by automatic Soxhlet extraction (Behr Labor-Technic extractor, Germany) of dried sample by repeated percolation in petroleum ether, under reflux conditions, followed by evaporation of solvent and measuring of the remaining lipid by gravimetric determination.

For protein determination, the method implied the measuring of nitrogen quantity, due to the fact that nitrogen is present in all amino acids, and then the use a multiplication factor (6.25 for meat and meat products) to calculate the quantity of protein. The used method was Kjeldahl digestion (Digestion System K-431, with a scrubber, BÜCHI Labortechnik AG, Switzerland) at high temperature with concentrated sulfuric acid, sodium sulfate and copper sulfate as catalyst, to convert nitrogenous substances in the meat to ammonium salts. Addition of concentrated alkali then converts the ammonium salts to free ammonia that is distilled with steam and collected in hydrochloric acid containing suitable colored pH indicators. The acid excess is back-titrated to neutral with sodium hydroxide solution.

RESULTS AND DISCUSSIONS

The oxidation of nitrite to nitrate in meat also explains why in meat products to which only nitrite has been added, nitrate will be found in considerable concentrations. The concentrations of nitrite and nitrate in investigated meat products are shown in Table 1 and Table 2, respectively.

Table 1

Crt. No.	Meat product	Concentration of nitrite, mg/kg		
		Minimum, mg/kg	Maximum, mg/kg	Mean, mg/kg
1	Minced meat	0.90	33.4	16.4
2	Sausage	8.66	51.2	25.8
3	Frankfurt	5.75	46.5	20.2
4	Salami	16.2	61.0	32.3

The concentrations of nitrite were below the maximum admitted level (MAL) in all studied samples, according to Romanian legislation (SP-C-401-98, SP-C-402-98, SP-C-701-98, SP-C-702-98, and SP-C-406-98). The value of MAL was set as 70 mg/kg in all cured meat products. The obtained mean concentrations of nitrite, in the investigated meat products, decreased in the following order: salami > sausage > Frankfurt > minced meat.

Table 2

Crt. No.	Meat product	Concentration of nitrate, mg/kg		
		Minimum, mg/kg	Maximum, mg/kg	Mean, mg/kg
1	Minced meat	0.72	26.3	11.6
2	Sausage	0.98	68.0	26.7
3	Frankfurt	1.48	22.4	12.9
4	Salami	1.45	54.2	21.9

The mean concentrations of nitrate, in the investigated meat products, decreased in the following order: sausage > salami > Frankfurt > minced meat. The concentrations of nitrate were lower than the concentrations of nitrite in minced meat, Frankfurt and salami and higher in sausage samples. European legislation (Council Directive 95/2/EC on food additives other than colors and sweeteners, Directive, 1995) set the MAL as a residual amount for nitrate in meat products, at 250 mg/kg. The obtained nitrate concentrations were below MAL, for all investigated meat samples.

The mean concentrations of nitrite obtained in this study were lower than those obtained by Hsu et al., 2009, for Frankfurt (83.9 mg/kg) and higher for minced meat and salami and the mean nitrate concentrations recorded in the present study were lower for Frankfurt (54.9 mg/kg), salami (142.5 mg/kg) and minced meat (18.7 mg/kg) (2). Also, mean concentrations of nitrite obtained in the present study were higher than those obtained by Akyuz et al., 2009, for salami (3.81 mg/kg) and sausage (7.66 mg/kg) and mean nitrate concentrations were lower for salami (123.35 mg/kg) and sausage (296.4 mg/kg) (11). The mean concentrations recorded in this paper were below the concentrations achieved by Siu et al., 1998, in salami samples for nitrite (108 mg/kg) and nitrate (98.5 mg/kg) (12).

The mean contents of moisture, sodium chloride, fat and protein for the cured meat products are shown in Table 3.

Table 3

Crt. No.	Meat product	Mean moisture content, %	Mean NaCl content, %	Mean fat content, %	Mean protein content, %
1	Minced meat	-	-	25.6	-
2	Sausage	46.2	1.45	30.8	15.2
3	Frankfurt	59.2	2.06	19.5	12.7
4	Salami	52.0	2.54	26.2	13.5

For minced meat, the contents of moisture, salt and protein were not regulated by Romanian legislation. Mean fat content varied in the range 23.0–31.45, with an average value of 25.6% and all the obtained values were below MAL (32%) set by Romanian legislation.

For sausage samples, the contents of moisture, salt, fat and protein ranged between 36.5-60.7%, 0.94-2.40%, 27.5-35.2% and 11.6-18.2, respectively. In one sample the content of lipids was higher than MAL (32%). The values of MAL for moisture and salt, and the minimum admitted level for protein set by the Romanian legislation were: 63%, 3%, and 11%, respectively.

In Frankfurt samples the values were in the range 50.4-75.2% for moisture, 1.38-2.73% for NaCl, 17.3-24.1% for lipids and 8.79-14.5% for protein content. In one sample, the moisture content was higher than MAL (70%) and in another sample the protein content was lower than the minimum admitted level (11%).

In salami samples the obtained values varied in this manner: 48.2-71.0% for moisture, 1.68-2.66% for salt, 23.6-36.8% for fat and 10.2-14.8% for protein content, respectively.

Similar to Frankfurt products, in two salami samples the moisture content was higher than MAL (68%) and in another sample the protein content was lower than the minimum admitted level (12%).

CONCLUSIONS

The contamination with nitrite/nitrate has the potential to alter the endocrine, nervous and immune systems of the organism, resulting in changes in gene expression and phenotypes, therefore the addition of nitrite/nitrate in processed meat must respect the maximum admitted concentrations for a safe human consumption.

In this study, the contents of nitrite and nitrate in processed meat product (minced meat, sausages, Frankfurt and salami) were determined using a spectrophotometric method following aqueous extraction and filtration for removing the interfering matrix components such as protein and fat-based substances. The studied spectrofotometric method represents a fast, reliable, sensitive and low cost analysis of nitrite and nitrate in cured meat product. The highest mean concentrations of nitrite (32.3 mg/kg) and nitrate (26.7 mg/kg) were registered in salami and sausages, respectively, and all the values were below MAL set by legislation.

Most of the analytes concentrations in studied meat products complied with the Romanian regulations, except for five samples where moisture, protein or lipid content were not conform to the legislation, lowering the nutritional quality of the products.

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STUDIES CONCERNING LEPTIN'S INFLUENCE ON THE SCC IN BUFFALO MILK

M.MIHAIU[1], ALEXANDRA LAPUSAN[1], , CRINA TEODORA CARSAI[2] , CARMEN JECAN[1], ROMOLICA MIHAIU[2] ,S.D. DAN[1]

[1)]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Mănăştur street No.3, 400372, Cluj-Napoca, Romania, e-mail: m.mihaiufmv@yahoo.com

[3)]Babes – Bolyai University, Faculty of Economics and Business Administration, Cluj-Napoca, Romania,[2)] University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnology

SUMMARY

The progress in animal breeding may be improved by combining traditional performance data with molecular genetic information. Leptin is a protein hormone expressed in the mammary gland of various species, including human (Smith-Kirwin et al., 1998), mouse (Aoki et al., 1999), cattle (Silva et al., 2002; Smith and Sheffield, 2002; Chelikani et al., 2003; Sayed-Ahmed et al., 2003; Bartha et al., 2005), and sheep (Laud et al., 1999; Bonnet et al., 2002). The influence of this hormone on milk production traits was studied at cattles (Buchanan et al., 2002),goats (Whitley N. C. et al. 2009),, sheep (Zhou H. et al. 2009). The presence of leptin in milk raises questions concerning the ability of the mammary epithelial cells to transfer leptin from the blood and/or to synthesize it, before its secretion. In order to gain a better insight and understanding of leptin's influence on milk's quality traits this paper focuses on the possible variations of the SCC according to the genetic polymorphism of this hormone. The method used in identifying leptin's genotype was PCR-RFLP, and Somatos-M for assessing the SCC. After the PCR we managed to identify the favorable allele C and T of leptin gene. This study confirms the researches done in this field which concluded also that this hormone may be a factor that influences production traits. The association between leptin polymorphism with milk's quality indicators such as SCC, suggests that this marker may be useful for selection based on molecular information.

Keywords: leptin, polymorphism, buffalo, milk, SCC.

MATERIALS AND METHODS

The research has taken into study 43 milk samples, collected and processed from buffalos raised in private households from Brasov and Salaj counties. The methods used for DNA extraction from milk's somatic cells, were the following: DNA extraction from blood using the solutions from the extraction kit; the DNA extraction from the somatic cells using the rapid extraction with PBS. After the DNA extraction we identified the electrophoresis profile of leptin's gene using the PCR - RFLP technique. This technique consists in repeated cycles of heating and cooling so as to melt and enzimatically replicate the DNA. The short fragments of DNA that have complementary sequences, along with a DNA polymerase region are the key components in allowing the selective and repeated amplification. By the PCR method we obtain a progress, in generated DNA this being used as a replication model, establishing in movement a chain reaction in which the DNA pattern is exponentially amplified. The PCR can be modified on a large scale so as to make a large amount of genetic manipulations.

The DNA extraction was done according to the following steps: 40µl raw milk was centrifuged at 2000 rotations/min for 30 minutes. After centrifugation the pallet was

immersed in one ml PBS(phosphate -buffer-saline), pH – 7,4, and centrifuged again at 4000 rot/minute-10 minutes, and then we suspended again the pallet in a 49 ml lyses solution (0,32 M sucrose, 10mMTris-HCl, 7,5mM MgCl1%, TritonX-100). Afterwards we centrifuged the pallet's nuclei at 2465gxg 10 minute and then washed them twice with 10ml NaCl and 0,025 M EDTA. The pallet was suspended again in 3 ml 10mM Tris-HCl solution, pH – 8, and 2mM EDTA, after which we added 100ml 10% SDS and 40ml proteinase K 910mg/ml). The nuclei's lysis was incubated for an hour at 65°C. After incubation we added 500ml NaCl, the precipitated proteins were centrifuged at 1800gxg 10 minutes. We added 6 ml isopropanol after which we centrifuged for 10 minutes at 1800gxg.. The pallet was dried at a room temperature and re dissolved in 500ml Tris-EDTA(10 Mm Tris-HCl, 1mM EDTA solution , pH 7,5. After following these steps the total quantity of the DNA extracted from milk was in between 14.2ng/µl-156.8 ng/µl. This method is not so indicated because it involves the using of some toxic, cancerous solvents like phenol – chloroform, isoamilic alcohol, etc.

The rapid extraction of the DNA from milk and colostrum was realised in the following way: 2 ml of milk from each sample were put in a tube and afterwards we added 0,4 ml PBS solution, then centrifuged it 4-5 times at 3000rpm for 20 minutes. After this step we left the samples in a bath water for 15 minutes time in which the somatic cells are broken down and freeing the DNA quantity. The DNA quantity obtained was then read at the Nanodrop spectrophotometer. The DNA extracted from milk using this method was in between 67ng/µl – 347 ng/µl.

After the DNA extraction we processed the samples according to the following protocol:

In an Eppendorf tube we put 16.2 µl water, 2.5µl buffer, 1µl DNTP, primers (amcrse): sense primer 1µl, antisense primer 1 µl; TAQ-polymerase 0.3µl, $MgCl_2$-1µl, extracted genomic DNA. We used the amplifying program at Termocycler that takes 1x95^{o}C→5 minutes (the alteration) the attaching of the primers in several stages at 40x95^{o}C→1 minute, at 40x64^{o}C→1 minute were the attachment takes place usually, and at 40x72^{o}C→1 minute. After this attachment protocol the final extraction takes place at 1x72C →10minutes.

The enzyme restriction was done with the Kpn21 (BspEi) enzyme and followed the next steps: we added H_2O 6,5ml, after which a tampon solution 2,5ml and afterwards Kpn21 (BspEi) enzyme 1µl.We obtained a reaction mix for the samples used and then we added 20µl PCR product applied by the PCR.

The enzyme has an activity at 55° C; the samples were put in the thermostat for 4 hours in a water bath. The restriction's role was to digest the amplified fragment from the leptin's gene in order to differentiate the implicated allele in the buffalo's milk quality.

The DNA sample's migration was made by an electrophoresis reaction in agars gel, for coloring and visualizing the products we used 2µl ethidium bromide.

In order to analyze the Somatic Cell Count number from these samples we used the Somatos M and Fossomatic machine.

RESULTS AND DISCUSSIONS

After the DNA extraction from milk we obtained values ranging between 14.2 ng/µl – 347ng/µl.

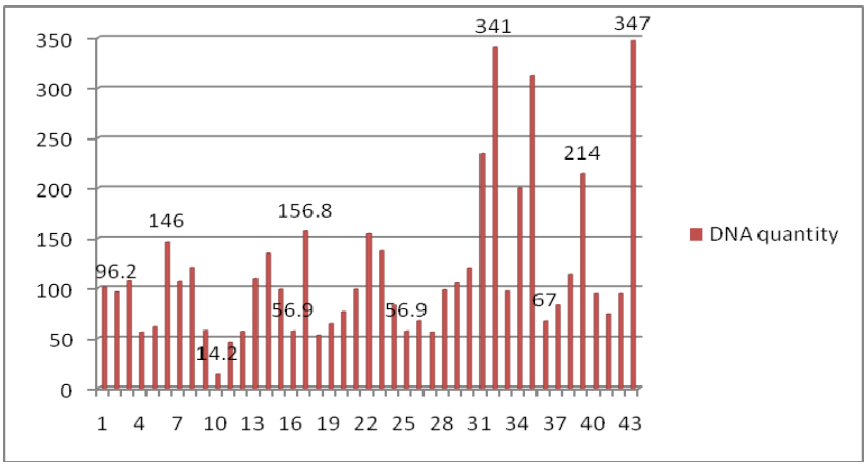

Fig.1. DNA quantity values obtained from the buffalo milk samples

In the majority of the samples we obtained the electrophoresis profile by gel migration and at the reading of the bands revealed in the gel we observed the appearance of a single clear band of 94 bp characteristic to TT genotype, two clear bands of 75 bp and 19 bp characteristic to CC genotype and three clear bands at 94 pb, 75 pb, 19 pb characteristic to CT genotype. Allele C from cytosine and allele T from thiamine are obtained by a cytosine-thiamine mutation. Following this mutation the two alleles are differentiated, representing three genotypes: CC, TT, and CT. According to the protocol described by Buchanan et al. (2002), after PCR reaction with specific primers from gene leptin we should obtain the following fragments: - the length of the amplified non restricted fragment should be of 94 bp and following the restriction with the Kpn2I restriction enzyme we should obtain the following fragments: TT with 94 bp the non restricted alleles by the enzyme, CT which will have the fragments 75 bp, 94 bp and 19 bp and CC genotype with 75 bp and 95 bp.

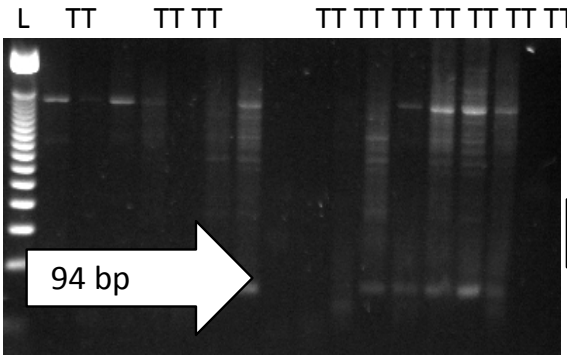

Fig.2: The electrophoresis profile of 94 base pair from leptin gene

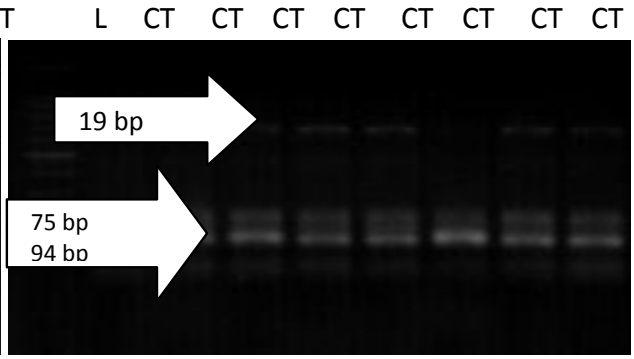

Fig.3: The electrophoresis profile of 94 bp, bp and 19 bp of leptin gene

L CC CC CC CC CC CC CC

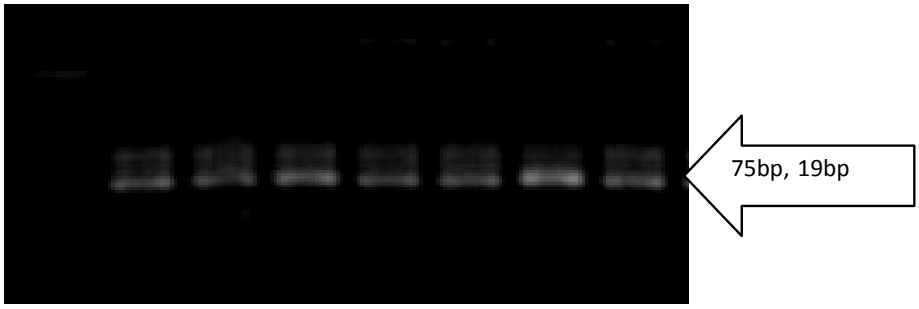

Fig.4: The electrophoresis profile of 75 bp and 19 bp of leptin gene

At the analyisis of the SCC we noticed that the differences in the values of the samples were significant. We mention the fact that all the samples were kept under the same temperature conditions and the SCC was analysed as soon as possible in order to obtain a representative value.

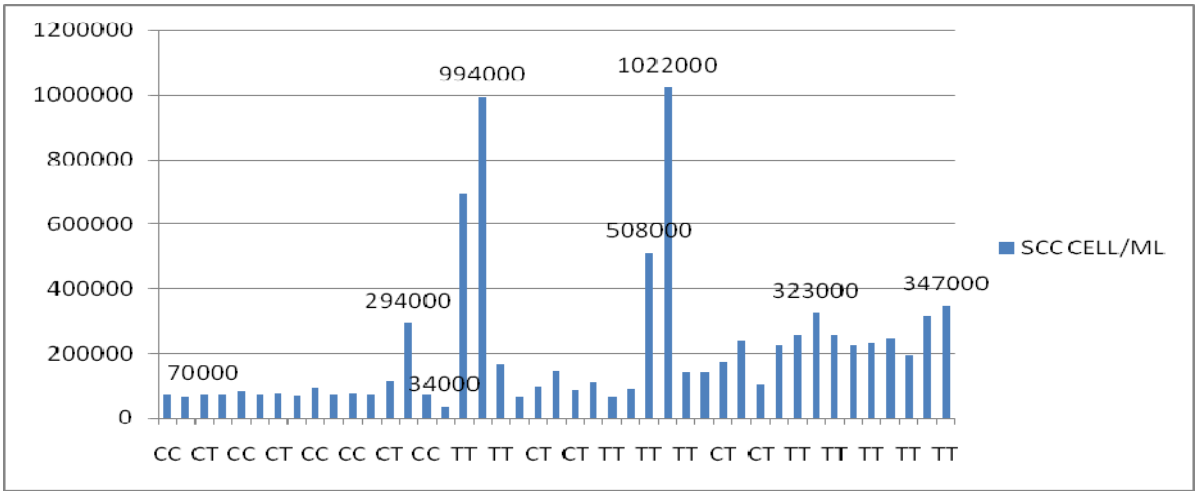

Fig. 5 The SCC values obtained from the buffalo milk samples

Comparing these SCC values to the genotypes of each individual, buffalos homozygous for the T allele demonstrated an increased number in SCC linear score in contrast with the buffaloes homozygous for the C allele, and heterozygous CT. In the field studies, TT genotype was found to be responsible for a higher milk yield and quality traits. Higher SCC can be symptomatic for mastitis infection but also this may reflect a possible increase in mastitis incidence associated with higher milk yield influenced by leptin or the role of leptin in modulating an immune response.

Our study confirms the researches done in this field which concluded also that this hormone may be a factor that influences production traits.

CONCLUSIONS

In the population studied the individuals homozygous for TT genotype are in majority, the CT and CC genotype being found only in 12 samples from 43 examined

The samples that had a higher SCC were correspondent to the ones with TT profile.

The association between leptin's polymorphism with milk's quality indicators such as SCC, suggests that this marker may be useful for selection based on molecular information.

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STUDY CONCERNING MOLDOVA RIVER WATER QUALITY IN NEAMT COUNTY

Elena Mitranescu[1], Catalina Lala[2], F. Furnaris[1], L. Tudor[1], Mariana Țerbea[3], D. Mitrănescu[4], Violeta Simion[5]

[1] University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 105 Spl. Independentei Street, Bucharest, Romania; mitranescuelena@gmail.com
[2] Roche Romania. SRL
[3] National Sanitary Veterinary and Food Safety Agency
[4] Penitentiaries General Department, Bucharest
[5] Spiru Haret University, Faculty of Veterinary Medicine

Abstract

Water is a complex environment where aquatic organisms live, whose chemical composition results from complex interactions between atmosphere, hydrosphere and biosphere.

Moldova River water quality, in Neamt County area, was established based on water samples collected from two sections: Timisesti and Roman.

From the collected samples there were assessed: physical indicators (temperature, pH), chemical indicators (dissolved oxygen, OBC_5, COC-Mn), nutrients ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$total phosphorus, total ammonia nitrogen), general ions, salinity (fixed residue, Cl^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, Fe, Mn), metals (Cr, Cu, Zn and dangerous substances - Cd, Hg, Ni, Pb), micro pollutants (cyanides, phenols, anionic detergents), and biological parameters.

Water quality depending on the values obtained for the followed parameters was ranged into five quality categories.

Physical-chemical parameters were assessed using NOVA 60 photo colorimeter and results interpretation in accordance with Order 161/2006.

Moldova River along Neamt County territory is ranged, according to the physical chemical parameters, within the class I throughout the analyzed territory, except for nutrients in section Timisesti and oxygen system in Roman section, which are suitable for Class II of quality.

The biological elements range Moldova River water within Class II of quality.

Moldova water pollution on Neamt County territory, in Roman section, is based on the alluvial deposits presence, the pollution of Roman city and the effluents of two wastewaters treatment plants.

Key words: quality parameters, water, maximum admitted limits

INTRODUCTION

Water is an essential element of life. Considered as an environmental factor, water has a fundamental role by its active participation in the physical and chemical processes from air and soil and by the involvement in the main atmospheric phenomena that determine different types of climate.

Water is used as source of raw materials, source of driving power in industry, transporting all the waste, being a working tool etc.

Providing quantitative and qualitative intake of water for human and animal consumption of natural water sources (underground, surface, and meteoric) and the vulnerability of water from these sources to different types of pollution require monitoring and assessing water quality.

In this context, monitoring and characterization activity of the aquatic environment state is an important tool in water integrated management, providing protection and sustainable use of the water as the main environmental factor.

MATERIALS AND METHODS

In this paper, Moldova River's water quality was assessed along Neamt County territory by taking water samples in two sections: Timisesti and Roman.
Assessing the ecological status of Moldova river water was done according to the five ecological states which express five classes of water quality: Class I - very good, class II - good, Class III - moderate, Class IV - low, Class V - poor.
Surface water quality assessment and classification in the 5 quality classes was done based on maximum admitted limits corresponding to each class as follows:
Class I indicates a low contamination level, class II a moderate contamination and Class III, IV and V reflect a contamination from moderate to very high.
For the assessment of Moldova River's water quality on chemical basis, the following groups of indicators were analyzed:
1. Physical indicators (temperature, pH)
2. Oxygen regime: (dissolved oxygen, OBC_5, COC-Mn)
3. Nutrients ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, total phosphorus, total ammonia nitrogen)
4. General ions, salinity (fixed residue, Cl-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, Fe, Mn)
5. Metals (Cr, Cu, Zn and dangerous substances - Cd, Hg, Ni, Pb)
6. Micro pollutants (cyanides, phenols, anionic detergents).
In order to establish the ecological status of Moldova River was used the saprob index calculation. Physical-chemical parameters were assessed using NOVA 60 photo colorimeter.
Results interpretation was in accordance with Order 161/2006.

Results and discussions

Table 1

The dynamics of biological indicators in monitoring sections of Moldova River's water in 2007

Control section	Date	Indicators					
		Phytoplankton		Phytobenthos		Zoobenthos	
		Taxons no.	No./l	Taxons no.	No./m²x10⁴	Taxons no.	No./m²
Timişeşti	***07.05***	27	1.070.000	14	2.850	3	20
	13.10	23	2.650.000	23	160.750	8	210
Average value		25	1.860.000	19	81.800	6	115
Roman	***12.03***	28	475.000	-	-	-	-
	08.05	30	740.000	18	11.400	5	45
	14.08	17	840.000	18	9.500	3	30
	14.10	20	1.725.000	28	62.000	5	105
Average value		24	945.000	21	27.630	4	60

Moldova River along Neamt county territory crosses the Moldavian Platform deposits which, geologically, are composed of relatively horizontal Sarmatian deposits.
The results of qualitative and quantitative analysis of Moldova River's phytoplankton, phytobenthos and zoobenthos in the 2 sections, Timisesti and Roman, are shown in Table 1 and Table 2.

Investigations have revealed the presence of 39 planktonic taxons in Timisesti section and 53 in Roman section, and zoobenthos: 8 species in samples from both sections.
The average values of the saprob index show a good ecological status for the analyzed river section, according to the biological quality elements.

Table 2

The dynamics of saprob index values in monitoring sections of Moldova River's water in 2007

Control section	Date	Phytoplankton			Phytobenthos			Macrozoobenthos		
		Saprob index	Class of quality	Ecological status	Saprob index	Class of quality	Ecological status	Saprob index	Class of quality	Ecological status
Timişeşti	***07.05***	1,83	II	Good	1,95	II	Good	2,5	III	Moderate
	13.10	1,70	I	Very good	1,56	I	Very good	1,89	II	Good
Average value		1,77	I	Very good	1,76	I	Very good	2,20	II	Good
Roman	***12.03***	1,95	II	Good						
	08.05	1,95	II	Good	2,21	II	Good	2,54	III	Moderate
	14.08	1,94	II	Good	2,43	III	Moderate	3	III	Moderate
	14.10	2,31	III	Moderate	2,03	II	Good	2,12	II	Good
Average value		2,04	II	Good	2,22	II	Good	2,55	III	Moderate

Moldova River water temperature and pH (average values) are shown in Table 3.

Table 3

Water temperature and pH average annual values for Moldova River – Neamt County

Control section	***No. of samples***	***Assessed parameters***		***Global quality class***
		Temperature (°C)	***pH***	
Timişeşti	220	13,2	8,2	I
Roman	232	10,5	8,3	I
Maximum admitted limit according to Order 161/2006		***Not defined***	***6,5 – 8,5***	

Water pH, according to the admitted limits, is considered close to the maximum limit.
Average values of the indicators representing oxygen regime group are presented in Table 4.

Table 4

Average values of the chemical indicators (Oxygenr regime - OR) in Moldova water - Neamt County

Sampling point	***No. samples***	***Assessed parameters***			***Global quality class***
		Dissolved oxygen (mg/l)	***CBO5 (mg/l)***	***CCO-Mn (mg/l)***	
Timişeşti	50	10,12	2,02	1,81	I
Roman	62	6,18	4,5	6,66	II
Maximum admitted limit according to Order 161/2006		***9***	***3***	***5***	***I***
		7	***5***	***10***	***II***
		5	***7***	***20***	***III***
		4	***20***	***50***	***IV***
		<4	***>20***	***>50***	***IV***

Analyzing data from the table it is noticed that, in terms of dissolved oxygen, biochemical and chemical oxygen consumption, water from Timisesti section is included in class I of quality and the water from Roman section in class II.
In Roman section, Moldova River's water suffers a slight pollution caused by the alluvial deposits, and the effluents from the two wastewater treatment plants - Petrotub si Danubina.
In Table 5 are shown the nutrients' average values in the 2 sections of Moldova River.

Table 5

Average values of nutrients in Moldavia River water, Neamt County

Sampling point		***Assessed parameters***					***Global quality class***
		$N\text{-}NH_4^+$ (mg/l)	***$N\text{-}NO_2^-$ (mg/l)***	***$N\text{-}NO_3^-$ (mg/l)***	***N total (mg/l)***	***P total (mg/l)***	
Timişeşti		1,05	0,03	2,17	5,88	0,36	II
Roman		0,38	0,007	0,09	1,5	0,0192	I
Maximum admitted limit according to Order 161/2006	***I***	***0,4***	***0,01***	***1***	***1,5***	***0,15***	***I***
	II	***0,8***	***0,03***	***3***	***7***	***0,4***	***II***
	III	***1,2***	***0,06***	***5,6***	***12,0***	***0,75***	***III***
	IV	***3,2***	***0,3***	***11,2***	***16,0***	***1,2***	***IV***
	V	***>3,2***	***>0,3***	***>11,2***	***>16,0***	***>1,2***	***IV***

According to the data in the table, nutrients (ammonia, nitrites, nitrates, nitrogen and total phosphorus) were recorded values corresponding to Class II of quality in Timisesti section and to Class I of quality in Roman section.

The average values of the indicators from the salinity group (fixed residue, chrome, sulphates, calcium, magnesium, sodium, iron, manganese) range Moldova River water from both sections within class I of quality (Table 6).

Table 6

The annual average values of physical-chemical parameter in Moldavia River

Control section	***Final class of quality***	***Assessed parametes***							
		Fixed residuum (mg/l)	***Cl^- (mg/l)***	***SO_4^{2-} (mg/l)***	***Ca^{2+} (mg/l)***	***Mg^{2+} (mg/l)***	***Na^+ (mg/l)***	***Fe (mg/l)***	***Mn (mg/l)***
Timişeşti	I	392	20,2	28,03	22,8	1,88	18,5	0,29	0,043
Roman	I	480	24,04	54	35,8	5,99	19	0,24	0,028
Maximum admitted limit according to Order 161/2006	I	500	25	60	50	12	25	0,3	0,05
	II	750	50	120	100	50	50	0,5	0,1
	III	1000	250	250	200	100	100	1,0	0,3
	IV	1300	300	300	300	200	200	2,0	1
	V	>1300	>300	>300	>300	>200	>200	>2,0	>1

For the metals chemical indicators group, respectively copper, chrome, zinc, cadmium, nickel and lead the average values are presented in Table 7. Values obtained for all parameters determined in the two sections range within Class I of quality.

Table 7

Average values of the metals in Moldova River water, Neamt County

Section	***Global quality class***	***Assessed parameters***					
		Cu (µg/l)	***Cr (µg/l)***	***Zn (µg/l)***	***Cd (µg/l)***	***Ni (µg/l)***	***Pb (µg/l)***
Timişeşti	I	14,02	18,66	55,22	0,50	6,55	4,88
Roman	I	13,62	18,92	82,17	0,32	7,22	4,22
Maximum admitted limit according to Order 161/2006	I	20	25	100	0,5	10	5
	II	30	50	200	1,0	25	10
	III	50	100	500	2,0	50	25
	IV	100	250	1000	5,0	100	50
	V	>100	>250	>1000	>5,0	>100	>50

From the micro pollutants group there were determined cyanides, phenols and anionic detergents. Micro pollutants' average values are provided in Table 8.

Table 8

Annual agerage values of MP (micro pollutants) from Moldova River, Neamt county

Section	***General qualuy classa***	***Indicatori determinați***		
		Cynides (µg/l)	***Phenols (µg/l)***	***Anionic detergetns (µg/l)***
Timişeşti	I	0,006	0,22	37,22
Roman	I	0,0028	0,55	37,1
Maximum admitted limit according to Order 161/2006	***I***	***0,01***	***1***	***100***
	II		***5***	***200***
	III		***20***	***300***
	IV		***50***	***500***
	IV		***>50***	***>500***

Analyzing the data from the table, it can be noticed that all three indicators assessed in both sections, the values obtained belong to class I of quality.

CONCLUSIONS

1. Average concentrations recorded for the indicators: oxygen regime, degree of mineralization and micro pollutants of Moldova River's water - Timisesti section - are appropriate to class I of quality, and for the nutrients to class II of quality.
2. Moldova River's water - Roman section - belongs to class I of quality regarding the nutrients, degree of mineralization and micro pollutants, while regarding the oxygen regime the water belongs to class II of quality.
3. Biological elements of quality (phytoplankton, phytobenthos and zoobenthos) frame the general water quality for both sections into class II.
4. Moldova River's water pollution in Roman section is based on both natural geological conditions (alluvial deposits) and the pollution caming from Roman city, and on the effluents of the two wastewater treatment plants Petrotub and Danubiana.

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RESEARCHES CONCERNING FISH WELFARE IN A FARM FROM SOUTHERN AREA OF ROMANIA

ELENA MITRANESCU[1], F. FURNARIS[1], L. TUDOR[1], MAGDA GONCIAROV[1], VIOLETA SIMION[2], S. BUSTANI[1], ADRIANA ORASANU[3]

[1] University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, mitranescuelena@gmail.com
[2] Spiru Haret University Bucharest, Faculty of Veterinary Medicine
[3] National Institute of Diagnosis and Animal Health

Abstract

In the modern aquaculture, fish optimum welfare implies ensuring rearing conditions which allow the fish to maintain homeostasis, to a normal somatic development and to be protected from stress. The present paper aims to assess fish welfare level in a fishery from southern area of Romania populated with common, silver and grass carp based on water chemical and microbiological quality and fish biochemical serum profile. There were harvested water samples from 3 ponds and there were established the physical-chemical parameters (pH, dissolved oxygen, residual chlorine, total phosphorum, sulphates, nitrates, nitrites, phenols, copper and total ferrum), as well as microbiological parameters (Aerobic Plate Count - APC, Fungal Plate Count - FPC and total coliforms). The water physical-chemical parameters analysis were done by using Spectroquant Nova 60 fotocolorimeter and the microbiological ones by using Compact Dry TC (Total count), YM (Yeast and Molds) or CF (total coliforms) rapid assays. In adittion, from each pond were harvested 2 fish from which there was collected blood by caudal vein puncture (CVP), 2 ml blood/fish. The serum biochemical profile has been established by using Vettest 8008, being analyzed 8 blood chemistry indicators: albumine, phosphatemia, uric acid, cholesterol, triglycerides, glucose, aspartate aminotransferase and alanine transaminase.

The researches led to the following conclusions: concerning chemical water quality, there were registered for chlorines exceeding of the admitted limits for carp rearing up to 10 times and for phenols the admitted limits from 1146/2002 Order (water in the fifth quality class); concerning the microbial load, total coliforms are 2.7 – 4.9 times higher than the reference value for second quality class water according to 1146/2002 Order; the water inapropriate parameters reflects in a serum profile modification: noticable is an exceeding of transaminases activity, most likely due to phenols toxicity.

Key words: fish, welfare, water parameters, biochemical serum profile

Fish farming is an age old activity and in practice from ancient times. Present day, this branch registered a remarkable development rate, due to the need to adopt more productive means to feed increasing Earth population.

Because fish capacity to feel pain was proved by many scientific researches, farming these species could not be conceived without assuring the optimum welfare level, which could be achieved only under a continuous monitoring.

If in other species of zootechnical interest there are present nowadays practical, numerical systems for animal a welfare assessment, which permit making comparisons between different field situations and livestocks, in fish such systems still are not structured. However, a fish welfare assessment system will include both ethological parameters (animal based) and rearing condition parameters (engineering based: water quality, fish density etc.), the latest being of main importance in assuring quality of fish life. The fish blood biochemical

profile analysis raises many problems and could not be yet applied on large scale for wellness screening in fisheries.

The present study aimed to assess welfare level in a fishery from southern area of Romania, populated with common carp, silver and grass carp, based on water chemical and microbiological quality and to relate water quality, as fish life environment, with biochemical serum profile.

MATERIALS AND METHODS

In order to assess the fish welfare, from the three ponds of the fishery were harvested water samples and there were analyzed physical-chemical parameters: pH, dissolved oxygen, residual chlorine, total phosphorum, sulphates, nitrates, nitrites, phenols, copper and total ferrum, as well as microbiological parameters: Aerobic Plate Count - APC, Fungal Plate Count - FPC and total coliforms. The physical chemical parameters analysis was done using Spectroquant Nova 60 fotocolorimeter and the results were compared with limits for carp rearing or with limits provided by 1146/2002 Order for surface waters quality.

In order to establish APC, FPC and total coliforms, there were used rapid assays, respectively Compact Dry TC (Total count), YM (Yeast and Molds) or CF (total coliforms). The results, namely total coliforms, because for APC and FPC are not provided limits in national law, were compared with the provisions of 1146/2002 Order.

The second part of the study implies establishing the serum biochemical profile. There were harvested by angling 2 fish (silver carp individuals: *Hypophthalmichthys molitrix*) from each pond and after Finquel anestezy (MS - 222) 100-200ppm concentration in water, they were emersed and blood was collected by caudal vein puncture approcah (CVP): from each fish maximum 2 ml of blood. After syneresis, there was established the biochemical profile on 1:10 diluted samples by using Vettest 8008. There were analyzed 8 blood chemistry indicators: albumine, phosphatemia, uric acid, cholesterol, triglycerides, glucose, aspartate aminotransferase and alanine transaminase.

The results were compared with the values in the literature, although often contradictory.

RESULTS AND DISCUSSIONS

The results of water physical chemical analyze are shown in the following table.

Table 1. Average values of water physical chemical parameters

Sampling point	Assessed parameters									
	pH	**O_2 (mg/l)**	**Cl_2 (mg/l)**	**P total (mg/l)**	**SO_4^{2-} (mg/l)**	**NO_3^- (mg/l)**	**NO_2^- (mg/l)**	**Phenols (mg/l)**	**Cu (mg/l)**	**Total Fe (mg/l)**
Fish pond 1	7.7	9.1	0.3	1	26	20	0.035	0.27	0.13	0.19
Fish pond 2	8.1	12	0.27	1	29	20	0.022	0.26	0.14	0.13
Fish pond 3	7.8	6.7	0.2	0.8	20	19	0.021	0.39	0.09	0.16
Maximum admitted limits for carps rearing	5.5-8	Min. 4	0.01-0.03	0.6-1	N/A*	50	0.2	6-17 ng/l†	0.3	2

* Values up to 80 mg/l framed the water quality in first class according the 1146/2002 Order

† Values higher than 0.05 mg/l (50 μg/l) framed the water quality in the fifth class according the 1146/2002 Order

The water meets the requirements for an optimum carp rearing for the most parameters with two exceptions: chlorines which exceed the admitted limits for carp rearing up to 10 times and phenols which registered exceeding even higher, framing the water within the fifth quality class according to 1146/2002 Order. The higher concentrations of phenols are in fishpond 2 and 3: 0.26 mg/l and respectively 0.39 mg/l.

This exceeding most likely affects fish welfare. Thus, a longtime exposure to 0.04 – 0.2 mg/l active chlorine concentration may result in nervous troubles, agitation, jumps outside the water, convulsions, lateral position, spasmodic mouth movements, fins uncoordinated moves, respirator troubles and eventually death. The skin and gills are covered with a thick mucus layer, could appear branchial congestion or haemorragies.

A nervous toxic to fish, phenols presence in ecosystem is due to the activity of chemical, petrol or pharmaceutical industries also could be related with the production and degradation of numerous pesticides. Phenol toxicity is related with two main processes: unspecified toxicity related with hydrophobocity of the individual compound and formation of free radicals.

Concerning the water microbiological parameters (table 2), there was noticed a 2.7 – 4.9 times exceeding of the reference value for second quality class water according to 1146/2002 Order.

Table 2

Average values of water microbiological parameters

Sampling point	**Assessed parameters**		
	Total coliforms (no./ml)	**APC (colonies/ml)**	**FPC (colonies/ml)**
Fish pond 1	490	500	51
Fish pond 2	270	495	21
Fish pond 3	315	525	25
Admitted limits according the 1146/2002 Order	500/100 ml: Quality class I 10000/100 ml: Quality class II Not provided: Quality class III-V	N/A	N/A

It was also being recorded a high number of colonies for aerobic plate count and fungal plate count. The high microbial load must be related to animal origin pollution.

Microorganisms find in water appropriate conditions to develop. They can have autochthon origin, forming specific biocenoses (planktonical, neustonical, benthal, epibiotical) and allochthon origin (those which come accidentally in the water from soil, carried by the wind or from precipitations' water, generated by men, animals or industrial activities or ubiquitary - living everywhere: in air, on soil or in water). From the autochthon microorganisms, could be mentioned bacteria, fungi, as well as protozoans and algae. In water are present all the physiological categories of bacteria (heterotrophic, autotrophic, chemoautotrophic etc.). The yeasts and molds from the water could result from soil or - the pathogenic ones - from ill organisms (plants or animals).

Table 3. Serum profile of the fish sampled in the assessed fishery

Blood parameter	Pond	Sample	Obtained value	Reference level
ALB Albumine g/dl	Fish pond 1	Sampe 1	1.	0.45 – 0.85
		Sample 2	0.	
	Fish pond 2	Sampe 1	2.	
		Sample 2	2.	
	Fish pond 3	Sampe 1	1.0	
		Sample 2	1.1	
PHOS Phosphatemia mg/dl	Fish pond 1	Sampe 1	19	6.2 – 9.3
		Sample 2	15.6	
	Fish pond 2	Sampe 1	22.1	
		Sample 2	20.7	
	Fish pond 3	Sampe 1	16.1	
		Sample 2	23.9	
URIC Uic acid mg/dl	Fish pond 1	Sampe 1	0.6	N/A
		Sample 2	0.8	
	Fish pond 2	Sampe 1	2.1	
		Sample 2	2.3	
	Fish pond 3	Sampe 1	0.9	
		Sample 2	0.4	
CHOL Cholesterol mg/dl	Fish pond 1	Sampe 1	278	150 – 240
		Sample 2	100	
	Fish pond 2	Sampe 1	242	
		Sample 2	243	
	Fish pond 3	Sampe 1	86	
		Sample 2	63	
TRIG Triglycerides mg/dl	Fish pond 1	Sampe 1	317	N/A
		Sample 2	92	
	Fish pond 2	Sampe 1	236	
		Sample 2	233	
	Fish pond 3	Sampe 1	94	
		Sample 2	98	
GLU Glucose mg/dl	Fish pond 1	Sampe 1	237	70 - 130
		Sample 2	113	
	Fish pond 2	Sampe 1	190	
		Sample 2	206	
	Fish pond 3	Sampe 1	250	
		Sample 2	153	
AST Apartate aminotransferase U/l	Fish pond 1	Sampe 1	29	23 – 42
		Sample 2	55	
	Fish pond 2	Sampe 1	25	
		Sample 2	29	
	Fish pond 3	Sampe 1	49	
		Sample 2	129	
ALT Aanine Transaminase U/l	Fish pond 1	Sampe 1	393	130 - 350
		Sample 2	473	
	Fish pond 2	Sampe 1	-	
		Sample 2	590	
	Fish pond 3	Sampe 1	620	
		Sample 2	-	

In table 3 there is shown the serum biochemical profile of silver carp individuals sampled in the three ponds of the fishery. We used as reference values the ones from the control batches in the literature, although often contradictory.

At the first hand, could be noticed a high variability of the results, this fact suggesting that individuals have different body conditions, some having surely poor body condition and welfare.

If the serum phosphate and albumine modifications is most likely caused by the intense muscular effort associated with fish collecting (by angling) and restraining, doubled by a mild hemoconcentration caused by maintaining the fish emersed, the transaminases and glucose increasing are caused by phenols toxicosis, having in view that the higher transaminases values: 620 UI/l ALT and 89 U/l AST mean value are registered for fish collected from third pond, the most contaminatend with phenols (0,39 mg/l).

These modifications of serum profile shows hepatopancreatic disorder, hence a poor fish welfare.

CONCLUSIONS

1. Concerning chemical water quality, there were registered for chlorines exceeding of the admitted limits for carp rearing up to 10 times and for phenols the admitted limits from 1146/2002 Order (water in the fifth quality class).
2. Concerning the microbial load, total coliforms are 2.7 – 4.9 times higher than the reference value for second quality class water according to 1146/2002 Order.
3. The water inappropriate parameters reflects in a serum profile modification: noticeable is an exceeding of transaminases activity, most likely due to phenols toxicity.
4. The fish welfare level is poor, so immediate action must be taken in order to improve it: water decontamination from phenols is essential.

ACKNOWLEDGMENTS

This study has been financed and is a part of ID_285 grant CNCSIS UEFISCSU, Idei 290/2007 contract: *Researches concerning the welfare of fish in farms, during transportation and in slaughtering units*

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RESEARCHES REGARDING THE SENSITIVITY TO ANTIBIOTICS OF SOME BACTERIAL STRAINS ISOLATED FROM MASTITIS COW MILK

G. C. Nadăş, N. Fiţ, F. Chirilă, S. Răpuntean, V. Rus
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca
3-5 Mănăştur Street, Faculty of Veterinary Medicine, gnadas@usamvcluj.ro

Abstract: This study considered 22 mastitis cow milk samples that were examined using bacterioscopic, bacteriologic and biochemical tests regarding the bacterial genera involved in mastitis etiology. For each isolate, the sensitivity to antibiotics such as Enrofloxacin, Oxitetracicline, Amoxicillin, Ceftiofur, Erythromycin, Amoxicillin Clavulanate and Tetra Delta using the diffusimethric standardized method were also determined. Bacteria genus most frequently isolated were represented by Staphylococcus spp. in 11 (50%) samples, followed by Streptococcus spp. in 9 (40,9%), Escherichia coli in 5 (22,7%), Bacillus spp. in 4 (18,18%), Micrococcus spp. in 1 (4,54%) and Candida spp. also in 1 sample. Regarding the sensitivity to antibiotics, the most efficient was Amoxicillin Clavulanate with an average of the inhibition area diameter of 21 mm followed by Enrofloxacin with 17,90 mm diameter, Amoxicillin with 17,68mm, Tetra Delta with 17,18mm, Erythromycin with 13,77mm. At the opposite pole situated Oxitetracicline with an average of 10,5 mm diameter and Ceftiofur with 6,5 mm.

Key words: mastitis, cow, bacteria, antibiotics.

INTRODUCTION

Antimicrobial agents are widely used for the treatment of bovine mastitis, respiratory tract infections and diarrhea in cattle. During acute infections and outbreaks of infectious disease in groups or herds it is important to use an effective antimicrobial treatment as early as possible. This empirical treatment is generally based on knowledge of the resistance pattern of the different bacterial pathogens to antimicrobial agents used in the particular animal species. Antimicrobial resistance is an increasingly important problem among several bacterial species causing infection in animals and humans in recent years. The problem for some bacterial species is so critical that there is few treatment options left.

The initial treatment of animals is commonly based on the experience regarding the expected resistance of the infectious agent. The fact that occurrence of antimicrobial resistance varies between countries and regions has the potential to complicate that matter. Furthermore, knowledge of expected resistance is limited by the small proportion of different bacterial pathogens from infected animals that actually are investigated for their antimicrobial resistance pattern.

The intensive use of antibiotics in both public health and animal husbandry has selected for antibiotic-resistant bacteria. Under antibiotic selective pressure, bacteria have the ability to develop and exchange resistance genes, making them nonsusceptible to the antimicrobial substances deployed. While antibiotic resistance has emerged in some important animal and human gram-positive pathogens, such as *Staphylococcus* and *Streptococcus* spp. and *Clostridium perfringens*, others, such as *Bacillus anthracis*, are currently still sensitive to antibiotics.

The aim of the study was the identification of the most efficient antibiotic that is to be used in case of acute mastitis, prior to the time elapsed for the isolation and sensibility tests are available.

MATERIALS AND METHODS

The researches took place between March 2009 and April 2010 within the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca. 22 mastitis cow milk samples collected in sterile tubes were cultured on glucose agar and blood agar, and for each sample the identification of the bacterial specie involved in mastitis etiology was made by microscopical, cultural and biochemical examination. For each bacteria isolated, the tests regarding its sensibility to antibiotics was realized using the diffusimethric method. The suspension from the tested germ at 1 tube of McFarland scale turbidity was sowed on a plate with glucose agar, then the surface dried, followed by the antibiotics positioning by the standardized method. The antibiotics used were represented by Enrofloxacin (ENR), Oxitetracicline (OXT), Amoxicillin (AML), Ceftiofur (CFT), Erythromycin (ERY), Amoxicillin Clavulanate (AMC) and Tetra Delta (NP-SN). After an incubation of 24 hours at 37°C the interpretation was made by measuring the inhibition areas around each micro disc.

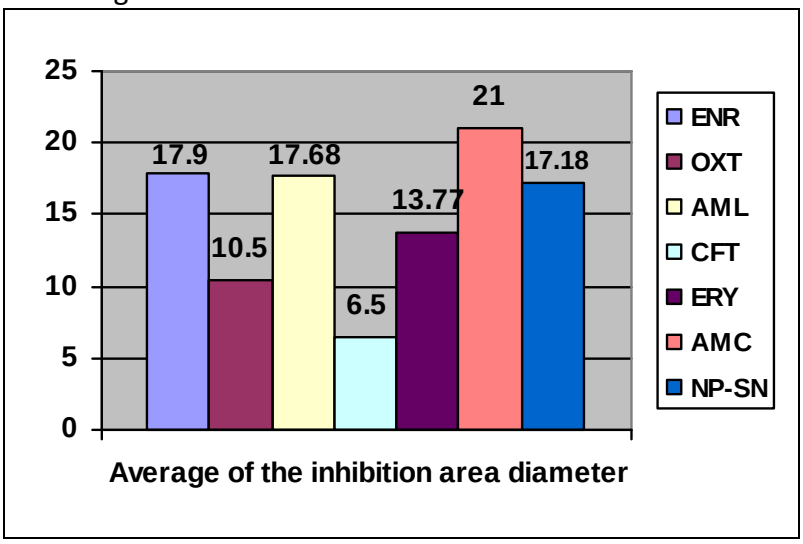
Results and discussions: The bacterial species isolated from the samples taken into study and their sensibilities to antibiotics are presented in table 1.

Table 1. Mastitis etiology and germs sensibility to antibiotics

Sample number	Mastitis etiology	Sensibility to antibiotics (inhibition area diameter in mm)						
		ENR	OXT	AML	CFT	ERY	AMC	NP-SN
1	Streptococcus spp. + B. cereus	16	28	R	R	18	20	12
2	Streptococcus spp.	12	13	18	R	R	20	R
3	Staphylococcus spp.	27	R	11	16	20	16	20
4	Streptococcus spp.	16	16	20	R	18	22	25
5	Staphylococcus spp.	18	19	30	18	24	31	32
6	Streptococcus spp.	18	R	20	14	28	24	28
7	Escherichia coli	25	R	15	R	R	17	R
8	E. coli + Staphylococcus spp.	18	15	14	16	17	18	R
9	E. coli + Streptococcus spp.	13	R	16	R	R	18	12
10	Streptococcus + Staphylococcus	13	13	20	16	20	25	16
11	Streptococcus + Staphylococcus	23	18	26	19	22	30	24
12	Escherichia coli	24	18	11	R	10	16	12
13	Staphylococcus spp.	15	R	15	R	R	18	15
14	Staphylococcus spp.	15	R	19	14	14	28	25
15	Staphylococcus spp.	17	R	16	R	R	18	20
16	E. coli + Candida spp.	R	R	18	R	16	22	24
17	Staphylococcus spp.	17	14	24	14	R	21	16
18	Bacillus spp. + Micrococcus spp.	27	22	R	R	18	R	26
19	Staphylococcus + Bacillus spp.	25	18	33	R	23	35	22
20	Streptococcus spp.	15	R	20	R	16	17	R
21	Staphylococcus + Bacillus spp.	20	15	11	R	20	16	16
22	Streptococcus spp.	20	22	32	16	19	30	23

Bacteria genus most frequently isolated were represented by *Staphylococcus spp.* in 11 (50%) samples, followed by *Streptococcus spp.* in 9 (40,9%), *Escherichia coli* in 5 (22,7%), *Bacillus spp.* in 4 (18,18%), *Micrococcus spp.* in 1 (4,54%) and *Candida spp*. also in 1 sample. The isolation of *Staphylococcus spp.* in 50% of the samples is not a surprise since the literature data is presenting the same isolation percentage. There is also the issue of isolating mostly in persistent bovine mastitis of small colonies of *Staphylococcus spp.,* so called dwarf colonies, important contributor to the prolonged survival of *Staphylococcus spp.* in some cases of mastitis.

Chart 1. Average of the inhibition area diameter for the antibiotics tested



As presented in chart 1, the most efficient antibiotic was Amoxicillin Clavulanate with an average of the inhibition area diameter of 21 mm, followed by Enrofloxacin with 17,90 mm diameter, Amoxicillin with 17,68mm, Tetra Delta with 17,18mm, and Erythromycin with 13,77mm. At the opposite pole situated Oxitetracicline with an average of 10,5 mm diameter and Ceftiofur with 6,5 mm.

CONCLUSIONS

1. Bacteria genus most frequently isolated from the samples of mastitis cow milk was *Staphylococcus spp.* in 11 (50%), followed by *Streptococcus spp.* in 40,9%, *Escherichia coli* in 22,7%, *Bacillus spp.* in 18,18%, *Micrococcus spp.* and *Candida spp*. in 1 (4,54%).
2. The best sensibility to antibiotics was observed for Amoxicillin Clavulanate followed by Enrofloxacin, Amoxicillin and Tetra Delta.

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EVALUATION OF PHOSPHORUS LEVELS IN LAMB BY LUCERNE HAY FEEDING TESTS

Aritina NEAGU, V.CRIVINEANU, G.V. GORAN
Facultatea de Medicină Veterinară Bucureşti,
Splaiul Independenței, nr. 105, sector 5, 050097, Bucureşti,
ggoran@fmvb.ro

ABSTRACT
The main goal of this study was monitoring the phosphorus levels in lamb by various lucerne hay feedlot feeding trials, as a result of fertilizing or not the soil. Fertilization the soil with phosphate influence the feeding value of the forage, determining improved performance of animals fed fertilized hay compared to unfertilized hay of lower phosphorus content and sometimes toxic accidents.
Hay produced on virgin sandy deserted soil which contained 0.10% phosphorus was unpalatable and produced poor animal gains, feed utilization and depressed serum phosphorus content. The addition of disodium phosphate to this hay improved animal gains, feed utilization and increased the blood serum phosphorus. Hay containing 0.15% phosphorus was apparently equal in feeding value to hay containing greater amounts of phosphorus. An increased retention of phosphorus was obtained by adding disodium phosphate to a low phosphorus hay in one test, in another, the addition of disodium phosphate to unfertilized hay resulted in increasing the phosphorus retention up to that of heavily fertilized hay.

Key words: phosphorus, fertilizer, poisoning, sheep

Phosphorus deficiency in soils and the forage produced there from and a phosphorosis in grazing animals has been widely recognized in many parts of the world. The use of phosphate fertilizers as a means of correcting soils low in phosphorus is a common practice. Some authors have summarized the literature on the effects of fertilization with phosphate on the feeding value of the forage, and they reported improved performance of animals fed fertilized hay compared to unfertilized hay of lower phosphorus content, and sometimes toxic accidents.

Fertilizer trials demonstrate marked yield increases when lucerne was fertilized with phosphorus-containing fertilizers. Farmers generally believe that the feeding value of the lucerne is improved as well as the yield as a result of the application of fertilizer. This paper reports a series of tests conducted at a private farm to explore differences in feeding value of lucerne produced on soils with various fertilization history.

MATERIAL AND METHODS

Chemical analyses and feedlot feeding trials were used to test the various feeds. To maintain continuity the various trials are reported chronologically, however, certain techniques were common to all the work and will be reported first to avoid repetition. In feeding trials all animal weights were obtained after 24 hours without feed and 12 hours without water. All animals were randomly assigned to treatments. Seven-day preliminary and 7-day collection periods were used. Phosphorus determinations in all material were based on ICP-OES method. Other chemical analyses were made by usual methods. Blood samples

collected from animals on test were refrigerated, the serum collected and transmitted for analysis.

First Sheep Feeding Test. In the first sheep test the hays that were high phosphorus hay produced at the farm from fertilized soil and the low phosphorus hay produced on the virgin deserted soil were ground and pelleted for the test. In addition, the low phosphorus unfertilized hay was supplemented with disodium phosphate to bring the phosphorus content up to the level found in the fertilized hay. An additional two lots were fed hay grown on a plot which had previously shown a phosphorus fertilizer response but had not been fertilized for five years. One lot was fed unsupplemented pelleted hay containing 0.15 % phosphorus. The other lot was fed the same hay but with sufficient disodium phosphate added to bring the phosphorus level up to the fertilized farm hay (23% P). Phosphorus and calcium levels of the pellets fed in this first sheep test are shown in table 1.

Second Sheep Feeding Test. As the results of the above trial were not conclusive another attempt was made to obtain hay from the area where the original deserted soil hay was grown. In the meantime two growing seasons had passed and the ownership of the land had changed. The new owner was most cooperative and set aside an area for experimental use. He understood that 45 kg of liquid phosphate (52% P) had been applied to the area two seasons before and knew that 90 kg had been applied the previous growing season.

Table 1

Composition of feeds used in first sheep feeding test (dry basis)

	Fertilized farm hay	**Unfertilized farm hay**	**Unfertilized farm hay + phosphorus**	**Unfertilized deserted soil hay**	**Unfertilized deserted soil hay + phosphorus**
P content of hay, %	0.23	0.15	0.22	0.10	0.23
Calcium, %	1.57	1.66	1.57	5.37	5.26

To obtain forage from this area of varying phosphorus contents, the area was divided into 3 plots. About half the area was left unfertilized; one-fourth was fertilized with 270 kg of treble super phosphate per acre and the remaining one-fourth with 105 kg of treble super phosphate. The third cutting of lucerne from the area was used for the second sheep feeding trial.

All plots were cut at the same time when the lucerne was at abrupt the one-tenth bloom stage. The hay was raked carefully so as to pick up as little sand as possible. The hay was then baled, ground and pelleted. After grinding, the hay from the unfertilized area was divided into two lots. One lot received added disodium phosphate to bring the phophorus content up to that of the hay grown on the plot receiving the largest amount of fertilizer. The composition of the pelleted feed is shown in table 7.

Table 2

Composition of feeds used in second sheep feeding test (dry basis)

	Heavily fertilized deserted soil hay**	**Lightly* fertilized deserted soil hay**	**Unfertilized deserted soil hay**	**Unfertilized deserted soil hay + phosphorus**
P content of hay, %	0.22	0.17	0.15	0.25
Calcium, %	1.40	1.30	1.20	1.10

* 105 kg treble super phosphate per acre.

** 270 kg treble super phosphate per acre.

RESULTS AND DISCUSSIONS

As pointed out in other publications, evaluation of forages from soils receiving differing treatments involves many complications. While phosphorus was the most obvious difference in the composition of the hays involved in this series of trials, other constituents of the feed also varied. The extreme unpalatability of this hay cannot be explained with the information available.

The addition of disodium phosphate to this hay before pelleting improved its palatability only slightly. The increased gains, feed efficiency and blood serum phosphorus levels resulting from the addition of the disodium phosphate clearly indicate that phosphorus was at least one of the reasons for the poor animal response to the original hay.

The lack of animal response to the addition of disodium phosphate to the unfertilized farm hay and the deserted soil hay in the second lamb feeding trial appears to indicate that 0.15% phosphorus is not limiting for growing lambs. The response to the disodium in the original deserted soil hay (first lamb feeding trial) suggests that 0.10% phosphorus is limiting. The daily intake of phosphorus on the deserted soil hay was much lower because of the lower feed intake. In the first lamb feeding trials the lambs on the feed containing 0.15% phosphorus ate 1,53 kg of feed per day to get approximately 2.31 g P daily or about 2.75 g per 45 kg body weight. In the second lamb feeding trial the lambs ate 1,77 kg of feed per day to get 2.67 g P daily or 4.09 g per 45 kg live weight. On the unfertilized desert hay in the first trial when the lambs only consumed 1 kg of the hay containing 0.10% phosphorus their daily intake was only 1.02 g or 1.46 g per 45 kg body weight. Some authors state that 0.15% is the minimum phosphorus level for fattening lambs and that 2.17 g daily per 45 kg live weight will meet their phosphorus requirement. It is doubtful if the depressed intake was due to a phosphorus deficiency in the animals, however, because the sheep showed an aversion to this hay immediately rather than after being fed on it long enough to develop a deficiency.

First Sheep Feeding Test. In addition to the differences in phosphorus content the unfertilized hay from the deserted soil was much higher in calcium (See also table 2). The results of the lamb feeding trial are shown in table 3. There was essentially no difference in the performance of the lambs on the two farm hays even with disodium phosphate added to the hay which was somewhat lower in phosphorus. This appears to indicate that under the conditions of this trial 0.15% phosphorus was adequate for the lambs.

The unfertilized deserted soil hay was unpalatable even in pelleted form. The addition of disodium phosphate increased its palatability only slightly as judged by the average daily

feed intake. Lambs fed this hay used their feed more efficiently than the lambs fed the same hay without phosphorus.

The average daily gain was significantly improved by the additional phosphorus, yet both lots receiving the deserted soil hay still gained very significantly less than the lambs fed the various forms of farm hay. The feed required per 45 kg of gain is markedly in favor of the lambs fed the farm hays, however, when the feed efficiency is expressed on the basis of the organic matter required per 45 kg of gain the deserted soil hay with the supplemental phosphorus compares favorably with the efficiency of the farm hay.

Table 3

Results of first sheep feeding test (112 days)

	Fertilized farm hay	**Unfertilized farm hay**	**Unfertilized farm hay + phosphorus**	**Unfertilized deserted soil hay**	**Unfertilized deserted soil hay + phosphorus**
Number of lambs	9	10	10	9	10
Av. initial weight, kg	29.2	28.2	27.9	28.44	28.35
Av. final weight, kg	51.57	47.47	47.83	34,56	39.46
Av. daily gain, kg	0.19	0.17	0.18	0.05[a]	0.09[b]
Av. daily feed, kg	1.55	1.53	1.56	1.01	1.04
Feed per 45 kg gain, kg	351	400	396	837	476
Salt consumption per lamb, kg	0.45	0.9	0.27	2.2	1.35
Blood serum phosphorus (mg/100 ml sg)					
Initial	6.48	6.45	6.09	6.20	5.67
Final	6.11	6.67	7.11	4.41[c]	7.86

[a] Gain significantly less (P<0.05) than unfertilized deserted soil hay plus phosphate
[b] Gain significantly less (P<0.01) than on farm hays
[c] Serum phosphorus significantly lower (P<0.01) than other lots.

The final blood serum phosphorus values for the lot fed the unfertilized desert hay were significantly lower than the values for the other lots. The addition of the disodium phosphate to this hay prevented this drop in blood phosphorus. The salt consumption of the various lots in this trial was measured. The salt consumption per lamb was markedly high in the lot fed the unsupplemented-unfertilized deserted soil hay. The addition of the disodium phosphate reduced the salt intake appreciably but it was still higher than in the lots fed the farm hay. It is interesting to note that the addition of disodium phosphate to the farm hay also reduced the salt intake compared to the lambs fed the same hay without the additional disodium phosphate. Perhaps the additional sodium in the disodium phosphate decreased the salt need of the sheep.

All sheep completed the trial on the farm hays without incident, but trouble was encountered in getting the sheep to eat a constant intake of the deserted soil hay either with or without the added phosphorus.

After repeated efforts two sheep finished on the unfertilized deserted soil hay and three finished on the same hay plus the disodium phosphate. The phosphorus retention by the lambs fed the deserted soil hay plus sodium phosphate was significantly higher than the retention by the lambs fed the other rations. The calcium retention was very significantly higher by the lambs fed the deserted soil unfertilized hay both with and without the supplemental phosphorus.

Second Sheep Feeding Test. The phosphorus content of this unfertilized hay was not as low as that obtained for the earlier experiment. Evidently the fertilizer applied the two seasons previously carried over enough to correct the very low level found in the original hay. The lower level of fertilizer only improved the phosphorus content slightly while the heavy application of fertilizer markedly increased the phosphorus content. In other respects the composition of the hay produced on the three plots varied only slightly. The calcium was much lower than in the hay from the same area used in the earlier test. The pelleted hays were fed to feeder lambs with the results shown in table 4. There was little, if any, difference in the response of the lambs to the four lots of feed.

Although the data show a slight advantage in favor of the unfertilized hay with the added phosphorus, the difference was not significant statistically. Much of the difference in gain could be accounted for by one lamb which gained poorly.

Table 4. Results of second sheep feeding test (64 days)

	Heavily fertilized deserted soil hay**	**Lightly* fertilized deserted soil hay**	**Unfertilized deserted soil hay**	**Unfertilized deserted soil hay + phosphorus**
Number of lambs	10	9	10	10
Av. initial weight, kg	34,4	33.1	33.1	32.6
Av. final weight, kg	45.3	44.9	43.6	45.5
Av. daily gain, kg	0.17	0.18	0.16	0.2
Av. daily feed, kg	1.89	1.93	1.77	1.9
Feed per 45 kg gain, kg	503	471	488	425
Blood serum phosphorus (mg/100 ml sg)				
Initial	7.5	7.4	7.3	7.5
Final	7.5	7.2	7.7	7.5

The phosphorus retention was again high in the lambs fed the feed supplemented with disodium phosphate. In this trial the retention by the lambs fed the heavily fertilized hay was also high, but very low in the lambs fed the hay from the plot that received a light

application of fertilizer. The calcium retention was very different from that found in the previous test.

In the first test large amounts of calcium were retained from the unfertilized hay with and without phosphorus added but in the second trial the unfertilized hay both with and without added phosphorus had negative balances.

CONCLUSIONS

1. Feeding trials with sheep were conducted on lucerne hay of varying phosphorus content to study its nutritive value.
2. Hay produced on virgin sandy deserted soil which contained 0.10% phosphorus was unpalatable and produced poor animal gains, feed utilization and depressed serum phosphorus content.
3. The addition of disodium phosphate to this hay improved animal gains, feed utilization and increased the blood serum phosphorus.
4. Hay containing 0.15% phosphorus was apparently equal in feeding value to hay containing greater amounts of phosphorus.
5. An increased retention of phosphorus was obtained by adding disodium phosphate to a low phosphorus hay in one test, in another, the addition of disodium phosphate to unfertilized hay resulted in increasing the phosphorus retention up to that of heavily fertilized hay.

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SOME INVESTIGATIONS IN CONSUMPTION SEA FISH (HERRING, MACKEREL)

O. Negrea [1], Camelia Raducu[1], L. Oana[2], Vioara Miresan[1], Z. Marchis[1], Gh. Mihai[1], F.Chirila[2], Ancuța Rotar[1]

[1]U.S.A.M.V. Cluj, Faculty Animal Sciences and Biotehnology, 3-5 Manastur street, 400372 Cluj-Napoca, Romania onegrea50@yahoo.com

[2]U.S.A.M.V. Cluj, Faculty of Veterinary Medicine,3-5 Manastur street, 400372 Cluj-Napoca, Romania

Abstract: The study was effected on a sample of 43 sea fish, uneviscerated and freeze, destined to human consumption (25 fish of mackerel species and 18 of herring species), proceeded from Markets, concerning incidence and intensity of acanthocephalosis in some morphological determinations for parasite species establishment and also lesions table, and puts in evidence some aspects. The incidence of acanthocephalosis in sea fish, on fish sample taken in study, presents a high percent in both species (76% mackerel and 83.3% herring). Under report of parasitism intensity, dominant are the reduced infestations, in both sea species (73% mackerel and 60% herring) followed by medium infestations (15.7% mackerel and 26.6% herring) and massive infestations (10.5% mackerel and 13.5% herring). Morphological determinations obtained for acanthocephal species establishment, regarding following parameters: body mean length 18.7 mm, cephalic tube mean length 1.93 mm, hook ranges 14, hook number on each range 20 – 25, also taken in view the body colorization pink to reddish and compared with speciality literature, establishes as acanthocephal parasite species **Rhadinorhynchus pristis**. Morpho-pathologic table puts in evidence lesions of haemorrhagic enteritis and haemorrhagic necrosis in pesthole to parasite fixation place and nodular lesions in muscle as a result of penetration of intestine wall by migration larva (acanthales).

Key words: acanthocephal, incidence, cephalic tube, hooks

MATERIAL AND METHODS

Investigations were effected on a sample of 43 consumption sea fish, uneviscerated and freeze in polyethylene bags 3 – 5 pieces / bag mackerel 25 pieces, herring 18 pieces), proceeded from Market type stores network in Cluj-Napoca. After thawing, with the help of scalpel general cavity is opened and it is dissected the intestine in length following by macroscopic exam and exam with magnifying glass intestine content and intestinal wall modifications, following extensivity level and intensity of parasitism with acanthocephales, lesions table and establishment of parasite species, with the help of anatomic clamp, parasite are sampling which are introduced in Petri plates for preservation in formol solution 10%.

For species establishment were effected morphologic determinations on a sample of 15 parasites taken from intestine, having in view: body color, parasite and cephalic tube length (biometers with millimeter ruler), number of hook ranges and total hook number on range (microscope, ob.3.2x).

In parasitism intensity appreciation with acanthophiles it was taken into account the number of parasites found in intestine, considering:

- Massive infestations: above 10 parasites/fish
- Medium infestation: between 5 – 10 parasites/fish
- Reduced infestation: under 5 parasites/fish

RESULTS AND DISCUSSIONS

Investigations done on a 43 consumption sea fish sample, uneviscerated and freeze , concerning the incidence and intestinal parasite intensity with species of acanthocephales, and also morphologic determinations for parasite species identification, respectively lesions table brought about, puts in evidence the following aspects:

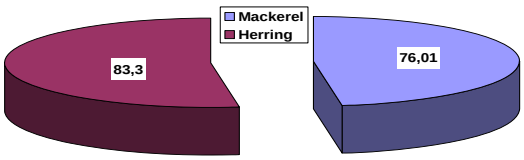
A. Parasite spreading with acanthocephales presents values which are shown in table 1 and graphic 1.

Table 1. **Parasite spreading with acanthocephales in consumption sea fish.**

Species	Number of fish examinated	From which	
		positive	%
Mackerel	25	19	76.0
Herring	18	15	83.3
total	43	34	79.0

Data presented in table 1. put in evidence a high level of acanthocephalosis incidence in sea fish, 76% in mackerel and 83.3% in herring, with an average of 79.0%. It must be mentioned the fact that high level of parasitosis in sea fish (mackerel, herring, hake, Thrachurus med., etc) is the result of biologic cycle of parasite. It is known the fact that acanthocephales are worms that in their cycle of development need 2 hosts (dixen cycle): one which is intermediate represented by crustaceae, aquatics amphipodes and isopodes and one which is definitive, represented by vertebrates, as are the fish, ichtyophages birds and mammals. Crustaceae takes from water the parasite eggs and into their body is formed the infestant larva - acanthela.

As these crustaceae represent the source of food for predatory fish, quantity consumed and especially the number of acantheles presented, favour the disease incidence. In the picture nearly we present the intestinal infestation variation with acanthocephales in consumption sea fish, function of species.



Picture1. Infestation variation with acanthocephales in consumption sea fish, on species.

B. Regarding infestation level with acanthocephales in consumption sea fish, the values obtained are presented in table 2.

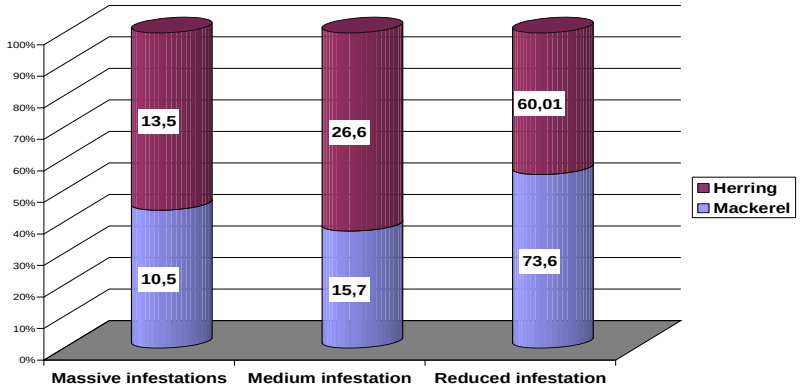
Table 2. **Infestation intensity with acanthocephales in sea fish, on species**

Species	Number of positive cases	From which					
		Low infestation		Medium infestation		Massive infestation	
		positive	%	positive	%	positive	%
Mackerel	19	16	73.6	3	15.7	2	10.5
Herring	15	9	60.0	4	26.6	2	13.5
total	34	23	67.6	7	20.5	4	11.9

Data presented in table 2 put in evidence different values of acanthocephalosis intensity level function of species, as shown:

- In mackerel species, on total fish taken in study are dominant low infestations (73.6%), followed by medium infestations (15.7%) and more reduced the massive infestations (10.5%)
- In herring species, on fish lot taken in study predominant are the low infestations (60.0%), followed by those medium (26.6%) and less the massive infestations (13.5%)

In graphic 2, are presented percent values of parasitism intensity with acanthocephales in species of sea fish.



Picture 2. Intensity level with acanthocephales in species of sea fish

C. Morphologic determinations to identify acanthocephales species parasites in intestine level (body biometers, color, number of hook ranges, etc), on 15 parasites sample, put in evidence the following aspects:

Table 3.

Morphologic determinations of acanthocephales species with intestinal localization in sea fish

Number of sample	Body size		Number of hook ranges	Number of hooks on range	Parasite color
	Body length (mm)	Cephalic tube length(mm)			
1	15.7	2.1	14	22	Pink - reddish
2	18.2	1.8	14	20	Pink - reddish
3	19.0	2.0	14	24	Pink - reddish
4	19.4	2.1	14	20	Pink - reddish
5	20.0	2.1	14	24	Pink - reddish
6	16.6	1.9	14	25	Pink -reddish
7	17.0	1.9	14	24	Pink - reddish
8	19.2	2.0	14	25	Pink - reddish
9	18.6	1.7	14	22	Pink - reddish
10	19.2	2.1	14	25	Pink - reddish
11	20.2	2.0	14	20	Pink - reddish
12	17.4	1.9	14	24	Pink - reddish
13	19.2	2.2	14	23	Pink -reddish
14	20.4	1.8	14	23	Pink - reddish
15	20.2	1.9	14	25	Pink - reddish
total	X 18.7	X 1.93	14	X 23	-

By data obtained and presented in table 3 are evaluated aspects regarding morphology of parasites found, with the purpose of a correct specification of parasite

acanthocephales species. Doing comparison of data obtained (average values), body length (18.7 mm), cephalic tube length (1.93 mm), number of hook ranges (14), number of hooks on range (23), color (pink – reddish), with those from speciality literature (Gabriela Munteanu and Dumitru Bogatu, 2003), we consider that acanthocephales species found in sea fish lot examined is ***Rhadinorhynchus pristis***. (picture 3).

Picture 3. Rhadinorhynchus pristis - cephalic tube (ob. 3.2X)

Concerning the morpho-pathologic table, majority of lesions are present to the level of intestinal mucous membrane, consequent to adult parasite fixation. Pathogenic role is due, in a great measure, to cephalic tube armed with cuticle thorns, which, through a mechanic action irritates, produces inflammations and ulcerates, sometimes, perforations of intestinal wall. It appears lesions of haemorrhagic enteritis and haemorrhagic-necrosis in focus, respectively lesions of hyperthrophic enteritis with a thickness of intestinal mucous, especially in pyloric appendices zone .

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ASPECTS REGARDING EPIZOOTIC METALINGUATULOSA IN MAIN DOMESTIC RUMINANT SPECIES

O. Negrea [1], Camelia Raducu[1], L. Oana[2], Vioara Miresan[1], Z. Marchis[1], V. Miclaus[1], F.Chirila[2], Ancuța Rotar[1]

[1]U.S.A.M.V. Cluj, Faculty Animal Sciences and Biotehnology, 3-5 Manastur street, 400372 Cluj-Napoca, Romania onegrea50@yahoo.com

[2]U.S.A.M.V. Cluj, Faculty of Veterinary Medicine,3-5 Manastur street, 400372 Cluj-Napoca, Romania

Abstracts. Research results on the incidence epizootiological mesenteric limphonodular linguatulosa, performed on 274 cattle, 43 buffaloes, 54 sheep and 38 goats from 4 counties of Transylvania (Cluj, Brasov, Sibiu, Mures), slaughtered in slaughterhouses and cutting point area, put in evidence a visceral linguatulosa incidence in cattle, with different values. The most increased percentage of infestation was reported in cattle in Cluj county (65.2 %), followed descending order by those in Mures county (58.8%) and Brasov county (42.5%), and the average of infestation is of 47.4%. In buffaloes, the incidence of viscerale linguatuloza is much lower than for cattle : from the 43 controled buffaloes, 25.5% were positive, the higher percentage being in Cluj (40.0 %) and the minimal in Brasov (17.8%). Extensivitaty of parasitism in sheep and goats show significant variations depending on species and area of origin. Thus the sheep infestation level was high in populations in Sibiu (45.4%) and low in those in Cluj (33.3%) and medium level was 40.7%. In contrast to goats is higher than the level of infestation of sheep with an average of 60.5%, being increased in goats in Sibiu (63.6%) than in Cluj (56.2%). Epizootiological data obtained reveals that the maximum sensitivity in metalinguatuloza is in goats (60.5%), followed by cattle (47.4%), sheep (40.7%) and buffaloes (25.5%). Parasitism intensivity in large ruminants, with preimaginale stages of L.serrata, checked on 141 positive mesenteric limphonodular masses, presents large differences depending on the species. Maximum infestation level is 47.8% to 18.1% in cattle over buffaloes Reduced infestations had mean values of cattle and 37.6% to 36.5% in buffaloes Infestations were on average 14.6% percent of the cattle. Buffaloes presents both extensivity and intensivity of parasitism, preimaginale forms of L.serrata, lower than other ruminants.

Key words: metalinguatulosis, incidens, lymph – nodes, ruminants, larva stages.

INTRODUCTION

Linguatuloza epizootic disease caused by adult forms of L serrata in carnivores located in the nasal passages and sinuses and larval forms from herbivores and some omnivores limphonodules located mainly in mesenteric, is less studied and researched the world, including in our country.

To date, no systematic research has been conducted on the epizootic parazitoze, consequently missing information on sources of infestation, the mechanisms of transmission and features state of receptivity or resistance of intermediate and final hosts. This prompted us to undertake a comprehensive study on the incidence metalinguatuloza in the main species of domestic ruminants and their responsiveness.

MATERIAL AND METHOD

Investigations regarding the incidence of domestic ruminants metaliguatuloza have been conducted on 274 cattle, 43 buffaloes, 54 sheep and 38 goats from the 4 counties of Transylvania (Cluj, Brasov, Sibiu, Mures), who were slaughtered in slaughterhouses and cutting points.

Steers were slaughtered Holstein breeds, Romanian Spotted and Pinzgau, aged 1 to 12 years by sex and state of maintenance different, bubalinele were common breed, aged 5 to 15 years, sheep, the race Turcan and Pan, aged 4 - years, and goats, the breed common with age 4 - 10 years.

In order to clarify the diagnosis of visceral linguatuloza were harvested after slaughter limphonodulare mesenteric masses, using methods of cutting into slices of watermelon and to assess the level of infestation with limfonodulara larval and nymph stages of the taurine and L serrata buffaloes settled three degrees of infestation:

- low infestation: 1 – 10 larva / mesenteric lymphonodular chain
- medium infestation: 11 – 25 larva / mesenteric lymphonodular chain
- masive infestation: above 26 larva/ mesenteric lymphonodular chain

RESULTS AND DISCUSSIONS

Epizootic study results on metalinguatuloza in ruminants, reveal different aspects related to the areas investigated and are given in Table 1.

Table 1.

Incidence in cattle metaliguatuloza lymphonodulare

Central zone	Animals examined (n)	From which positive	Infestation percentage %
Cluj	23	15	65.2
Brasov	200	85	42.5
Mures	51	30	58.8
TOTAL	274	130	47.4

Different analysis of data submitted is found that the incidence of mesenteric linguatuloza on cattle lymphonodulare show different values depending on the origin.

So, the highest frequency of infestation of 62.5% is in Cluj county, and the smallest, from 42.5% in Brasov county. Extensivity infestations level in Mures county is 58.8% and the overall mean infestation is 47.4% The level extensivity metalinguatuloza limfonodulare mesenteric may be largely influenced by the thermal variation. Thus, in the counties of Cluj and Mures, thermal regime is moderate with small variations in temperature, with mild winters, in Brasov county thermal regime presents special variations, harsh winters with frosts and repeated thaws, that influence different eggs resistance capacity of L. serrata. Linguatuloza lymphonodulare extensivity variation in cattle in correlation with the area of origin is shown in Fig.1

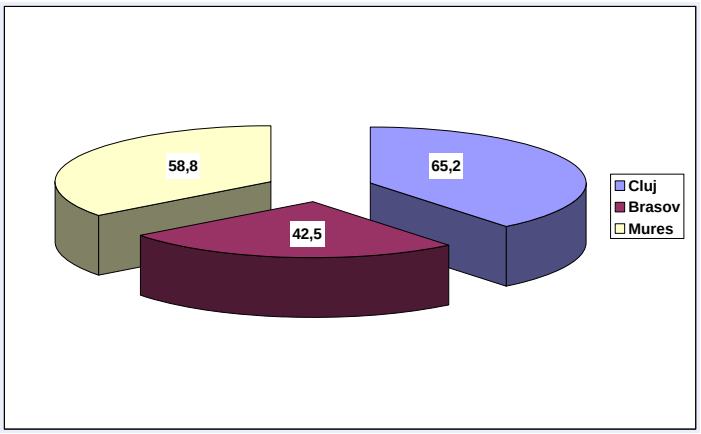

Fig.1. Visceral linguatulosa variation in cattle

Lymphonodulare mesenteric linguatulosa incidence in buffaloes in slaughterhouses of the 2 counties is presented in Table 2.

Table 2.

Visceral linguatulosa extensivety in buffaloes

County controlled	Animals examined	From which positive	% infestation
Cluj	15	6	40.0
Brasov	28	5	17.8
TOTAL	43	11	25.5

Be noted that the buffaloes killed linguatulosa lymphonodulare presents much lower values compared to cattle, average extensivity being 25.5%.

However there are significant differences according to area of origin, with variations from 40.0% extensivity in Cluj county, up from 17.8% in Brasov county.

Suppose that the climate factor, already noted, with variations of temperature and humidity different, pasture areas with much moisture (Olt meadow), zoning growth in buffalo of Fagaras, in the villages surrounding Olt River flood plains, are reducing much the resistance capacity of L.serrata eggs and implicit, the intermediate host contamination. Linguatulosa lymphonodulare extensivity variation in buffalo is shown in Fig.2.

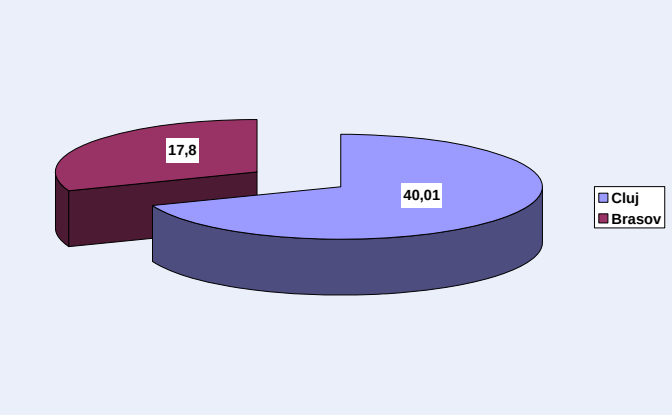

Fig.2. Lymphonodulare linguatulosa incidence in buffalo

In sheep and goats, results of investigations carried out on actual slaughtered in abattoirs in the counties of Cluj and Sibiu, the incidence linguatuloza visceral mesenteric, are presented in Table 3.

Table 3.

Lymphonodulare linguatulosa incidence in sheep and goats

County controlled	Sheeps			Goats		
	Number of samples	From which positive	%	Number of samples	From which positive	%
Cluj	21	7	33.3	16	9	56.2
Sibiu	33	15	45.4	22	14	63.6
TOTAL	54	22	40.7	38	23	60.5

The data presented are found with important differences related to the incidence of parasitic, depending on species and area. Thus, the average values of infestation in sheep are 40.7%, slightly higher in Sibiu county (45.4%) and lowest in Cluj county (33.3%). The average goat infestation is higher than the sheep, of 60.5%; there are significant value differences between the 2 county: 63.6% to 56.2% in Sibiu and respective in Cluj. In temperate climate conditions in our country, when sheep and goats are grazed almost throughout the year, there is a constant risk of contamination with eggs L.serrata. With this occurs also the sensitivity of species. In general, sheep and goats are more susceptible to infestation polyparasitary compared with steers and buffaloes. Metalinguatulosa incidence in sheep and goats is shown in Fig.3.

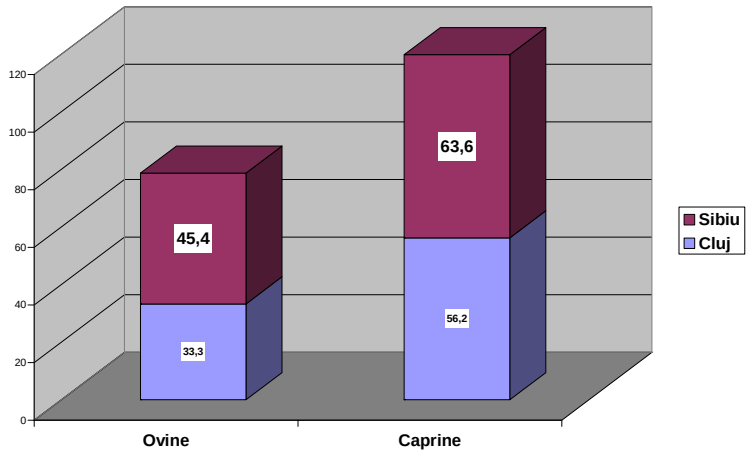

Fig.3. Metalinguatulosa incidence in sheep and goats

Results on the degree of parasitism with intensivity of larval and nymph stages, examination of 141 consecutive positive mesenteric mass lymphonodulare in cattle and buffalo, are given in Table 4.

Table 4.

Parasitism intensivity with larval and nymph stages L.serrata in cattle and buffaloes

Host species	Number of samples	From which					
		Low infestation	%	Medium infestation	%	High infestation	%
cattle	130	49	37,6	20	14,6	61	47,8
buffalo	11	4	36,5	5	45,4	2	18,1

Following examination of the 141 masses lymphonodulare mesenteric, metalinguatuloza positive, the two species taken in the study, results revealed the following: of the 130 tables mesenteric limfonodulare of cattle were identified with 49 masses lymphonodulare, with low infestation (37.6%), 20 masses lymphonodulare with moderate infestation (14.6%) and 61 masses lymphonodulare with massive infestation (47.8%), whom lymphonodules were hypertrophied of soft consistency and in section is running a creamy purulent mass rich in viable larvae.

Buffaloes were found at a low infestation in 4 cases (36.5%), a moderate infestation in 5 cases (45.4%) and massive infestation in 2 cases (18.1%)

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APPLICATIONS OF BIOMEDICAL INFORMATICS AND TELEMEDICINE

T. NICA, V. CRIVINEANU
Faculty of Veterinary Medicine, Bucureşti
Str. Splaiul Independenței, nr. 105, tudornica@rdslink.ro

Abstract: Biomedical informatics is an emerging discipline underlying the acquisition, maintenance, retrieval and application of knowledge and information in research, education, health-related basic sciences, clinical disciplines with computer science, statistics, engineering, mathematics, information technologies and management. Telemedicine is a rapidly developing application of clinical medicine where medical information is transferred through interactive audiovisual media for the purpose of consulting, and sometimes remote medical procedures or examinations. Biomedical and Health Informatics is its interdisciplinary nature: It connects computer science, medicine, biology and health care, and provides a synergy that goes beyond anything that researchers in any single domain can provide.

Keywords: biomedical informatics telemedicine e-health

OBJECTIVES

Health informatics or medical informatics is the intersection of information science, computer science and health care. It deals with the resources, devices, and methods required to optimize the acquisition, storage, retrieval, and use of information in health and biomedicine. Health informatics tools include not only computers but also clinical guidelines, formal medical terminologies, and information and communication systems. It is applied to the areas of nursing, clinical care, dentistry, pharmacy, public health and (bio)medical research.

Biomedical informatics coalesces the related fields of Medical Informatics and Bioinformatics. Health Informatics contains subsets such as Telemedicine, Clinical Informatics, and Dental Informatics, Pharmaceutical Informatics, Nursing Informatics and Public Health Informatics.

Biomedical informatics also provides the tools and skills needed for the development and application of new technology for improving patient care, medical education, health sciences and management for healthcare/hospital systems.

Central to both medical informatics and bioinformatics is the collection and analysis of information. While medical informatics is more concerned with structures and algorithms for the manipulation of the data and how it can be applied in healthcare, bioinformatics is more concerned with the data itself and its biological implications.

MATERIALS AND METHODS

Telemedicine (e-health) has been defined as the use of telecommunication to provide diagnostic and therapeutic medical information between patient and doctor without either of them having to travel. It is the use of information and communication technology (ICT) to deliver health services, expertise and information over distance.

The basic requirements for telemedicine include:

- use of information and communication technology, such as the Internet, is required for long distance health services delivery.
- expert advice must be available at site being referred to.
- sufficient description of the problem being referred or services required from a medical opinion.
- adequate time should be given for assessment of all requests.

The amount of time of would be required for a response from the expert from the other side would depend on the type of telemedicine being practised. There are basically three types of telemedicine:

Store-and-Forward Telemedicine: This is usually used for transferring digital images from one location to another. Images are captured with digital cameras or still video, and sent to another location. They are attached with a summary of the case in text (usually by email). This type of telemedicine is usually not used in emergency situations, as specialist review cases when convenient. There is no limit to the physical distance between the origin and the destination of the image.

Real-time video linking: This method is used when a 'face-to-face' consultation is necessary. It is usually between the patient and their provider in one location and a specialist in another location. Specialized video conferencing equipment at both locations allows a 'real-time' consultation to take place. High bandwidth communication channels (ISDN lines) are necessary, although this type of telemedicine is now within reach of the desktop computers connected to the Internet.

Telemedicine through Remote Network Monitoring. An RMON implementation typically operates in a client/server model. Monitoring devices (commonly called "probes" in this context) contain software agents that collect information and analyze packets. These probes act as servers and the Network Management applications that communicate with them act as clients. The probes have more responsibility for data collection and processing, which reduces SNMP traffic and the processing load of the clients and information is only transmitted to the management application when required, instead of continuous polling.

It is possible to combine any of the three to suit the needs of the organization.

The hospital information systems were first developed in the 1960s and have been an essential part in hospital information management and administration. Early systems consisted of large central computers connected to by dumb terminals, which are now being replaced by networked microcomputers. The systems were used to manage patient finance and hospital inventory.

A hospital information systems (HIS) is a computer system that is designed to manage all the hospital's medical and administrative information in order to enable health professional perform their jobs effectively and efficiently.

Hospital information systems now focus on the integration of all clinical, financial and administrative applications and thus could also be called an integrated hospital information processing systems (IHIPS).

Components of a hospital information system consist of two or more of the following:

- Clinical Information System
- Financial Information System
- Laboratory Information System
- Nursing Information Systems
- Pharmacy Information System
- Picture Archiving Communication System

- Radiology Information System

No hospital information system can be regarded as a success unless it has the full participation of its users. Thus human and social factors would have to be considered in its design, more often than not, they can be easily addressed by providing adequate training and education about the system.

A Clinical Information System (CIS) is a computer based system that is designed for collecting, storing, manipulating and making available clinical information important to the healthcare delivery process.

Clinical Information Systems provide a clinical data repository that stores clinical data such as the patient's history of illness and the interactions with care providers. The repository encodes information capable of helping physicians decide about the patient's condition, treatment options, and wellness activities as well as the status of decisions, actions undertaken and other relevant information that could help in performing those actions.

Some of the areas addressed by Clinical Information Systems are:

- Clinical Decision Support: This provides users with the tools to acquire, manipulate, apply and display appropriate information to aid in the making of correct, timely and evidence-based clinical decisions.
- Electronic Medical Records (EMRs): this contains information about the patient, from their personal details, such as their name, age, address and sex to details of every aspect of care given by the hospital (from routine visits to major operations)
- Training and Research: Patient information can be made available to physicians for the purpose of training and research. Data mining of the information stored in databases could provide insights into disease states and how best to manage them.

An electronic health record is an evolving concept defined as a systematic collection of electronic health information about individual patients or populations. It is a record in digital format that is capable of being shared across different health care settings, by being embedded in network-connected enterprise-wide information systems. Such records may include a whole range of data in comprehensive or summary form, including demographics, medical history, medication and allergies, immunization status, laboratory test results, radiology images, and billing information.

Its purpose can be understood as a complete record patient encounters that allows to automate and streamline workflow in health care settings and to increase safety through evidence-based decision support, quality management, and outcomes reporting.

Information Technology applications are now becoming commonplace in **veterinary practices** that are moving towards becoming paperless as they implement electronic medical records (EMRs) for their patients.

EMRs provide veterinaries with the ability to provide standardized clinical patient records and reports and the opportunity for providing their clients with continuous care education. Some EMRs come with a graphical module that provides charts that clients can take home and see the improvement in the weight of their pets due to treatment or change in diet.

EMRs can come as standalone applications or part of a veterinary practice management system, which provides veterinaries with tools for managing all aspects of the practice, such as billing, printing out of reminders and handouts, decision support and outcomes analysis.

RESULTS, DISCUSSIONS AND CONCLUSIONS

Some of the benefits of telemedicine include the fact that it can lead to improved access to medical care for geographically or socio-economically isolated patients. Also it prevents unnecessary patient travel to secondary or tertiary health care centers, promotes education, prevention, clinical trials and other programs, facilitates community education concerning pertinent health care topics.

Telemedicine can assist preservation of the patient's current provider-to-patient relationship while facilitating access to specialty care and enhances continuing medical education and support for rural-based providers and remove the obstacle of professional isolation. It can effectively utilize medical resources by creating an integrated network of primary, secondary and tertiary care and may help secure the financial stability of rural hospitals and allow patients to obtain medical consultation in their own community.

However, telemedicine also may suffer from a few barriers in its path. Local licensure rules may prohibit a physician from consulting in another area. Reimbursements are slow to come through because of complicated rules and requirements. Cultural resistance, fear of malpractice and the cost and the exclusiveness of the appropriate telecommunications technology are another hindrance. Also regular telephone lines do not supply adequate bandwidth for most telemedical applications. Many rural areas do not have high bandwidth telecommunications access required.

Regarding the Clinical Information Systems, research has been done to show their value and these have highlighted not just the benefits but also the barriers that might be faced by hospitals who implement such systems.

Some of the benefits are:

Easy Access to Patient Data - Clinical Information Systems can provide convenient access to medical records at all points of care. This is especially beneficial at ambulatory points, hence enhancing continuity of care. Internet-based access improves the ability to remotely access such data.

Structured Information - The clinical information captured in Clinical Information Systems is well organised, thus making it easier to maintain and quicker to search through for relevant information. The information is also legible, making it less likely that mistakes would be made due to illegible writing.

Improved Drug Prescription and Patient Safety - Clinical Information Systems improve drug dosing and this leads to the reduction of adverse drug interactions while promoting more appropriate pharmaceutical utilisation.

Despite the benefits being offered by Clinical Information Systems, they are not without the barriers that prevent them from being rolled out in every hospital. These include some of the following:

Initial cost of acquisition - the high cost of basic infrastructure of clinical information technology can be a stumbling block to many healthcare organisations.

Privacy and Security - there are still huge concerns in the healthcare industry about the privacy of patient data on computer systems and how to keep such information secure. The HIPAA and Data Protection Act passed by respective governments in the US and the UK were introduced to address some of these concerns.

Clinician Resistance - clinicians usually have 10-20 minutes to see their patients and if their interactions with a CIS during these sessions proves to be counterintuitive by taking up more time than is necessary, there is bound to resistance to it use.

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BUILDING AND MANAGING AN ONLINE DATABASE OF THE MOST IMPORTANT TOXICOSES IN VETERINARY MEDICINE

T. NICA, V. CRIVINEANU
Faculty of Veterinary Medicine, Bucureşti
Str. Splaiul Independenței, nr. 105, tudornica@rdslink.ro

Abstract : *The objective of this paper is to present a software program designed as an online database consisting of the most important poisonings a veterinarian can encounter in his activity. A short description of the the main components of a computer system is made, followed by the presentation of the database and the interface itself. The program can be accessed online by any interested person, but it is aimed for the use of veterinary clinicians and students mostly.*

Keywords: *online database veterinarian toxicology*

OBJECTIVES

Our target was to build and implement a computer program for diagnosis and emergency therapy in veterinary medicine poisonings. To this end, the first step was compiling a database that stores information about the main poisonings a veterinarian may face in practice. This database is constructed on-line to reap the benefits of Internet communication, both in terms of accessibility to information and opportunity for development and continuous improvement.

MATERIALS AND METHODS

A **computer system** comprises of the computer itself (hardware platform), the programs running on that computer (software component) and the communication network. These three components must be in perfect harmony and work integrated to serve their intended purpose.

Hardware platform. The computing era as we know it kicked off in 1951 with the release of the first commercially successful electronic computer known as UNIVAC 1.

Todays computers are much smaller than any of the earlier computers produced and are no longer beyond the reach of small to medium sized organisation. Till date, computers have been making strides in not only getting smaller (portable microcomputers and handheld computers) but also increasing in processing power. This has led in to an increase in the sophistication of software programs and invariably an increase in the functionality of health information systems.

Those systems are now used in carrying out almost all functions within a hospital (patient appointment and staff scheduling, electronic prescriptions, decision support, insurance billing and electronic medical records).

Software component. At first, computers were programmed by calculating circuits that were manually wired together for mathematical calculations to be performed.

This was followed by the release of the first software by the University of Manchester in England. Series of computer programs followed suit and could be loaded onto the computer through the use of memory tapes and punch cards.

However it was not until the 1990s with the introduction of the Internet, as we know it today and network computing that the islands of information within healthcare organisations could now be integrated to give an almost seamless flow of information.

Although it was now possible for computers within different parts of the hospital or even different hospitals could talk to one another, the data collected by different software packages from different manufacturers were stored in incompatible formats. The concerns raised by the lack of communication between various medical information location points led to the introduction of the Electronic Data Interchange (EDI). EDI defines how the communications (called messages) should occur between different systems, and its primary concern is the content of the messages and not how the message is carried across the network.

In terms of costs, unlike the hardware that is in a continuous decline in prices of manufacturing and purchasing, the software component of a computer is more expensive (domestic computer software are sometimes two or even three times more expensive than the computer itself, exponentially increasing the price of software systems for companies and large computer networks).

The computer network. A network is a group of computers or computer like devices that communicate to one another using a common transmission medium. Computers send requests and data through the transmission medium, usually network cables or optical fibres. Wireless networks use radio waves as the mode of transmission and are becoming increasingly common.

Resources made available on the network are accessible by the users of the networks, this include documents, images, video and audio clips. It is also possible to share software across the network and this can lead to a reduction in the maintenance of software on each computer on the network.

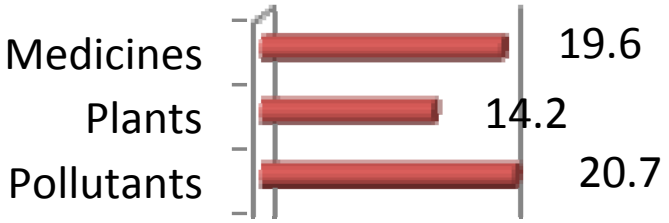
Devices on a network communicate to another by breaking down data into groups of electrical pulses which are known as packets. These packets contain information about the address of the source of origin of the packet and its destination. It is this information that is used by the network equipment to ensure that packet gets to its intended recipient.

The **Internet** is a global system of interconnected computer networks that use the standard Internet Protocol Suite (TCP/IP) to serve billions of users worldwide. It is a network of networks that consists of millions of private, public, academic, business, and government networks of local to global scope that are linked by a broad array of electronic and optical networking technologies. The Internet carries a vast array of information resources and services, most notably the inter-linked hypertext documents of the **World Wide Web** (WWW) and the infrastructure to support electronic mail.

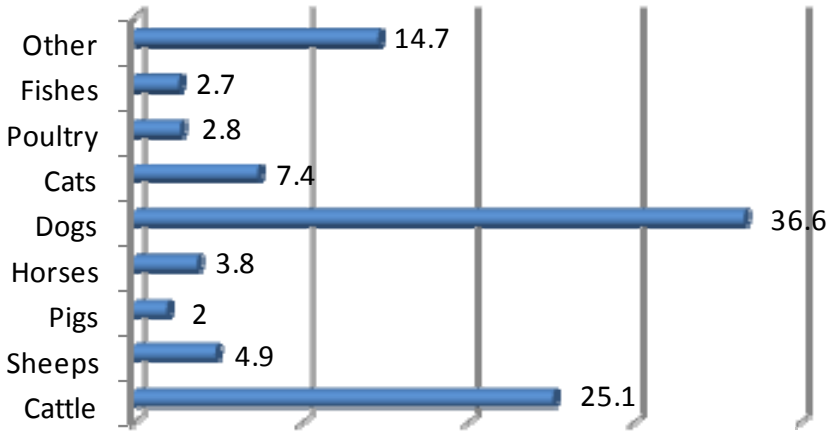
Our program was developed on the WWW platform using PHP web programming language, while database creation and management was done through MySQL relational system.

Selection of toxicoses. The selection of the most important poisonings that were placed in the database was made using statistical data from specialized centers in EU countries, such as the National Center for Toxicological Veterinary Information from Lyon. These organizations provide immediate access to any information in the field of veterinary clinical toxicology, both in France and in other neighboring areas.

According to the studies they carried out, after the centralization of over 38,000 cases have been relevant conclusions on the poisonings incidence and the main species affected. Thus it was found that 45.5% of poisoning cases were due to pesticides, industrial pollutants followed by 20.7%, medicines by 19.6% and 14.2% in the proportion of plants.



Regarding animal species most affected were the dogs (36.6%) and the cattle (25.1%).



Using this type of information, we could move to the proposed composition of the database, with the mention that it will be constantly enriched with new data and updated with the latest research in the field.

RESULTS AND DISCUSSIONS

The program can be accessed by any interested person equipped with a computer terminal and internet connection. The link between the user and the database is made through a friendly graphical interface and ergonomic appearance.

The user can easily access information about any animal poisonings, which are classified alphabetically, according to the species of animals affected or the type of etiological agent and the clinical syndrome produced.

Once selected a toxicosis, the visitor is presented with a document that contains data about the species most affected, etiology, pathogenesis, clinical manifestations, lesions, prognosis and treatment of such poisoning. The datas presented are structured so they are quick and easy to study.

Another important component of the program is the ability to query the database through a similar questionnaire used in the Faculty Clinics Medical Records, which includes data on the animal, clinical course and clinical signs observed. Thus, the user is guided to a correct differential diagnosis and as an emergency treatment instituted as quickly.

Entering and editing information in the database is made through a separate address which is restricted, based on an account password.

CONCLUSIONS

The program is intended for the use of veterinary clinicians and students who wish to inform themselves in the veterinary toxicology field. It also can address pet owners for their own knowledge, noting that, in case of emergencies, the main medical priority is still a swift visit to a veterinary clinic.

In the European Community, the number of users of these types of programs is constantly growing, thanks to the many benefits - structured information source, with rapid access and continuous data updating.

In addition to advantages in clinical and teaching fields, the program's ability to gather and submit regular statistical data on the database queries is also beneficial in scientific research, with multiple uses in the animal and environmental surveillance.

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ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* STRAINS ISOLATED FROM BROILERS SKIN

Isabela NICORESCU, Maria CRIVINEANU
Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei,
050097, Bucharest, Romania, isabela_nicorescu@yahoo.com

Abstract
The use of antimicrobial agents in animal production or for the treatment and control of infectious diseases in animals has strong public health consequences by creating a reservoir of resistant bacteria and bacteria-borne resistance genes that can be passed on to humans.
In this study, 36 strains of Campylobacter jejuni and 22 strains of Campylobacter coli isolated from broilers skin samples were tested for antimicrobial susceptibility. The samples were collected as part of a study on the prevalence and antimicrobial resistance of Campylobacter spp. in broilers carcasses at retail level. Microorganisms were tested for their ability to produce visible growth in microtiter plate wells holding identical volumes of broth with a defined inoculum, in the presence of antimicrobial agent solutions in incrementally two-fold increasing concentrations. The tests were performed under standardized conditions.
The obtained results showed that the most frequent resistance of Campylobacter strains was detected to tetracycline and ciprofloxacin; thus, the resistance to tetracycline was 38.8 % for C. jejuni, while for C. coli the resistance was 72.7 %. The resistance to ciprofloxacin was 53.6 % for C. jejuni and 63.6 % for C. coli. A lower level of resistance was found to gentamicin (C. jejuni 5.5 % and C. coli 4.5 %), streptomycin (C. jejuni 2.7 %) and erythromycin (C. jejuni 5.5 %).
The prevalence of antimicrobial resistance differed between C. coli and C. jejuni, noting a higher resistance of C. coli. The high prevalence of ciprofloxacin and tetracycline resistance found in Campylobacter strains isolated from broilers is extremely worrying; this situation could have important therapeutic implications.

Key words: C. coli, C. jejuni, antimicrobial resistance, broilers.

Thermophilic *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as one of the most frequent causes of acute diarrheal disease in humans throughout the world. *Campylobacter* infections usually occur following ingestion of improperly handled or cooked food. Campylobacteriosis is considered a zoonotic disease, and animals such as poultry represent the principal reservoir for *Campylobacter* (5).

Antibiotics are used in human and veterinary medicine for treatment and prevention of infections and as growth promoters in food animals. Their increased use has resulted in the increased incidence and levels of antibiotic resistance to many enteric bacteria and encouraged the persistence and transfer of antibiotic-resistance determinants in microbial genomes (2, 4).

Antibiotic resistance is an increasing problem in some pathogens and the capability of multidrug resistance is a concern. Some authorities regard food animals as the primary source of antibiotic resistance genes present in human food-borne pathogens, whereas the others as the major source of the problem regard imprudent use of antibiotics in humans. In case of zoonotic bacteria like campylobacters, the use of antimicrobials in animals may determine drug-resistant bacterial populations, which represent a potential threat to the consumer. When a pathogen has already acquired antibiotic resistance, there is no need for gene transfer or development of drug resistance for the pathogen to be difficult to control clinically (1, 7).

Due to the often self-limiting diarrhea in humans, antibiotic treatment is normally not required. Nevertheless, antibiotic treatment is indicated for severe and prolonged enteritis, septicemia and for persons at risk such as very young or immuno-compromised patients. Besides erythromycin, which is an antibiotic of choice, fluoroquinolones and tetracycline are commonly used. Particularly fluoroquinolones are first-line drugs for empiric therapy of acute diarrhea, as they are effective against most major pathogens causing bacterial enteritis. Therefore, most cases of campylobacteriosis receiving antibiotic treatment will initially be treated with fluoroquinolones. Worrying is the fact that in recent years a rapidly increasing proportion of *Campylobacter* isolated from humans and animals all over the world were found to be resistant to fluoroquinolones (3).

The objective of this study was to determine the prevalence of antibiotic resistant *Campylobacter* strains in broilers skin samples at retail level.

MATERIALS AND METHODS

The following antimicrobial agents were tested for bacterial resistance: ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin and tetracycline. The MIC determination was done using broth dilution method, as recommended by Clinical and Laboratory Standards Institute (CLSI) (8, 9). To accomplish this purpose it was used "The Sensititre susceptibility plates for *Campylobacter*" by Trek Diagnostic System. Microorganisms were tested for their ability to produce visible growth in microtitre plate wells holding identical volumes of broth with a defined inoculum in the presence of antimicrobial agent solution in incrementally two-fold increasing concentrations. The tests were performed under standardized conditions so that the results to be reproducible.

A total of 36 strains of *Campylobacter jejuni* and 22 strains of *Campylobacter coli* isolated from broiler skin samples were tested for antimicrobial susceptibility. Isolation of *Camylobacter spp*. was performed in accordance with the SR EN ISO 10272-1/2006. Samples were prepared aseptically by weighing 25 g into a sterile stomacher bag and then 225 ml of Bolton broth was added. After incubation for 48 hours at 41.5˚C in microaerobic atmosphere, one loopful of enrichment broth was streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated for 48 hours at 41.5˚C in microaerobic atmosphere. The confirmation of *Campylobacter spp*. and then the identification of the species were performed by biochemical tests (12).

After species determination, *Campylobacter* strains were incubated on Columbia agar with sheep blood in a microaerophilic atmosphere for 24 hours at 41.5˚C. From each plate, colonies were taken and dissolved completely in 4 ml saline water so the read value on the McFarland scale to be 0.5, which represents approximately 1-2 x 10^8 cfu/ml. From this suspension there were transferred 50 µl to 10 ml of Muller-Hinton broth with lysed horse blood, the mix was homogenized and then inoculated each 100µl per well in the panel. Inoculum was approximately 5 x 10^5 cfu/ml. After that, all wells were covered with a perforated adhesive seal and the samples were introduced to incubation in an atmosphere containing 10% CO_2, 5% O_2 and 85% N_2 for 48 hours. The microaerophilic atmosphere was obtained using gas generation kits (11).

After incubation, the results were read using the Sensititre manual. The MIC is recorded as the lowest concentration of antimicrobial agent that inhibits visible growth. Growth appears as turbidity or as a deposit of cells at the bottom of a well (11).

The epidemiological cut-off values are established by EUCAST (the European Committee on Antimicrobial Susceptibility Testing); in case of *Campylobacter*, the cut-off

points are different for *Campylobacter jejuni* and *Campylobacter coli*. These values and the range tested for each antimicrobial agent are presented in table 1 (11).

Table 1

Range of antimicrobials tested for *Campylobacter jejuni* and *Campylobacter coli* and cut-off values applied for the classification of strains as resistant

Antimicrobial agent	**Abbreviation**	**Range tested (µg/ml)**	**Cut-off value (µg/ml) R >**	
			Campylobacter jejuni	***Campylobacter coli***
Tetracycline	TET	0,25 – 16	2	2
Erythromycin	ERY	0,5 – 32	4	16
Streptomycin	STR	1 – 16	2	4
Gentamicin	GEN	0,12 – 16	1	2
Ciprofloxacin	CIP	0,06 – 4	1	1
Nalidixic acid	NAL	2 - 64	16	32

RESULTS AND DISCUSSIONS

In total, 58 *Campylobacter* strains were tested for antimicrobial resistance; 20 strains (34.48%) were sensitive to all tested antibiotics, while 38 strains (65.52%) were resistant to at least one of the antibiotics. Four strains were resistant to four or more of the tested antibiotics; these isolates were considered multiresistant. None of the isolates was resistant to all six agents tested. The results of antimicrobial susceptibility testing for *C. jejuni* and *C. coli* strains isolated from broilers skin are shown in tables 2 and 3.

Table 2

Antimicrobial resistance in *Campylobacter jejuni* (n = 36) from broiler skin
Distribution (%) of MIC values (µg/ml)

Substance	**Resistance (%)**	**Distribution (%) of MIC values (µg/ml)**										
		0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Tetracycline	38.8			27.6	0.5		27.7	25	8.3	5.5		
Erythromycin	5.5				83.3	11.1			5.5			
Streptomycin	2.7					88.8	8.3		2.7			
Gentamicin	5.5		47.2	25	22.2			2.7		2.7		
Ciprofloxacin	58.3				19.4	22.2	8.3		50			
Nalidixic acid	5.5						44.4	30.5	16.6	2.7		5.5

Bold vertical lines denote microbiological cut-off values. White fields denote range of dilution tested for each antimicrobial agent.

Table 3

Antimicrobial resistance in *Campylobacter coli* (n = 22) from broiler skin
Distribution (%) of MIC values (µg/ml)

Substance	**Resistance (%)**	**Distribution (%) of MIC values (µg/ml)**										
		0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Tetracycline	72.7			13.6		13.6			18.1	13.6	40.9	
Erythromycin	22.7				54.5		22.7				9.09	13.6
Streptomycin	13.6					31.8	36.3	18.1			13.6	
Gentamicin	4.5		45.4		36.3	13.6			4.5			
Ciprofloxacin	63.6	13.6		22.7			9.09	13.6	40.9			
Nalidixic acid	9.09						18.1	31.8	40.9			9.09

Bold vertical lines denote microbiological cut-off values. White fields denote range of dilution tested for each antimicrobial agent.

In our study, most frequency of resistance of *Campylobacter* strains was detected to tetracycline and ciprofloxacin; thus the resistance to tetracycline was 38.8 % for *C. jejuni*, while for *C. coli* the resistance was 72.7 %. The resistance to ciprofloxacin was 58.3 % for *C. jejuni* and 63.6 % for *C. coli*. By comparison, these values are similar to those reported by European Food Safety Authority (EFSA) (10). It is interesting that resistance is quite high to ciprofloxacin, considering that studies carried out up to 1987 had shown that strains resistant to fluoroquinolones were practically nonexistent. The use of quinolones, mainly enrofloxacin, in veterinary practice, has been correlated with the increase resistance in *Campylobacter* strains (6).

Differences were observed in the frequency of resistance among *C. coli* compared to *C. jejuni*: *C. coli* strains were more likely to be erythromycin-resistant compared to *C. jejuni* (22.7 % compared with 5.5 %). *C. coli* were also more likely to be tetracycline, streptomycin and ciprofloxacin-resistant compared to *C. jejuni* (72.7 % compared with 38.8 % for tetracycline, 13.6 % compared 2.7 % for streptomycin and 63.6 % compared with 58.3 % for ciprofloxacin). The lowest levels of resistance were found to gentamicin (*C. jejuni* 5.5 % and *C. coli* 4.5 %), streptomycin (*C. jejuni* 2.7 %) and erythromycin (*C. jejuni* 5.5 %).

CONCLUSIONS

1. Tetracycline, ciprofloxacin and erythromycin resistance was present in *Campylobacter* strains isolated from broilers skin.
2. The prevalence of resistance differed between *C. coli* and *C. jejuni*, noting a higher resistance of *C. coli*.
3. The high prevalence of ciprofloxacin and tetracycline resistance found in *Campylobacter* strains isolated from broilers is extremely worrisome; this situation could have important therapeutic implications.

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INCIDENCE OF SALMONELLA SPP. IN A POULTRY SLAUGHTERING UNIT

MIHAI OBADĂ, ALINA VLAD SABIE, MIHAI CARP- CARARE
University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iaşi
Faculty of Veterinary Medicine

Abstract
Salmonella spp. on poultry carcasses was identified using standard ISO SR EN 6579/AC/2006. A number of 60 samples were analyzed in the 2009-2010 period. The samples were collected from the poultry carcasses and tested by classical bacteriological methods.The proportion for poultry carcasses contaminated with Salmonella spp. was 5%.
Key words: slaughtering unit, Salmonella spp., poultry

INTRODUCTION

The diseases originating in bacteria from genera *Salmonella* represent for the majority of countries with high animal raising industry one of the most important sanitary-veterinary problems due to financial loses and also due to the implication in human health of food borne diseases because of the consumption of contaminated animal products.

Progress in the last decades in the technology of processing, preservation and manipulation have only partially removed the possibility of contamination with pathogenic microorganisms, and consumer claims about the microbiological quality of poultry carcasses grew increasingly. Consumers are more careful when they are buying poultry carcasses, more sensitive to smell, flavor and color. It could say that, currently the microbiological quality of poultry carcasses has become a major factor in its marketing.

Poultry carcasses is an important source of human contamination with *Salmonella spp*. *Salmonella enteritidis* and *Salmonella typhimurium* are serovars most frequently isolated from poultry carcasses and in cases of food poisoning in humans (EFSA Journal, 2004).

MATERIALS AND METHODS

In 2009-2010 were collected a total of 60 samples in a slaughtering unit. The samples were obtained under sterile conditions by taking neck skin, weighing 25 grams for each sample. Carcase microbiological assessment was made according to programs of the National Agency for Sanitary Veterinary and Food Safety : ISO SR EN 6579/AC/2006, which states that identification of microorganisms like *Salmonella spp*. from poultry carcasses will be made on 25 grams samples of neck skin. According to this standard in 25 g sample *Salmonella spp.* has to be absent. In order not to suffer further contamination, skin fragments were collected with sterile scissors and were lodged in special sterile bag (Stomacher 305/175 mm) kept on ice since sampling until to the laboratory, where they were processed.

1.Pre-enrichment phase consisted of inoculation of samples in a neselective liquid medium (BPW- buffered peptone water) and incubation at 37°C for 18 h.

Procedure : were seeded in 225ml pre-warmed BPW 25g product. (Koyuncu Sevinc et al., 2009).

2.Enrichment phase was made on two liquid selective enrichment mediums. Enrichment consisted of two selective media inoculated with the culture from pre-enrichment medium; thus used as selective enrichment medium, Rappaport-Vassilidis with soy broth (RVS broth), incubation was done at 40.5 to 42.5°C for 24 h and for medium Muller-Kaufmann tetrathionate broth with Novobiocin (MKTTn), incubation was done at 37°C for 24 hours.

3.Isolation phase consisted of the sowing of a selective solid mediu with cultures obtained in enrichment phase.

It was used the following medium : dezoxicolat xylose lysine agar (XLD agar) - incubation at 37°C and examined after 24 h.

Only the characteristic colonies developed on the selective isolation media were taken into considered. The characteristic colonies of *Salmonella spp*. grown on XLD agar have a black center and a transparent light area of red colour due to color change indicator.

4.Confirmation phase consisted in election of the presumptive colonies of *Salmonella spp.* and determination of serological and biochemical characteristics, being tested one characteristic colony from each plate.

To obtain a faster result, suspicious colonies from selective media were inoculated on mediums politrope: TSI (triple sugar iron), MIU (mobility, indole, urea), MILF.

TSI agar – having in regard the way how the medium is poured into the tube (slope and column) the sloping surface of agar was seeded by striate and column by puncture. It was incubated at 37°C for 24 h and were interpreted medium changes as follows:

a) column.
   - yellow – glucozo-positive (glucose fermentation)
   - red or unchanged – glucozo-negative (not ferment glucose)
   - black - the formation of hydrogen sulfide;
   - bubble or crack - gas formation from glucose,
b) inclined surface medium
   - yellow - lactozo/zaharozo-positive;
   - red or unchanged - lactozo/zaharozo-negative ;

Characteristic cultures of *Salmonella spp.* showed that inclined surface medium is alkaline (red) and the average medium is acid (yellow) with gas (bubbles) and hydrogen sulfide formation.

Urea agar – was seeded the inclined surface of medium by striate. Was incubated at 37°C for 24 h. If the reaction is positive, breaking urea molecule release ammonia, which turns the color of phenol red to pink and later to dark red.

L-lysine decarboxylation medium - liquid medium were inoculated immediately below the surface. Was incubated at 37°C for 24 h. Turbidity and purple coloration after incubation showed a positive reaction (yellow-negative).

Medium for indole reaction - was inoculated a tube containing 5 ml of medium tryptone/tryptophan with suspected colony. It was incubated at 37°C for 24 h. After incubation 1 ml Kovacs' reagent was added ; forming a red ring indicates a positive reaction.

Research regardind the presence of antigens "O" *Salmonella* was performed by slide agglutination with appropriate sera from pure cultures and after removal of autoagglutinated strains. (Apostu Sorin, 2006, Vol III)

Serological confirmation was made using serum anti-*Salmonella* "O" polyvalent C, F, G, H.

Indications: Serological identification of Salmonella strains belonging to serogroups CO, FO, GO, HO is made using Kauffmann–White scheme. Identification of a suspected strain of *Salmonella* was made using the slide agglutination reaction.

Slide agglutination reaction : agglutinative serum was diluted in sterile isotonic solution of saline (SPS) as indicated on vial strength. On microscope slide is put a drop of diluted immune serum and beside him a drop of sterile saline. Bacteriological loop is performed using an suspension of a bacterial colony identified biochemical old 18 to 20 hours on 2% agar medium in a drop of sterile saline. Were excluded from serotyping *Salmonella* strains form □□R□□ which agglutinate spontaneously with sterile saline.

If the suspension is homogeneous the procedure is the same in case of drop of serum agglutinative. If agglutination is positive, white granules appear in 1-3 minutes.

Faster detection method was made on chromogen Rambach medium, on which the characteristic colonies of *Salmonella* species are red.

Faster detection of *Salmonella* spp in food was made with Singlepath Salmonella used for qualitative determination.

Singlepath immunochromatographic *Salmonella* is a test based on antibodies labeled with gold. Test device has an circular area where is put the culture suspension and an oval test (T) and an control window (C).

Test sample was subject to an enrichment broth medium Rappaport - Vassiliadis for 24 hours at 37°C, then the tube with medium was subjected to boiling water bath for 15 min. It was left to cool at room temperature, and transferred using a pipette 150 ml in the circular test. Reading was done in 20 minutes and test positivity was indicated by the emergence of a distinct red lines, similar to that in control.

RESULTS AND DISCUSSION

Although *Salmonella* spp is recognized as the most important pathogens associated to poultry is not known precisely the disease incidence in humans due to poultry. (D'Aoust, 1989)

The incidence of bacteria of the genus *Salmonella* in and on birds ranging from one geographical area to another, the dates ranging between 4-100%. The incidence is influenced by factors such as growing conditions and climate. There are countries like Sweden where the birds are free from *Salmonella* spp, situation reached after application of government control programs and measures by poultry farmers and meat processors.

For selective isolation were used two solid media, one highly selective (XLD) 24 hours at 37°C and one less selective (Istrati-Meitert), on which colonies appear green.

Biochemical confirmation was done on T.S.I., M.I.L.F. and API 10S.

Serological confirmation was made using serum anti-*Salmonella* "O" polyvalent C, F, G, H.

In slaughterhouses which slaughter large numbers of birds are frequent cross-contamination due to the small space between carcasses placed on conveer and the balance of carcasses allowing the contact of each other. (Crețu Carmen and col., 2008)

Faster isolation from food was made using Glisa test and Rambach chromogen medium, and the identified was easy to do heaving in regard that chromogen medium allow to obtain characteristic colonies.

The proportion for poultry carcasses contaminated with *Salmonella spp.* was 5%. (Table 1)

Table 1

***Salmonella spp. values/* 25g neck skin from poultry carcasses**

Samples	Nr. of samples	Positive samples		Negative samples	
		Nr.	%	Nr.	%
Poultry carcasses	60	3	**5 %**	57	**95 %**

After Committee WHO / FAO data, were identified based on antigenic study over 2400 (2435) of serotypes. Their spread in the world does not recognize regional boundaries and with few exceptions, have little host specificity.

On the other hand, is widely accepted that no longer can draw a clear line between *Salmonella* pathogenic for humans and those found in animals. Therefore the issues raised by *Salmonella* infections in animals and humans can be treated only as a whole.(Cason J.A.,1997, D'Aoust J-Y., 1989)

Between different types of *Salmonella* and organisms in which they have penetrated, different relationships are established. It seems difficult to explain why, quite frequently, even in cases of highly pathogenic serotypes, the relationship between organism and germs, remain in state of strictly epiphyte, more or less long.

Salmonella enterica serovar enteritidis was the predominant serovar in outbreaks with *Salmonella* in 2007. Registration percentage was 60.2% in case of outbreaks of *Salmonella enterica* and *serovar typhimurium* showed a rate of 10.7%. (EFSA Journal, 2009)

In 2008, salmonellosis was again the second most often reported zoonotic disease in humans accounting for 131,468 confirmed human cases. The statistically significant decreasing trend in the notification rate of the salmonellosis cases continued in the European Union for the fifth consecutive year. In particular, the human cases caused by *S. Enteritidis* decreased markedly in 2008, while an increase in *S. Typhimurium* cases was observed.

In foodstuffs, *Salmonella* was most often detected in fresh broiler, turkey and pig meat, on average at levels of 5.1%, 5.6% and 0.7%, respectively.

Overall, in the EU, *S. Enteritidis* and *S. Typhimurium* are the serovars most frequently associated with human illness. Human *S. Enteritidis* cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *S. Typhimurium* cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat. (EFSA Journal, 2010)

CONCLUSIONS

1. The 60 samples were examined under sterile conditions, taking neck skin, weighing 25 grams for each sample on poultry carcasses, determining the incidence of *Salmonella spp.*
2. In poultry carcases, *Salmonella spp.* was detected at level of 5%.
3. The percentage is similar to that found in EU.

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THE PROTECTING EFFECT OF A POLYPHENOLIC EXTRACT OBTAINED FROM SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*) FRUITS AGAINST PHOTO-OXIDATION OF DIFFERENT VEGETAL OILS

Camelia PAPUC, V. NICORESCU, Corina DURDUN, Gh. GORAN, Carmen CRIVINEANU

Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei, 050097, Bucharest, Romania, cami_papuc@yahoo.com

Abstract

The stability of oils is commonly affected by photo-oxidation processes, which yields both primary and secondary oxidation compounds. In this study, it was investigated the ability of alcoholic extracts obtained from sea buckthorn fruits (Hippophae rhamnoides) to act as antioxidants in photo-oxidation process of some commercial vegetal oils. The experiments were carried on rafinated sunflower oil, virgin olive oil and cold pressed maize germs oil. The oxidative stability of oils added with sea buckthorn extract was determined after exposure 30 minutes at UV light. Peroxide values, the formation of conjugated dienes and malondialdehyde and free fatty acids assay were studied in order to estimate the oxidation process. Peroxide values and free fatty acids were investigated by volumetric assays; conjugated dienes were evaluated by spectrophotometry at 232 nm, while the formation of malondialdehyde was investigated by thiobarbituric acid assay. The experiments showed that sea buckthorn alcoholic extract protected the oils against photo-oxidation process. The obtained results indicate the fact that sea buckthorn extracts could be used in food industry as natural antioxidants in order to replace partially or totally synthetic antioxidants.

Key words: sea buckthorn, photo-oxidation, peroxide value, conjugated dienes, malondialdehyde, acid value.

Lipid oxidation caused by UV radiation is named photo-oxidation, and involves the interaction of unsaturated lipids with singlet oxygen. Oxygen excited state ($^1O_2$), singlet oxygen, is produced when light interacts with oxygen in the presence of pigments such as chlorophyll and riboflavin, named sensitizers:

$$\text{sens} \xrightarrow{\textit{light}} \text{sens}^*$$
$$\text{sens}^* + {}^3O_2 \longrightarrow \text{sens} + {}^1O_2$$

Singlet oxygen species generates photo-oxidation of unsaturated lipids that involves the attack at double bond of a singlet oxygen (5).

In case of photo-oxidation, it has been postulated that the double bond within a fatty acid molecule may be capable of capturing outside source of energy, such as heat and light, to reach a critical excitation level (4). Upon reaching the critical excitation level, the double bond may break, thus giving rise to a free radical species, which may in turn generate more free radicals.

Photo-oxidation, which is a natural oxidation and chemical degradation processes of edible oils, also result in rancidity of food items, whereby these processes convert fatty acid esters of oils into free fatty acids. Furthermore, the free fatty acids also give rise to smell that is observed in many vegetable oils over time (1).

Photo-oxidation cannot be stopped by the synthetic antioxidants such butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) or the natural antioxidant tocopherols (vitamin E). Photo-oxidation may be interrupted by introducing molecules which reacts more quickly with singlet oxygen than unsaturated lipids.

Plant polyphenols are toxicity-free antioxidants that have the capacity to annihilate free radicals which initiate and propagate lipid peroxidation processes and, from this reason, it is thought that polyphenols can partially or totally replace synthetic antioxidants (2, 3, 6, 7, 8).

The aim of this study was to demonstrate photo-oxidation protecting effects of a polyphenolic extract obtained from sea buckthorn fruits on different commercial oils.

MATERIALS AND METHODS

Obtaining of polyphenolic extract. In order to obtain vegetal extract, the dry vegetal material was ground and then subdued to a solid-liquid extraction with ethanol 60% (v:v).

Oils. The experiments were carried on commercial rafinated sunflower oil, virgin olive oil and cold pressed maize germs oil. Oils were treated with vegetal alcoholic extracts in relation of 100:1 (v:v). The oxidative stability of oils added with sea buckthorn extract was determined after exposure at UV light (254 nm) for 30 min. All samples and control systems were evaluated in triplicate.

Determination of peroxide value. Peroxide values were determined according to Official American Oil Chemist's Society (AOCS) methods (AOCS, 1985). The values were expressed as meq of peroxide O_2 /kg oil.

Determination of conjugated dienes. Conjugated dienes formed through photo-oxidation of oil samples were determined according to the analytical methods described by IUPAC (IUPAC, 1979). The content of conjugated dienes were expressed as absorptivity at 232 nm of 1% oils in 2,2,4-trimethylpentane.

Determination of malondialdehide. A modified thiobarbituric acid reactive substances (TBARS) method was used to measure the antioxidant activity of alcoholic extracts. 50 µl alcoholic extract was added to 1.95 ml TBA-TCA (thiobarbituric acid – trichloroacetic acid) solution. The mixture was heated in a 100ºC water bath for 30 min and cooled at room temperature. After the addition of 2 ml butanol, the mixture was mixed and centrifuged at 2000 rpm for 15 min. The butanol layer was separated and the absorbance at 532 nm was measured (9).

Determination of acid value. Acid value was determined according to Official American Oil Chemist's Society (AOCS) methods (AOCS, 1985). The values were expressed as mg KOH/g oil.

RESULTS AND DISCUSSIONS

Control oil samples had different reactions to photo-oxidation by exposure for 30 min at 254 nm UV radiation; this fact is due to the variable content in unsaturated lipids and also to the variable contents in tocopherols of the oils. Oil samples added with sea buckthorn alcoholic extract exhibited photo-oxidation protection for all tested oils.

Peroxide value. The peroxide value was taken as a measure of primary oxidation compounds produced in the photo-oxidation of oils samples. The obtained results showed that sea buckthorn alcoholic extract protected against photo-oxidation all oil samples (Fig. 1). Sea buckthorn extract offered the best protection against lipid peroxidation in case of sunflower oil; for this oil, peroxide value was 23 times higher after UV exposure in case of control samples, while in case of sunflower oil added with sea buckthorn extract peroxide value was

8.8 times higher after UV exposure. Good results were obtained also for maize germs oil; peroxide value was 14 times higher for control samples and 10 times higher for sea buckthorn added samples after UV exposure. In case of virgin olive oil, peroxidation process was less intense, probably due to its lower content in linoleic and linolenic acids.

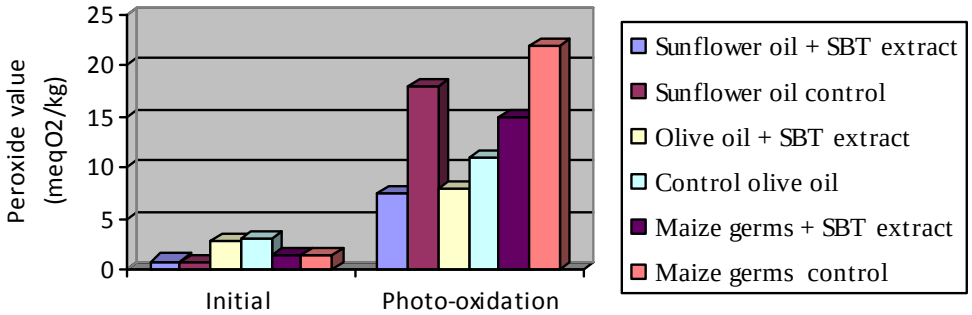

Fig. 1. Relationship between peroxide value and oils exposure at 254 nm UV radiation for 30 minutes

Conjugated dienes. Oxidation can cause double bond position shifts in polyunsaturated fatty acids. In this process, conjugated dienes and trienes structures are formed, and this can be indicated by changes in UV absorption (10, 11). The changes in the absorptivity at 232 nm of all systems are presented in Fig. 2. The increase of absorptivity at 232 nm was progressive for all control oils, proportionaly with polyunsaturated fatty acids content. Sea buckthorn alcoholic extract determined a decrease of conjugated dienes forming ratio for all oils. The obtained results are similar to the ones recorded for peroxide value; the lowest conjugated dienes formation process was observed in case of olive oil, while the best protection against photo-oxidation was recorded in case of sunflower oil added with sea buckthorn extract.

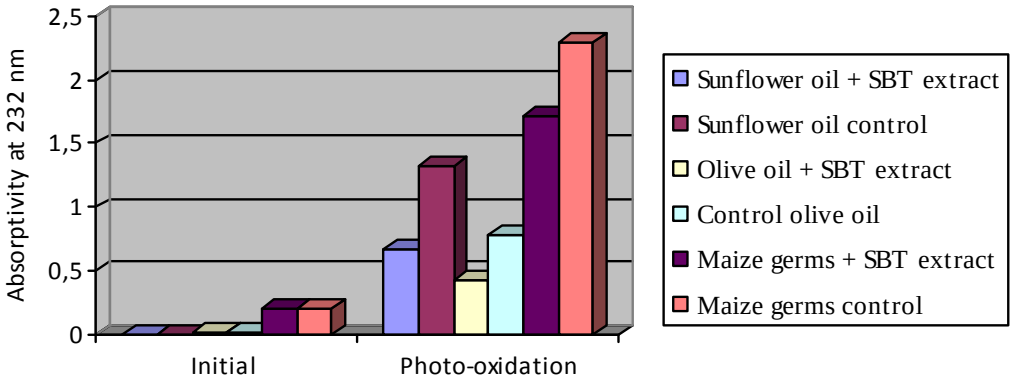

Fig. 2. Conjugated dienes assay. Relationship between absorptivity at 232 nm and oils exposure at 254 nm UV radiation for 30 minutes

Malondialdehyde. This dialdehyde is preferentially formed by autoxidation of fatty acids with two or more double bonds. In this study, malondialdehyde forming was inhibited by seabuckthorn alcoolic extracts in all oils exposed at UV radiation (Fig. 3).

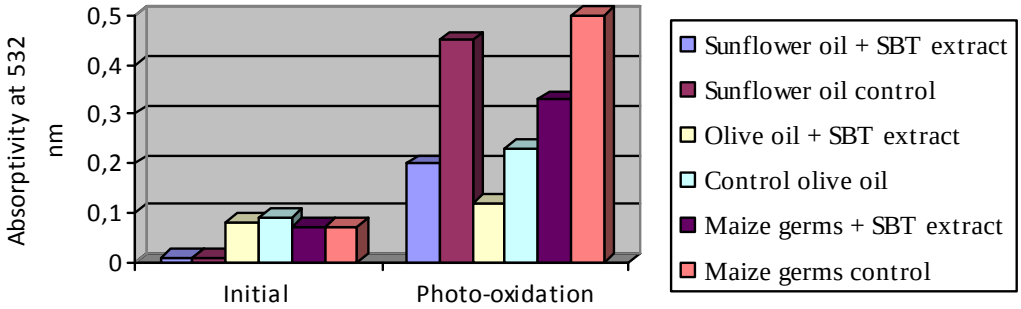

Fig. 3. Malondialdehyde assay. Relationship between absorptivity at 532 nm and oils exposure at 254 nm UV radiation for 30 minutes

Acid value. Photo-oxidation of oils generates a multitude of compounds, such as acids with short chain molecule, formed by the oxidation of aldehydes. The changes in acid values for all samples are shown in Fig. 4.

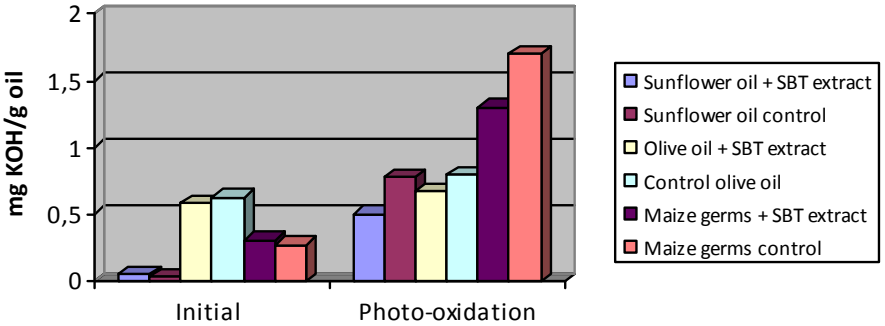

Fig. 4. Relationship between acid value and oils exposure at 254 nm UV radiation for 30 minutes

The results obtained in this study demonstrate that sea buckthorn alcoholic extract contains compounds that act as antioxidants in photo-oxidation by quenching singlet oxygen or triplet sensitizer or free radicals generated in photo-oxidation.

CONCLUSIONS

1. Sea buckthorn alcoholic extract protects vegetal oils against photo-oxidation.
2. Sea buckthorn alcoholic extract had the highest degree of protection against photo-oxidation in case of sunflower oil.
3. The less susceptible to photo-oxidation is olive oil.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, PNII – IDEI program, project number ID_256/2007.

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OBSERVATIONS ON AVIAN INFLUENZA

T.C. PATACHE[1],M. ARSENE,[2] ARSENE MARINELA[2]
1. DSVSA – Galați; 2. DSVSA – Tulcea.

Summary
Observations on a flock of 268 poultry from two private farms on avian flu, have found a morbidity rate of % 2,46.
Clinically, the birds presented adynamy, deep depression associated with horiplumation, loss of appetite, excessive thirst, cyanosis and chin ridge, dyspnea and noisy breathing, swelling of head and neck, rapid death.
Necropsy performed showed cyanosis ridge, chins, ears, look internal-bleeding and bleeding bodies point of the serous, of the proventricol and intestine.

***Key words**: inquiry, flu, birds, lesions.*

INTRODUCTION

Formerly referred to as fever typical European horse, bird plague, typhus exudation of chicken, bird, fish or avian influenza is a virus disease of birds known for its high mortality rate. Avian influenza viral nature of the disease was highlighted in 1955 (Savu and col, 2006), when he was involved in producing disease virus isolated. Avian influenza affects a wide variety of domestic and wild birds, the infection evolved multiple forms, of varying severity, from expressions subclinical or asymptomatic to serious developments, fatal, depending on virulent strain of avian host sensitivity involved and affected. Followed this observation of clinical signs and gross lesions in the bird flu, according to the extensive growth, free.

MATERIAL AND METHODS

Epidemiological observations, clinical and anatomo-patological were made in 2009, a total of 268 poultry, represented by 130 chickens, 20 turkeys, 14 guinea fowls, 40 ducks, 32 geese and 46 pigeons. The birds were reared in extensive, free, and belonged to two private holdings, located near a pond, where there were diseases and mortality.

RESULTS AND DISCUSSION

Epidemiological surveys result that private holdings which have been recorded cases of illness and sudden death in chickens, are located close to ponds. The birds were grown in the extensive free access, especially ducks and geese, in the water sometimes coliving the wild, many of them migratory. Consider a source of infection, with a high probability that was represented by wild palmipedele (ducks and geese) were infected in contact directly or indirectly with the flocks of these birds.

Of the 268 poultry were sickened seven chickens, representing % 2,46 (Table 1).

Table 1

The results of the clinical examination

Household	Total Birds	From which	No. birds	That got sick	
				Nr.	%.
1.	154	Hens	75	4	5,33
		Turkeys	11	-	-
		Guinea fowls	5	-	-
		Ducks	21	-	-
		Geese	17	-	-
		Pigeons	25	-	-
2	115	Hens	45	3	6,33
		Turkeys	9	-	-
		Guinea fowls	6	-	-
		Ducks	19	-	-
		Geese	15	-	-
		Pigeons	21	-	-
T o t a l	**268**		**268**	**7**	**2,46**

Mentioned that the illness and mortality were recorded only in chickens, although these side by side with other species (turkeys, guinea fowl) susceptible to infection.

The first signs of bird flu in flocks examined evolution was the emergence of sudden death in two chickens. Clinical adynamyc birds and deep depression associated with horiplumation, loss of appetite and excessive thirst, cyanosis ridge, chins and skin, dyspnea and noisy breathing, swelling of head and neck, lacrimal hypersecretion and rapid death.

Necropsy performed on the seven hens dying ridge showed cyanosis, chins and ears, congestive, hemorrhagic appearance of organs, the proventricol hemorrhagy, congestion and the presence of patches and intestinal mucosa.

CONCLUSIONS

From observations of bird flu following conclusions:

1. Contact birds (ducks and geese) to domestic migration favored the penetration of the virus in livestock holdings receptive population.
2. Of the 268 poultry examined, seven were sick chickens, which is 2.46%.
3. Registration disease only in chickens, confirming their increased sensitivity compared to other poultry.
4. Clinically they presented adynamy, prostate, horiplumation, loss of appetite and swelling of connective tissue of head neck region.
5. The necropsy has revealed the appearance of hemorrhagic dead bird, the proventricular and enterital hemorrhagy.

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MORPHOLOGICAL QUALITY INFLUENCE OF SHEEP OOCYTES ON *IN VITRO* MATURATION

Anamaria Petrean, I. Groza, L. Bogdan, Simona Ciupe, Sorana Matei, AL. Pop

Universitatea de Ştiinţe Agricole şi Medicină Veterinară Cluj-Napoca
Facultatea de Medicină Veterinară
Disciplina de Reproducţie, Obstetrică şi Ginecologie Vetrinară
Calea Mănăştur, nr. 3-5, Cluj-Napoca, România, anamariapetrean@yahoo.com

ABSTRACT

The study was carried out to investigate the relationship between the development potential and the diameter of ovine oocytes. Ovaries collected from a local abattoir were transported to the laboratory within 1-3h of slaughter. The oocytes from follicles (2–6 mm in diameter) were recovered by aspiration and stored in a preincubated (at 38.6 °C, 5% CO2 and maximum humidity) hepes-modified TCM 199 solution. Good quality oocytes (evenly granulated cytoplasm with at least two layers of surrounding cumulus cells) were selected and subjected to culture in TCM 199 supplemented with 0.05 IU/ml FSH, 1 IU/ml hCG and 1 µg/ml E2 (OCM; oocyte culture medium). Before culturing, the selected oocytes (n = 270) were divided into three categories based on diameter: (a) d<70 µm; (b) d=70–90 µm; and (c) d>90 µm. In each category half of the oocytes were denuded of the cumulus cells (denuded oocytes - DO) and the rest remained intact (cumulus enclosed oocytes - CEO). All of the oocytes were cultured in OCM for a period of 26–27 h. After the incubation period a morphological examination was performed under stereomicroscope along with the evaluation of the diameter under inverted microscope.The percentage of matured oocytes was 66,66%, 77,77%, and 84,44% for the cumulus enclosed oocytes with diameters of <100, 100–110, >110 µm, respectively. The corresponding values for the denuded oocytes were 62,22%, 75,55% and 77,77%. The rate of maturation in cumulus enclosed oocytes was higher than that of denuded oocytes in all the size categories. The results suggest that in sheep the antral follicles ranging from 2 to 6 mm contain fully grown oocytes which, despite their varaiabilty in diameter, show good competence for in vitro nuclear maturation.

Keywords: sheep, oocyte, diameter, “in vitro” maturation;

INTRODUCTION

There are many criteria for the selection of good quality oocytes in order to improve the in vitro maturation of oocytes in different farm animal species. Some criteria are, the size of the follicle [*Pavlok et al., 1992; Blondin and Sirard, 1995*], level of atresia [*de Wit and Kruip, 2001*] and progesterone concentration in the follicular fluid [*Hazeleger et al., 1995, Groza, 1996*]. It has been shown that the oocyte developmental competence is also related to the morphology of the cumulus–oocyte-complexes (COCs) [*Blondin and Sirard, 1995*], morphology of the corona radiata cells [*Laurincik et al., 1996*] and size of the oocytes [*Raghu et al., 2002*]. Oocyte size can generally be used as an indicator of oocyte growth, as there is an intensive synthesis of RNA during this phase that causes an increase in size.

MATERIALS AND METHODS

Sheep ovaries (n = 70) were collected during the non-breeding season (April to June) from a local slaughterhouse and transported to the laboratory in saline at 30–35 °C in a thermos flask within 1–3 h after collection. The ovaries were washed three times with prewarmed fresh saline (37 °C), and all visible antral follicles with a 2–6 mm diameter aspirated, using a 20-G needle connected to a 2 ml syringe (figure 1). Prior to aspiration, syringes were filled with 0.5 ml pre-incubated Hepes-modified TCM supplemented with 50 IU/ml heparin.

Figure 1 – Oocytes recovery using aspiration technique

The contents of syringe were transfered into a collection tube and after sedimentation, the follicular aspirant was poured into a Petri dish and observed under stereomicroscope and then evaluated under an inverted microscope equipped with a video camera (Nikon). The number and the quality of oocytes was determined according to their morphological characteristics:

- the aspect and size of zona pellucida (integrity, thickness);
- the aspect of cumulus cells (number of cells- layers, compactness);
- the aspect of cytoplasm (homogenous , heterogeneous, granular);
- the aspect of perivitelin space (uniforme, irregular).

Only oocytes surrounded by more than three layers of unexpanded cumulus cells, with integrity of pellucida zone, homogenous cytoplasm and uniforme perivitelin space were recovered and selected for *"in vitro"* maturation (IVM) (figure 2).

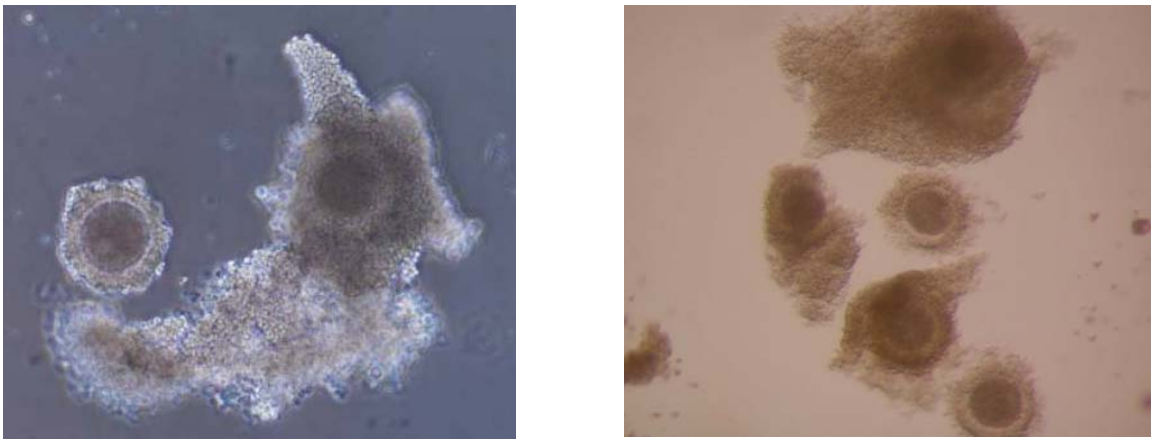


Figure 2 – Good quality oocytes selected for"in vitro" maturation

Prior to maturation, the size of each oocyte was measured using the Motic Images Plus software which comes with the inverted microscope. The selected oocytes (n = 270) were allotted into three categories based on diameter: (a) d <70 µm, (b) d = 70–90 µm, and (c) d >90 µm. Before culturing, cumulus cells were removed by pipetting half of the oocytes, in each size category. This classification was performed to evaluate the possible effect of oocyte diameter on IVM and the influence of the oocyte and cumulus cells interaction.

In each category half of the oocytes were kept intact (CEO: cumulus enclosed oocytes) and the rest were denuded of the cumulus cells (DO: denuded oocytes). The CEO and DO was randomly distributed in maturation droplets, 15 oocytes per 50 µl, covered by sterile paraffin oil (Sigma Chemical) in 35 mm Falcon dishes. The oocytes were incubated under an atmosphere of 5% CO_2, 95% air with 100% humidity at 38,6 °C for 26–27 h. The oocyte culture medium (OCM) consisted of bicarbonate-buffered TCM 199 with l-glutamine supplemented with 0,05 IU/ml FSH, 1 IU/ml hCG, 1 µg/ml E2, 100 µl/ml penicillin, 100 µg/ml streptomycin, 20% FCS (Sigma Chemical), and 0,2 mM Na-Pyrovate. To establish the degree of IVM a morphological examination was performed under stereomicroscope along with the evaluation of the diameter under inverted microscope.

RESULTS AND DISCUSSIONS

Morphological evaluation of oocytes is often the most intimidating part of IVF technique being an initial step, something which starts off. This stage is necessary due to the fact that using bad quality oocytes with no chance of development for fertilization is unjustified.

After the application of aspiration technique a total number of 394 oocytes were obtained from 213 follicles, with an average of 5,63 oocytes/ovary.

From 394 sheep oocytes recovered, 270 oocytes (68,53%) were included in the *„cultivable”* quality category as they presented more than 3 cumulus cells layers, homogenous cytoplasm, intact pellucid membrane and uniforme perivitelin space. The other 124 oocytes (31,47%) were considered *„non-cultivable”* as they presented heterogeheous/granular cytoplasm, ruptures of zona pellucid, irregular perivitelin space. Only good quality oocytes (n=270) were used for *“in vitro”* maturation protocol, divided into 3 categories (n=90) depending on their diameter: (a) d <70 µm, (b) d = 70-90 µm, and (c) d >90 µm (figure 3).

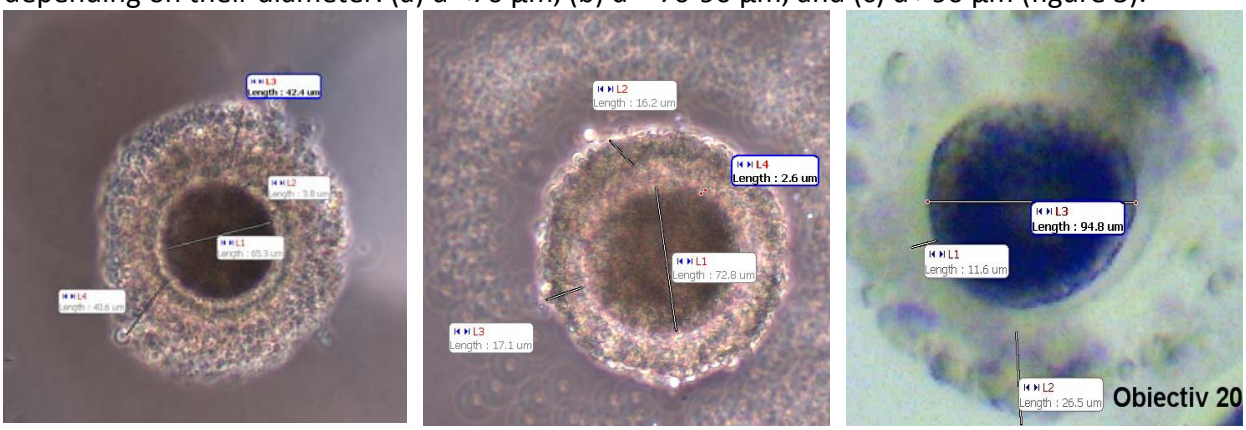


Figure 3 – Classification of ovine oocytes based on diameter:
a) d <70 µm, b) d=70–90 µm, c) d >90 µm

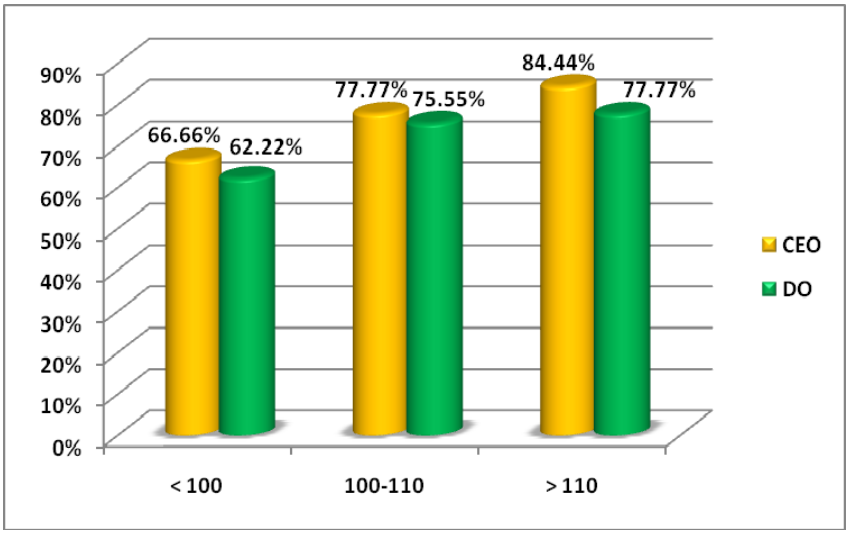
There was heterogeneity in the oocyte diameter obtained from visible follicles with a diameter between 2 and 6 mm. The oocyte diameters were in the range of 65,3–98 µm for the smallest and largest oocytes, respectively.

As set out in table 1, relationship was detected between oocyte size and *„in vitro"* maturation in the cumulus enclosed and denuded oocytes. In both CEO and DO the percentage of maturated oocytes was higher in the largest oocytes (>90 µm): 84,44% and 77,77%.

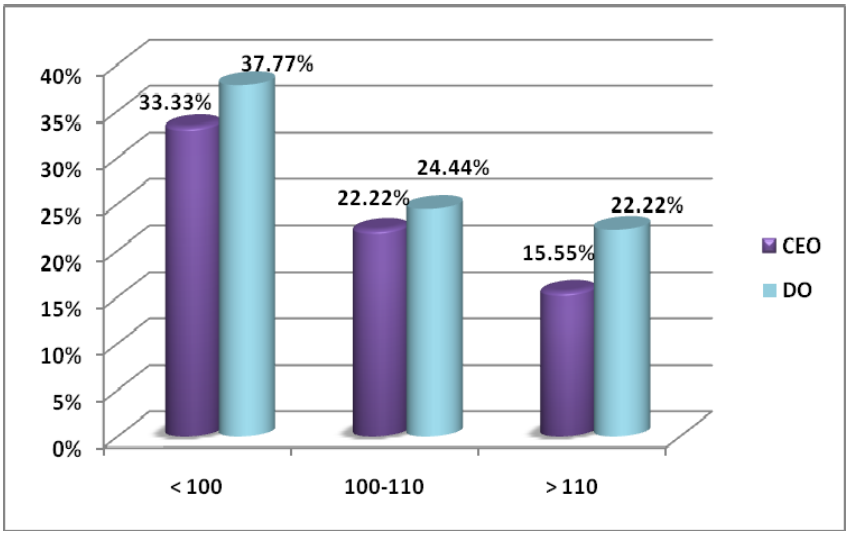
At the end of the maturation period the oocytes diameter increased in both groups: 66,66% vs. 62,22% oocytes presented d<100; 77,77% vs. 75,55% oocytes presented d=100–110; 84,44% vs. 77,77% oocytes presented d>110. As expected, the percentage of maturated oocytes of CEO in all size categories was higher than DO (graphic 1, graphic 2).

Table 1 - Ovine oocyte distribution in size categories after "in vitro"maturation

Exp. Group	Oocytes diameter (µm)		Cultured oocytes (n)	Maturated oocytes	Degenerated oocytes
	before IVM	after IVM			
CEO	<70	<100	45	66,66% (30 oocytes)	33,33% (15 oocytes)
	70–90	100–110	45	77,77% (35 oocytes)	22,22% (10 oocytes)
	>90	>110	45	84,44% (38 oocytes)	15,55% (7 oocytes)
DO	<70	<100	45	62,22% (28 oocytes)	37,77% (17 oocytes)
	70–90	100–110	45	75,55% (34 oocytes)	24,44% (11 oocytes)
	>90	>110	45	77,77% (35 oocytes)	22,22% (10 oocytes)



Graphic 1 – Percentage of maturated oocytes depending on their diameter



Graphic 2 – Percentage of degenerated oocytes depending on their diameter

The quality of oocytes from follicles of different sizes may be the main source of variation in the IVM process. The heterogeneity in oocyte diameters, regardless of follicular size, has been reported in many species (Motlik and Fulka, 1986, Fair et al., 1995). In the present study, however, the observed heterogeneity in diameter of the oocytes isolated from antral follicles, ranging from 2 to 6 mm, was similar than those reported in the same or other species (Motlik Fulka, 1986, Laurincik et al., 1996, Raghu et al., 2002).

In several species, the oocyte ability to resume and complete meiosis in vitro has been attributed to the oocyte diameter (Motlik and Fulka, 1986, Fair et al., 1995). The smaller oocytes tend to follow an abnormal path of meiotic maturation, resulting in disturbances in the maturation process (Pavlok et al., 1992). In cattle, some data have indicated a clear relationship between oocyte diameter and developmental competence. In sheep, there is a relationship between follicular size and oocyte diameter, as oocyte diameter influences the meiotic progression.

In the current study, despite the variation in oocyte diameter, the data showed relationship between the size of oocytes and its ability to reach maturation. It seems that the micro-environmental condition of sheep oocytes in peripheral follicles (2–6 mm in diameter) is the main indicator regarding their progression through meiosis, regardless of the oocyte size.

Regarding the effect of oocyte diameter and its relationship with the presence of cumulus cells, the data showed that in the absence of cumulus cells (DO) there was no significant difference in maturation between denuded oocytes in the different size categories. The importance of cumulus cells on *"in vitro"* maturation of the oocyte emerged in this study, with results that are in agreement with those reported by other researchers.

CONCLUSIONS

1. After the application of aspiration technique a total number of 394 oocytes were obtained from 213 follicles, with an average of 5,63 oocytes/ovary.
2. From the total of 394 sheep oocytes recovered by aspiration method 270 (68,53%) were considered *"cultivable"* while 124 (31,47%) were considered *"non-cultivable".*

3. In sheep, follicles ranging from 2 to 6 mm, present heterogeneity in the oocyte size (diameters were in the range of 65,3–98 µm for the smallest and largest oocytes, respectively).
4. In both CEO and DO the percentage of maturated oocytes was higher in the largest oocytes (>90 µm): 84,44% and 77,77%.
5. At the end of the maturation period the oocytes diameter increased in both groups: 66,66% vs. 62,22% oocytes presented d<100; 77,77% vs. 75,55% oocytes presented d=100–110; 84,44% vs. 77,77% oocytes presented d>110.

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CREATING BEST PRACTICE GUIDES-BASIC REQUIREMENT FOR ACHIEVING SAFE AND HEALTHY FOOD

R. POPA[1], MAGDA GONCIAROV[2], L. TUDOR[2] , DOINA LUPU[1]
[1]Sanitary Veterinary Direction and for the Food Safety,
[2]U.S.A.M.V.,Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei, District 5, Bucharest, 050097, magdagonciarov@yahoo.com

SUMMARY
GMP/GHP sites covers the basic requirements of hygiene and processing to ensure safe and healthy food production.
These requirements relate to infrastructure and equipment, raw materials, production control, waste management, pest control, sanitation procedures, quality health water, maintaining the cold chain, health personnel, sanitation personnel training. When properly implemented, these rules contribute to the control .Officers possibilities of contamination, failure to comply with hygiene principles governing these areas.
Key words: guide, European Commission, Codex Alimentarius .

Member States of the European Union encourage the establishment of national guides to good hygiene practice and application of HACCP principles. It is encouraged the dissemination and use of national guidelines and of the Community. However, food business operators may use these guides on a voluntary basis.

National guidelines are being drawn as guides to good practice, they are made and distributed by the food business:

- in consultation with representatives of parties whose interests may be seriously affected, such as competent authorities and consumer associations;
- compliance with codes of practice applicable to the Codex Alimentarius.

Member States shall transmit national guides and this establishes and operates a system of registration of these guides and make it available to Member States.

Community Guidelines, prior to completion of Community guides to good hygiene practice and application of HACCP principles, the Commission shall consult the Standing Committee guides, scope and theme.

In establishing the Community guidelines, the Commission shall ensure that they are prepared and distributed:

- by or in consultation with representatives of the resort community of food sectors, including SME's and other stakeholders such as consumer associations;
- in collaboration with parties whose interests may be seriously affected, including competent authorities;
- taking in account of codes of practice applicable to the Codex Alimentarius.

Commission invites the Committee on the Food Chain and Animal Health to regularly review all guidelines made. This analysis has the purpose to preserve practical guidelines and to take account of scientific and technical progress.

National and Community guides shall provide guidance on good practice to combat health risks in primacy production and related activities.(1)

Guides to good health practice must include adequate information on the risks involved in primacy production and related activities and measures to combat such risks,

including the relevant set of national and Community law or national and community programs. These risks and measures may include, for example:

- control of mycotoxin contamination, heavy metals and radioactive substances;
- use of water, organic waste and fertilizers;
- correct and appropriate use of veterinary drugs and feed additives and their traceability;
- preparation, storage, use and traceability and litter;
- proper disposal of dead animals, waste and litter;
- protective measures designed to prevent the introduction of contagious diseases transmissible to humans through food, and must notify the competent authority;
- procedures, practices and methods to ensure that foods are produced, handled, packaged, stored and transported in adequate health, including effective sanitation and pest;
- hygiene measures for slaughter and breeding animals;
- measures on record keeping.(3)

The official checks shall be made on the basis of risks based on risk classification in the production of food.

Inspection bodies have a responsibility to classify units of food, using raw materials of animal origin and/or non-animal based production activities and the actual risk associated with existing work. Classification units are the basis for programming the control activity.

Guides of good sanitary practice is a simple mean, but effective, to overcome difficulties that may arise in certain food establishments to implement HACCP procedure detailed. Representatives of various sectors of food and, on particular, of those sectors where many food establishments have difficulty in developing HACCP procedures, should consider these guidelines, and authorities should encourage sector representatives to develop such guidelines .Needed to assist the development of these guides to good practice for those food sectors that are insufficient or poorly organized.

Using of the guides to good practice may help food establishments to control hazards and demonstrate compliance. They can be applied by any food sector and, especially, where food is handled according to procedures well known that are often part of the training of operators in the usual sectors, such as:

- restaurants, including food facilities of transport, such as on ships;
- catering sectors delivering prepared a central unit;
- the bakery and confectionery;
- retail stores, including butchers. (5)

For such units may be sufficient for the guides to good practice to describe a simple and practical methods to control hazards, not mandatory to enter the details of the nature of the hazards and identify critical control points. These guidelines should, however, cover all significant hazards in a unit and must clearly define the procedures to control these hazards and corrective actions to be taken in case of occurrence of problems.

Such guidelines may also emphasize the potential hazards associated with certain foods (egg raw eggs and the occurrence of salmonella in them) and food contamination control methods (egg purchase these raw eggs from a reliable source, and the combination time/temperature processing).

Best practice guidelines have been developed and evaluated by competent authorities for many food sectors, They are usually a combination of good hygiene practices (GHP) and HACCP- based elements and include, for example:

- practice guidelines for the implementation of mandatory requirements;
- requirements for a raw materials;
- a hazard analysis;

- pre-establishment of critical control points in the preparation, manufacture or processing of food identifying hazards and specific control requirements;
- preventive hygiene measures to be taken when handling sensitive and perishable products (such as, for example, products ready for consumption);
- development of several measures, if prepared food for groups of consumers with increased susceptibility to illness (children, elderly, etc..)
- the need for documentation and records;
- protocols for data validation of life.(3)

A special type of guide to good practice is a generic HACCP guide.

General guide may suggest common hazards and controls of certain food activities and help the manager or the HACCP team in making food safety procedures or methods and appropriate record keeping.

Food operators must be aware that there may be other hazards, egg. those associated with the unit or process location that apply and that such hazards can not be expected in a generic HACCP guide. When used generic HACCP guides exist, however, the necessity of further examination for the possible presence of these hazards and their control methods. In those areas where there is a similarity between the activities, where the manufacturing process is linear and where prevalence may be high hazard, generic guidelines may be appropriate, egg.:

- for slaughterhouses, establishments handling fishery products, dairy units, etc..
- to units using standard food processing procedures, such as food preservation, liquid food pasteurization, freezing/ quick-freezing food, etc.(4)

CONCLUSIONS

1. GMP/GHP cover the basic requirements of hygiene and processing to ensure safe and healthy food production.
2. European Union states encourage preparing national guides to good hygiene practice and application of HACCP principles. It is encouraged the dissemination and use of national guidelines and of the Community. However, food business operators may use these guides on a voluntary basis.
3. National and community guides must provide guidance on good practice to combat health risks in primary production and related activities.
4. General guide may suggest common hazards and controls of certain food activities and help the manager or the HACCP team in making food safety procedures or methods and appropriate record keeping.

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STUDY ON THE RISK ANALYSIS (IDENTIFICATION OF HAZARDS, THEIR EVALUATION, IDENTIFYING PREVENTIVE MEASURES TO CONTROL RISK)

RAREŞ POPA[1], MAGDA GONCIAROV[2], LAURENȚIU TUDOR[2] , DOINA LUPU[1]
[1]Sanitary Veterinary Direction and for the Food Safety,
[2]U.S.A.M.V.,Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei, District 5, Bucharest, 050097, magdagonciarov@yahoo.com

Summary
The risk, according to NACMF (National Advisory Committee on Microbiological Criteria of Foods) is any element of physical biological or chemical may pose a threat to consumer health.
Basic terms used when discussing HACCP and risk assessment are:
Hazard and risk. The terms are not used with the same meaning in the literature. Sometimes the term hazard is defined or used to refer to the cause of an adverse, sometimes defined having the meaning of negative result, which is a concern.
K**ey words:** danger, risk, contamination

Risk is something defined as the probability or possibility of occurrence of an undesirable effect, sometimes defined as including both elements of probability and impact. Sometimes it can be defined to include elements of probability and impact, but is used to refer only probability.

It was suggested that it would be appropriate that the term hazard is used only to designate an agent or an action that might cause adverse effects (egg. pathogen, toxins or chemicals having the food as a source).

Performing risk analysis is a key stage of the HACCP system, an inadequate analysis of hazards that can lead to wrong design of a HACCP plan. This stage involves technical expertise and scientific documentation in various fields to correctly identify all potential hazards. An important role in identifying hazards has the HACCP team members with experience in product microbiology, hygiene and product technology.

The contaminants mean those bodies or elements carrying the role of microbiological agents or vectors, which may at the same time with achieving or penetration into food, to contaminate it with infectious agents which it bears.

Classification factors for contamination:

A. Biotic factors (direct)
- subjects or carriers (stool, excretions, secretions, hair, feathers, appendages, etc.).
- Healthy subjects role-vector (tail, horns, hair, feathers, appendages, etc...).

B. Abiotic factors (indirect)
- technological factors (equipment, vehicles, packages, tools, installations, etc.)
- environmental factors (water, dust, pollen, plant debris, etc...)
- other factors (wood, glass, metal, plaster, etc.) (4).

Ways of contaminating food:

1. Primary contamination type: animal product is contaminated before slaughter, milking, etc... By direct transmission of the pathogen (milk obtained from an animal that evolved a subclinical staphylococcal mastitis is a milk – raw material infected with staphylococcus);

2. Secondary contamination type:

a) through factors that depend on the sick animal (stool, hair, secretions, excretions, appendages that come in contact with food, milk obtained from cattle that evolves Colibacilosys and where hygiene conditions are not complied with the milking, may be contaminated with coli bacillus through stool);

b) through factors from a healthy animal, but contaminated by a sick animal (hair, appendages, feathers) (milk obtained from a healthy animal may become contaminated if the hair gets into the milk that was contaminated by sneezing or coughing by a neighboring animal, sick or a carrier of bacillus of tuberculosis);

c) through factors that depend on the role of vector animals (rodents, birds or insects or infected with the role of vectors that come into contact with food) (A pig carcass properly stored for drying may be contaminated throughout the stool by a rodent who has Yersinioza). (1)

Risks of contamination:

- primary risk - is in direct relationship with primary contamination factors;
- secondary risk - is in direct relationship with factors secondary contamination.

The risk analysis includes:

a. identifying hazards related to food at all stages of manufacture;

b. assessing severity (consequences of exposure to the risk factor) and the likelihood of such hazards;

c. identifying the existing preventive measures necessary to control these hazards.

Risks associated with food can be classified into three categories: biological, chemical and physical.

Depending on the hazards involved, biological pathogens can be classified as: bacterial, viral, parasitological, fungal and prion.

Bacteriological risks are often associated with raw materials from which it is produced meat, including animals that are major components. Bacteriological hazards can reach food during meat processing, from those who are involved in processing; the processing environment is made by other ingredients in the products, or even due to processing.

Identification of bacteriological hazards that may be subject to production is clearly a difficult task, much of pathogens may be associated with meat: Salmonella, Campylobacter jejune, Escherichia coli, Listeria monocytogenes, Clostridium botulinum, Staphylococcus aureus and Yersinia enterocolitica.

In developing the HACCP plan is necessary to comply with the requirement of three biological hazards:

- Destroying, removing or reducing risks;
- Prevent recontamination;
- Halting the development of microorganisms and toxins production.

The heat treatments applied to products (pasteurization, refrigeration, freezing, etc...) Microorganisms may be destroyed or inhibited.

Temperature, pH, water activity (a_w), time, substrate rich in nutrients, preservatives, the presence or absence of oxygen are factors that influence the development of pathogens. These factors influence differently depending on the species bacterial microorganisms.

Dangerous microorganisms and parasites were classified according to severity risk that has three groups: severe risk, moderate risk of extensive spread, moderate risks with limited distribution.

Severe risks:

Clostridium botulinum, Salmonella typhi, Salmonella paratiphi A, B, Tenia solium, hepatitis A, Trichinella spiralis;

Moderate-risk:

Listeria monocytogenes, Norwalk virus family, rotaviruses, Ascaris lumbricoides;

Moderate to high-risk spread:

Staphylococcus aureus, Clostridium perfringens, Giardia lambilia;

Alimentary infections are the result of eating foods that contain live pathogens, harmful to health (Salmonella, Listeria monocytogenes).

Food-origin toxic infections are diseases that can be caused by factors combined, like the consumption of bacterial loaded food and / or their metabolic products.

Infectious diseases (tuberculosis, brucellosis, etc...) Can occur in ingesting food containing a small number of pathogens, their multiplication is not required in the food. (2)

Chemical dangers may be caused by a natural process that occurs in food or which may occur during processing. Substances in this category are intentionally or unintentionally added during growth, harvest, storage, processing, and distribution and could include elements of feed, water, medicines, pesticides, chemicals (lubricants, cleaning solutions, paint, etc...).

Chemicals used in food production can be toxic at a certain level, causing illness or death from consumer storage (preservatives, dyes, pigments). Natural chemical hazards can be: aflatoxins, mycotoxins, allergens, etc... Chemical hazards may involve illness accumulation in the body or even death by ingesting a dangerous dose. Application of the HACCP system is necessary to control hazards. Allergens are proteins whose consumption can cause an immune response of the body in different forms of the light phase, redness, itching to the worst, namely anaphylaxis. Some foods can cause allergic reactions, most often involved are the proteins in soy, wheat, peanuts, nuts, eggs, milk, shellfish, and sesame seeds. Since the allergic reaction can be caused by very small amounts of allergen, public authorities require manufacturers to properly label the products with mandatory indication of a possible allergen.

Polycyclic aromatic hydrocarbons (PAHs) are substances produced during the smoking process, as a food preservative.

Accumulation of PAHs in foods that reek of smoke depends on how the natures of the fuel, temperature, smoke, the process, packaging and coating structure.

A smoking temperature of 20-25 ° C does not significantly influence the production of PAHs. Temperatures of 400-1000 ° C can cause a linear increase of PAH.

Nitrates and nitrites, sodium or potassium, additives in specific color printing and the preservation of food products are currently used technology of meat. A possible chemical risk is misuse of sodium nitrite which has a toxic chemical, being a factor to be considered in risk analysis.

In healthy adult bodies nitrites and nitrates are absorbed in the gastrointestinal tract. The most exposed category to nitrites poisoning is infants up to 3 months.

In calculating the content of nitrates and nitrites on the finished product, regulated by law, is considered conservation with smoke that may be another source of nitrates and nitrites.

Maintain control of chemical risks can be achieved by:

- Pre Reception (specifying the composition of raw materials, checking quality certificates issued by the supplier, etc...)
- Check before use (setting the purpose for which chemicals will be used and verify the quantity used, ensuring proper labeling of substances used);

- Monitoring of the storage and handling to avoid conditions favorable for the development of toxic substances) and
- Inventory of existing chemical substances, their strict records and registration of substances, dosage and how to use them.

Natural Hazards refers to a natural component of food which may occur unexpectedly injury or illness of the person who consumed food. Foreign materials like glass, metal and plastic are the most familiar natural hazards which could occur if equipment or production process was not properly checked during processing.

There are many causes that may contribute to the occurrence of natural hazards in food including contaminated materials, maintenance or poor design of equipment or resource materials used in packaging may be contaminated or negligence of employees.

Natural hazards can be easily prevented through good working practices. In this may take measures to prevent the major physical threat. (3)

CONCLUSIONS

1. The risk, as NACMF says (National Advisory Committee on Microbiological Criteria of Foods) is any element of physical biological or chemical may pose a threat to consumer health.
2. The risk is sometimes defined as the probability or possibility of occurrence of an undesirable effect, sometimes defined as including both elements of probability and impact. Sometimes it can be defined to include elements of probability and impact how, but is used to refer only probability.
3. Making the risk analysis is a key stage of the HACCP system, an inadequate analysis of hazards can lead to wrong design of a HACCP plan.
4. Through contaminants means those bodies or elements carry the role of microbiological agents or vectors, which may once to achieve or penetration into food, contaminating them with infectious agents whom it bears.

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4. **Regulation (EC) no. 854/2004** of the European Parliament and EU Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin for human consumption;

THE ASSESSMENT OF HYGIENE IN DAIRY COWSHEDS WITH TIE–STALLS IN TRANSYLVANIA

SILVANA POPESCU, CRISTIN BORDA, MARINA SPINU, R. STEFAN, CARMEN D. SANDRU, EVA A. LAZAR
Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj-Napoca, 3-5 Manastur street Romania; popescusilvana@yahoo.com

The aim of this study was to assess the hygiene level in dairy cowsheds with tie-stall housing, in Transylvania. We evaluated 20 tie-stall barns (1376 dairy cows) for body hygiene of cows through the method proposed by Cook. The obtained results were statistically processed. The distribution of scores of 3 and 4 differed in the three body regions of the cows and among cowsheds. The mean value for the udder area was 28.15%, for upper leg and flank 44.11% and for lower leg 34.24%. The multiple comparison Tukey-Kramer test showed significant differences between the mean proportions of scores of 3 and 4 in the udder area and in the upper legs and flank ($p < 0.001$) and in the lower legs and the upper legs and flank ($p < 0.01$), respectively. Positive correlations between hygiene levels of the three body regions; namely udder and lower legs ($r_s = 0.88$, $p < 0,0001$), udder and upper legs and flank ($r_s = 0.85$, $p < 0.0001$) and also lower legs and upper legs and flank ($r_s = 0.91$, $p < 0.0001$) were demonstrated. Significant negative correlations between the length of the beds and the cleanliness of legs ($r_s = -0.47$, $p < 0.01$; $r_s = -0.56$, $p < 0.05$) and udder ($r_s = -0.62$, $p<0.01$) were demonstrated too. The obtained results indicate poor hygiene in the investigated sheds, with negative influence on dairy cattle health and welfare.

Keywords: dairy cow, hygiene score, cleanliness, body hygiene, dirtiness

The environment in which dairy cows are kept have decisive effect on their health and welfare. A clean and comfortable barn represents the key to maintaining health and longevity of dairy cows. The appraisal of the barns hygiene level can be done through several assessment systems based on the quantification of the manure soiling in different body regions of the cows (Chaplin et al., 2000; De Rosa et al., 2003; Faye and Barnouin, 1985; Hughes, 2001). However, apart from their value in scientific research, the majority of these systems failed as practical hygiene monitoring tools at farm level (Cook, 2002). A practical system for quantifying the hygiene at farm level is the one proposed by Cook (2002). The hygiene scoring system (Cook, 2002) is considered to be an effective tool in showing the need for remedial measures for the existing deficiencies in the hygiene management. For a scoring system to be useful both for veterinarians and farmers, the significance of manure contamination in different body areas must be understood and the dirtiness level compared to an established standard, derived either from the contamination level of the same farm in time, or from the data obtained in some similar farms (Cook, 2002). For the hygiene scoring to be taken seriously, the farmer must understand what the costs of keeping animals in a dirty environment are. For dairy cows the outcome of low hygiene is a high risk of mastitis and worsening of lameness.

The connections between cowshed hygiene, clean cows and low number of somatic cells in mixed milk were highlighted in several studies (Barkema et al., 1999; Barkema and

Schukken, 2003; Bodoh et al., 1976). A high hygiene standard indicates a limited exposure to the pathogens of environmental mastitis and constitutes a basic aspect of food safety, hygiene protocols and quality.

Several researches indicate also that cows housed in humid conditions or in ones contaminated by dejections are much more prone to infectious foot diseases, such as foot rot (interdigital necrobacillosis), heel horn erosion and hairy heel warts (papillomatous digital dermatitis) (Bergsten, 1997; Cook and Cutler, 1995). The cleanliness of different body regions (hind legs, udder) of the cows is associated with the surface of the beds and bedding type, housing techniques and the lenght of the beds (Bergsten and Pettersson 1992; Chaplin et al. 2000; Cook 2002). The available literature suggests that tie-stall cowsheds are cleaner than free-stall ones, due to the fact that the former have no manure-covered alleys (Cook 2002, Cook and Nordlund 2009).

The aim of this work was to assess the general hygiene of dairy cows kept in tie-stalls based on cows' body hygiene in cowsheds. The significance of the association between the length of the bed and hind legs and udder cleanliness was also determined.

MATERIALS AND METHODS

The investigations of this study were made between November – December 2009 in 20 dairy cowsheds (with 32-113 cows/shed) with tie-stalls, in Transylvania. Each cowshed was visited once. All the cowsheds were closed, with solid flooring. The cows were kept on short beds (1.6 -1.8 m lenght) in 60% of the cowsheds and on medium sized beds (1.8 - 2.2 m lenght) in 35% of the cowsheds. Long beds (2.4 – 3 m lenght) were found in only one cowshed. Most predominant breeds were the Romanian Spotted Cattle and Holstein Friesian, with an average milk production of 10-23 liters/cow/day. All of the assessed animals were adult cows, between 3 and 9 years of age, with body weights between 350 and 500 kg. In 90% of the cowsheds, small amounts of bedding (straw, sawdust, wood shavings) were used on the cows' bed. Only one cowshed had mechanical ventilation, all the others were naturally ventilated. In 70% of the investigated cowsheds the manure removal was done manually (once or twice a day).

The hygiene of the farms was assessed based on the cows' body hygiene, using the system (hygiene scoring system) proposed by Cook (2002). Three body regions were assessed: lower leg, udder and flank and upper leg, awarding points (from 1 to 4), depending on dejection soiling of these areas. For each area a different score was assigned. At the end, the proportion of scores of 3 and 4 (too dirty) was calculated for the three body regions of the cows from each barn and their mean percentage within all of the assessed cowsheds. All the cows (1376) from the 20 cowsheds included in the study were evaluated. In each cowshed the length of beds was measured for all cows. The results obtained were statistically processed with the software GraphPad InStat version 3 (GraphPad Software Inc. USA). Tukey-Kramer Multiple Comparisons Test-One-way ANOVA was used to compare the data and for determination of the correlations, the Spearman's rank correlation coefficient (r_s) was calculated.

RESULTS AND DISCUSSIONS

The obtained results following the evaluations of the cows' body hygiene in the 20 cowsheds are shown in Table 1. The proportion of scores of 3 and 4 is different in the three body areas of the cows. There were significant differences among the farms, as well. Thus, at udder level, a mean value of 28.15% was recorded, ranging from 12.76% to 46.66%, at the

lower leg level there were values between 15.95% and 46.66% with a mean value of 34.24% and at upper leg and flank level the proportion varied between 25.53% and 56.81%, with a mean value of 44.11 %.

Tukey-Kramer Multiple Comparisons Test showed significant differences between the average proportion of the scores of 3 and 4 at udder level and upper leg and flank ($p < 0.001$) and between the areas of the lower leg and upper leg and flank ($p < 0.001$) (Table 2). Positive correlations between the level of hygiene of the three body regions, namely, the udder and lower leg ($r_s = 0.88$, $p < 0.0001$), udder and upper leg and flank ($r_s = 0.85$, $p < 0.0001$) and, also, lower leg area and upper leg and flank ($r_s = 0.91$, $p < 0.0001$) (Table 3), were demonstrated. The correlations between the length of stall beds and the cleanliness of the hind legs and the udder are given in Table 4. The length of the stall-beds is negatively correlated with the hygiene of lower leg ($r_s = -0.47$, $p < 0.01$), upper leg and flank ($r_s = -0.56$, $p < 0.05$) and udder ($r_s = -0.62$, $p < 0.01$).

Table 1. Mean proportion of 3 and 4 hygiene scores for udder, lower leg and upper leg and flank body areas in 20 dairy cowsheds

Cowsheds	Proportion of Hygiene Scores 3 and 4		
	Udder	Lower Leg	Upper Leg and Flank
1	31.25	41.66	52.08
2	28.00	34.00	40.00
3	28.57	34.28	42.85
4	28.00	32.00	44.00
5	40.00	40.00	52.00
6	38.46	42.85	53.57
7	12.76	15.95	25.53
8	15.30	30.61	32.65
9	27.24	35.35	42.42
10	38.63	45.45	56.81
11	35.00	35.00	40.00
12	14.28	21.42	32.14
13	17.07	19.51	36.58
14	36.11	41.66	55.55
15	46.66	46.66	53.33
16	21.42	26.19	38.09
17	30.76	42.30	53.84
18	26.92	38.46	46.15
19	21.87	28.12	37.50
20	27.77	33.33	47.22
Mean	28.15	34.24	44.11

The hygiene scoring system was devised by Cook (2002) in order to quantify hygiene at farm level and to evaluate improvements needed in hygiene management. This system is considered as a remedial tool for the existing deficiencies. The author believes that a quantitative approach in the hygiene assessment of cows gives a more efficient meaning to conveying to the farmer the message that the cows are too dirty, compared with a qualitative appraisal. Besides, the recording of the scores by body region allows more accurate

recommendations on how to keep the cows clean. Because a mean score of hygiene level for each body area does not present any importance for the farmer, only the proportion of the scores indicating the "too dirty" condition is considered, namely the 3 and 4 scores.

Table 2. Tukey-Kramer Multiple Comparisons Test (One-way ANOVA) for soiling degree of the body areas of Udder, Lower Leg, Upper Leg and Flank

Comparison	Mean difference	q	P value
Udder vs Lower Leg	-6.08	3.043	ns p > 0.05
Udder vs Upper Leg and Flank	-15.96	7.981	*** p < 0.001
Lower Leg vs Upper Leg and Flank	-9.87	4.938	** p < 0.01

If the value of q is greater than 3.405 then the p value is less than 0.05, p = statistical significance,

ns = not significant, **= considered very significant, ***= considered extremely significant.

Table 3. Correlations between the hygiene degree in the three body regions: udder, lower leg, upper leg and flank, in 20 dairy cowsheds

Correlation	n	Correlation coefficient (Spearman's r)	95% Confidence Interval	p value
Udder and lower leg	20	0.88	0.73 to 0.95	p<0.0001
Udder and upper leg and flank	20	0.85	0.66 to 0.94	p<0.0001
Lower leg an upper leg and flank	20	0.91	0.78 to 0.96	p<0.0001

p = statistical significance, p < 0.0001 considered extremely significant, n = number of cowsheds investigated

Table 4. Correlations between the lenght of the stalls and the dirtiness degree in the three body regions of assessed cows

Correlation	n	Correlation coefficient (Spearman's r)	95% Confidence interval	p value
Stall's lenght and hygiene of the lower leg	20	-0.47	-0.76 to -0.02	0.0096**
Stall's lenght and hygiene of upper leg and flank	20	-0.56	-0.81 to 0.14	0.0334*
Stall's lenght and hygiene of the udder	20	-0.62	-0.83 to -0.23	0.0035**

p = statistical significance, * = p < 0.05 considered significant, ** = p < 0.01 considered very significant, n = number of cowsheds investigated

The high percentage of the 3 and 4 scores indicates a precarious, unacceptable hygiene level of the cowsheds, with severe consequences on health, productions and welfare of dairy cows.

In the investigated sheds the proportion of 3 and 4 scores is different for the three body areas of the cows, the differences being statistically significant between the areas udder vs upper leg and flank lower leg vs upper leg and flank (Table 2). The mean proportion of the 3 and 4 scores in the three body regions (Table 1) is higher than Cook's findings (2002) in his assessment of

20 dairy cattle farms in Wisconsin, with tie-stall and free-stall housing. He found that, on average the proportion of cows in tie-stalls considered to be “too dirty” was, by zone, as follows: 20% for udder, 25% for lower leg zone, 30% for upper leg and flank zone. However, these figures are higher than those found in an other study where, while the scoring systems were similar, the scores were not identical (Zurbrigg et al., 2005). Within 317 study farms, 8% had more than 15% of the cows with significantly dirty udders and 46% had more than 15% of the herd with significantly dirty hind limbs. Poor hygiene is a big problem in tie-stalls as the cow is both eating and lying in the same stall and the claws are often standing in manure.

In our study the dirtiest area of the animals was the upper leg and flank, followed by the region of the lower leg and the udder, respectively (Table 1). The obtained results are in agreement with the existing data in the literature, stating than the cows kept in tie-stalls have higher scores in the body region of the upper leg and flank than the ones in free breeding because of lying down in the dejections laid in stalls (Cook 2002). This body region can also get soiled in poorly maintained stalls presenting elements splashed with dejections or through the movements of the dirty tail around the hind section. In 70% of the investigated cowsheds the manure removal was done manually, leading to a deficient cowshed hygiene and vitiated air.

Similar to other researches (Schreiner and Ruegg 2003; Zurbrigg 2005), the present study shows that the cleanliness of the udders and of the legs are positively correlated (Table 3).

The cleanliness of the udder and of the hind legs is associated with the stall surface and with the bedding materials used, with the housing techniques and the stall dimensions (Bergsten and Pettersson, 1992; Chaplin et al., 2000; Cook, 2002). Significant correlations were demonstrated between the stall length and the hind legs and udder cleanliness (Table 4), in agreement with the results of other studies (Anderson, 2003; Zurbrigg et al., 2005). The high degree of soiling of the cows is mainly due to their housing in short stalls (60% of the assessed cowsheds had short stalls), being not properly cleaned. This can be explained by the fact that shorter stalls force the cows to lie diagonally when they want to be with all four legs inside the stall or makes them lay their hind legs on the manure evacuation alley. The cows lying diagonally on the beds have higher chances to urinate and defecate on the back part of the stall. If cows lie down before the stalls are cleaned, their hind legs become dirty. The cows lying with their hind legs on the manure evacuation alley may present large amounts of dirt and dejections attached to their legs. When these cows are in decubitus, the dirt and dejections are transferred to the stall, dirtying the bedding to a certain extent. Both the dirty udders and the dirty legs were correlated with an increased number of lame cows (Alban et al., 1996; Zurbrigg, 2005). There are several possible explanations for these correlations (Sprecher et al., 1997). In order to ameliorate the pain, lame cows spend more time in decubitus and thus they have more chances to lie in dejections (Kloosterman, 1997). Cows with severe lameness may be reluctant to rise once they are lying down and thus they can defecate and urinate in decubitus (Herlin, 1997). Both situations will result in a wet and dirty back segment of the stall, increasing the risk for those cows to have dirty hind legs and udders. A third possible explanation could be linked to management problems. Stall cleaning, periodic trimming and, generally, the periodic care of the hoofs can be seen by some farmers as lower priorities.

Relationships between udder hygiene and outbreak of mastitis episodes were also demonstrated. It has been long known that the rate of new infections is increasing with the bacterial density at the level of the teats. Several researches showed correlations between hygienic housing, clean cows and lower numbers of somatic cells present in the mixed milk at farm level (Barkema and Schukken, 2003; Bodoh et al., 1976). In one of the studies an

environmental sanitation indicator based on the amount of dejections present on the cow body and in its environs constituted a prediction element for the occurrence of coliform bacteria mastitis (Bartlettet al., 1992). The results of the investigation made by Cook (2002) demonstrated a significant correlation between the proportion of the udders rated with 3 and 4 in each farm, observed over a 6 months period, and the incidence of mastitis.

CONCLUSIONS

The results obtained show poor hygiene in the investigated cowsheds, with negative influence on dairy cows health and welfare. The dirtiness of the three body regions demonstrated in the study is mainly caused by the disregarding of the recommandations for daily cleaning and bedding change in the cowsheds but also by the improper stall lenght in the majority of the assessed cowsheds. The exposure of the cows to dirt, mud and dejections constitutes the premise for an increased percentage of subclinical and clinical mastitis and lameness.

ACKNOWLEDGMENT

This work was supported by CNCSIS –UEFISCSU, project number 1095 PNII – IDEI 1492/2009.

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THE LEVEL OF MICROCLIMATE PARAMETERS IN DAIRY CATTLE BARNS WITH TIE STALLS

SILVANA POPESCU[1], CRISTIN BORDA[1], IULIANA C HEGEDUS[2], R. STEFAN[1], EVA A. LAZAR[1]

, [1]Faculty of Veterinary Medicine,
[2]Faculty of Animal Husbandry and Biotechnologies in Agriculture; popescusilvana@yahoo.com

The aim of this study was to determine the microclimate parameters' level in tie-stall houses for dairy cows and to compare it with the recommended values of our country. A number of 15 dairy cattle tie-stall barns were investigated, in Transylvania, over the period of November - December 2009. The measurements were made in the morning and in the evening in each of the barns. The obtained data were statistically processed with the SPSS version 17 software. Excepting the ammonia concentrations, no significant differences were demonstrated by the Mann-Whitney test ($p>0.05$), between the morning and the evening determinations. The air's temperature had very similar mean values in the morning and in the evening, 11.82°C and 11.22°C, respectively. The air's humidity was higher in the morning, 83.10%, comparing with its evening value, 79.73%, and the airflow velocity had a mean value of 0.34 m/s in the morning and in the evening as well. All of the investigated barns were poorly illuminated, the mean intensity of the light was 14.46 lx. The ammonia's mean concentration was higher in the morning, 24.73 ppm, than at the evening determination, 15.13 ppm, the difference being statistically significant ($p<0.05$). The carbon dioxide was of 1213.3 ppm in the morning and 740.00 ppm in the evening, and the hydrogen sulphide was present in none of the investigated barns. The obtained results indicate inappropriate microclimate in the majority of the investigated barns with important divergences of the parameters from the recommended values for the dairy cows.

Keywords: air temperature, relative humidity, ammonia, carbon dioxide

Microclimate is important that determine air quality in dairy barns. Providing good air quality in farm animal housing is important for health and welfare of farm animals and staff and for the outdoor environment of farming enterprises (Seedorf and Hartung 1999, Radon et al. 2002). Microclimate is the local environment around a dairy cow where the climate may differ from the surrounding areas of the dairy building. The microclimate, or surrounding air, contains oxygen for the cow's metabolism and is the medium for the transport of excess heat, water vapors, gases emitted by the animals, gases from the decomposition of manure, and other particulate matter. The important microclimate parameters that affect air quality in dairy buildings include temperature, relative humidity, air velocity, noise and lighting as well as gases such as oxygen, carbon dioxide, methane, ammonia, hydrogen sulphide, and nitrous oxide. Others include dust and microorganisms found in air.

Temperature is an environmental parameter that can affect the health, welfare, and production efficiency of dairy cows, and thus the profitability of dairy production. The design of dairy buildings influences indoor temperature and, for that matter, the dairy cows themselves.

Dairy cows continuously produce heat and moisture. When moisture evaporates from the skin of cows, the cow's surface temperature decreases because of evaporative heat loss. A relative humidity (RH) over 90% at high indoor temperature will induce heat stress in dairy cows due to restricted evaporative heat losses. However, an excessively low RH results in excessively dry bedding material in the dairy building and increases dust and the incidence of lung diseases in dairy cows (Seedorf et al. 1998). Moreover, high relative humidity increases the rate of deterioration of building materials in the dairy building (De Belie et al. 2001a-c).
High gaseous concentrations in animal buildings affect the welfare of animals, workers and the life span of the buildings themselves (De Belie et al. 2001a-c, Radon et al. 2002). Carbon dioxide, methane, ammonia, hydrogen sulphide, and nitrous oxide are the most prominent gases found in dairy buildings. When gases produced in concentrated dairy production escape from the buildings, they contribute to environmental problems such as global warming, acid rain, and upsetting the nutrient balance in the environment (Anderson et al. 2003).
The aim of this study was to determine the microclimate parameters' level in tie-stall houses for dairy cows and to compare it with the recommended values of our country.

MATERIALS AND METHODS

This study investigated 15 dairy cattle tie-stall barns (30-100 dairy cows/barn), in Transylvania in the time interval November - December 2009. All of the barns were closed, with solid flooring. The manure cleaning was made by manual handling (in 80% of the barns) or mechanically (in 20% of the barns). The cattle houses had only natural ventilation systems, unorganized (in 12 of the barns) and organized (in 3 barns). The cow houses usually had short stalls (80% of barns). In 20% of the barns there were medium long beds. In 80% of the houses bedding was used (straw, sawdust). The cows were kept tied in the barns during the cold season (pasturing in the rest of the year, in daytime) or permanently (without pasturing). Each barn was visited once for the study. The temperature, the relative humidity, the air flow velocity, the lighting intensity, the ammonia, carbon dioxide and hydrogen sulphide concentrations in the air, were measured in the morning (at 5-6 a.m.) and in the evening (at 7-8 p.m.) in three different points of the barn. The mean values of the determined parameters were calculated for each barn.
Air temperature, relative humidity and air flow velocity in the barns were determined using a Testo 400 – GmbH & Co device. The lighting intensity was measured with an Extech electronic lux meter. Ammonia, carbon dioxide and hydrogen sulphide concentrations were determined by air sampling with Dräger – Multiwarn II (Dräger Safety, Germany) device.
The obtained data were statistically processed with the SPSS version 17 software and were compared with the maximal admitted values for dairy cattle houses in our country. The descriptive statistical indicators were calculated (mean, standard deviation, median, minimum and maximum) for the measured parameters. In order to compare values the Mann-Witney non-parametric test was used.

RESULTS AND DISCUSSIONS

The obtained results of the measurements made in the morning and in the evening in the 15 dairy cattle barns with tie-stalls are shown in Tables 1-2. Excepting the ammonia concentrations, no significant differences were demonstrated by the Mann-Whitney test ($p>0.05$), between the morning and the evening determinations.

Temperature had similar mean values at the two measurements (in the morning and in the evening), in accordance with those recommended for dairy cattle barns. Yet the maximal recorded value was higher than the recommended optimal temperature. Various recommendations for temperature conditions for keeping dairy cows appear in literature (CIGR 1994, Seedorf et al. 1998, Teye et al. 2008). In Romania the recommended optimal temperature for dairy cows ranges between 10 and 14 C° (NSVFSA 2007). Temperature is an environmental parameter that can affect the health, welfare, and production efficiency of dairy cows, and thus the profitability of dairy production. The thermal environment around a dairy cow varies according to the complex interactions between environmental conditions and animal-related factors. Furthermore, factors such as the dairy breed and age, structural design, floor type, stocking rate, and nutrition also influence how the thermal conditions in the building affect individual animals. Under certain optimum environmental conditions, dairy cows are not only comfortable, but produce higher outputs.

Table 1. Descriptive statistic analysis for microclimate parameters in the 15 investigated dairy cattle barns, in the morning.

Parameter	n	Mean	SD	Median	Minimum	Maximum	95% C I From To
Temperature (C˚)	15	11.82	4.56	10.900	7.00	19.70	9.29 14.34
Relative humidity (%)	15	83.10	9.39	85.400	65.50	94.85	77.90 88.30
Air flow velocity (m/s)	15	0.34	0.04	0.3400	0.29	0.400	0.32 0.36
Light (lx)	15	14.46	6.51	12.000	7.00	28.500	10.85 18.07
Ammonia (ppm)	15	24.73	7.86	28.000	9.00	35.000	20.37 29.09
Carbon dioxide (ppm)	15	1213.3	836.55	1000.0	100.00	2000.0	750.02 1676
Hydrogen sulphide (ppm)	15	0	0	0	0	0	0

n = number of barns, CI = confidence interval, SD = standard deviation

The measured relative humidity had in the morning (83.10%) and also in the evening (79.73%) mean values exceeding the optimal value for dairy cattle barns. In a study realised by Teye et al. (2008) in dairy cows' barns in Finland and Estonia, the relative humidity varied from 38% to 92%. For the relative humidity in animal buildings CIGR (1984) recommends maximum and minimum values as a function of indoor temperature. In Romania, the recommended optimum relative humidity for dairy cows is from 60% to 75% (NSVFSA 2007). Relative humidity in the dairy buildings exceeded the recommended values when the ventilation was inadequate. High relative humidity during the cold seasons is a major problem in most of the dairy buildings. A well-insulated roof is needed in naturally ventilated dairy buildings. Adequate roof insulation can not only prevent the condensation of moisture at roof level, which leads to rust and mould in dairy buildings, but also improve the exchange of air in the building.

The velocity of the air flow had the same mean values in the morning and also in the evening, corresponding to those recommended for dairy cattle barns. The maximal recorded values were slightly higher (0.4 m/s). The results are in conformity with those obtained by other researchers in their studies (Teye et al. 2008).

Table 2. Descriptive statistic analysis for microclimate parameters in the 15 investigated dairy cattle barns, in the evening.

Parameter	n	Mean	SD	Median	Minimum	Maximum	95% C I From To
Temperature (‘C)	15	11.22	3.86	10.70	6.30	16.70	9.08 13.36
Relative humidity (%)	15	79.73	8.77	82.00	61.50	94.00	74.87 84.59
Air flow velocity(m/s)	15	0.34	0.04	0.33	0.280	0.400	0.31 0.36
Light (lx)	15	14.46	6.51	12.00	7.00	28.50	10.85 18.07
Ammonia (ppm)	15	15.13	7.39	15.00	5.00	27.00	11.03 19.22
Carbon dioxide (ppm)	15	740.00	604.51	500.00	100.00	2000.0	405.20 1074.8
Hydrogen sulphide (ppm)	15	0	0	0	0	0	0

n = number of barns, CI = confidence interval, SD = standard deviation

All of the investigated barns were poorly illuminated; the mean value of lighting intensity was 14.46 lx. The light levels were about 4 times lower than the minimal recommended illuminance for the dairy cows (50-60 lx). Studies ruled in the United States revealed increased milk productions up to 16% in the cows which benefitted of prolonged illumination at a higher light intensity (Dahl et al. 2000). The conclusion of these studies is that cows should be exposed daily to 16-18 hours of light per day (more than 200 lx), in order to meet the benfits of a long photoperiod.

The ammonia was found in each of the investigated barns, in the morning as well as in the evening (tables 1-2). Its concentration is higher than those described in scientific literature (6-10 ppm) (Groot Koerkamp et al. 1998, Seedorf and Hartung 1999, Demmers et al. 2001, Jungbluth et al. 2001, Anderson et al. 2003). Clark and McQuitty (1987) studied the air quality in six Alberta commercial dairy barns and found that the NH_3 was present in all six barns and the overall mean values ranged from 7 to 20 ppm. Groot Koerkamp et al. (1998) investigated concentrations and emissions of ammonia in different livestock buildings in England, the Netherlands, Denmark and Germany. The highest ammonia concentration in cattle houses was found in Germany (22.7 ppm), with mean values in different countries varying between 0.9 ppm and 7.1 ppm. Another investigation of ammonia concentrations in livestock buildings in Germany found a mean value of 6.4 ppm in cow houses (Seedorf and Hartung 1999). In a more recent study conducted in dairy cow barns in Finland and Estonia, the ammonia

concentrations varied between 0 and 64 ppm (Teye et al. 2008). The maximal reported value was higher than our study's highest value (27 ppm). High ammonia concentrations are usually found in closed buildings. Ammonia from closed buildings for dairy cattle is produced in large quantities through urea hydrolysis in urine (Muck and Steenhuis 1981). In the majority of the studied barns the ammonia concentration was higher in the morning, the Mann-Whitney Test demonstrating very significant differences between the values in the morning and evening measurements (p=0.0019). These results highlights the importance of ammonia measurements in the barns' air early in the morning, before aerating, in order to find out the real concentration not only of ammonia, but also of other harmful gases, which affect cows during the night. During the day, because of the opened doors and windows, the ammonia concentration is usually lower in the dairy cattle houses. The indoor ammonia concentration depends on the flooring type, bedding material, animals' age, the microclimate factors, the manure evacuating system, the frequency of cleaning and on the animals' diet (Groot Koerkamp et al. 1998). In our study, the barns with high ammonia concentrations were poorly ventilated and dirty. In 60% of the investigated barns the ammonia concentration exceeded the admitted treshold value of our country (26 ppm). NH_3 levels in animal housing can exceed 25 ppm when lower winter ventilation rates are used and can reach 40 ppm in poorly ventilated buildings (Groot Koerkamp et al. 1998). Ammonia is considered the most significant pollutant in the air of the cattle barns, due its irritating effect on respiratory epithelium (Marschang 1973). At concentrations less than 100 ppm and in a poorly ventilated facility, ammonia appears to affect pulmonary function in cattle.

The carbon dioxide's concentration varied in the investigated barns, the morning values being higher than those measured in the evening, but this difference was not statistically significant (P>0,05). In all of the barns the carbon dioxide concentration was below the maximal admitted limit value for dairy cattle houses. The main source of carbon dioxide in dairy buildings is from respiration. Minor proportions (6.1%) are produced through the degradation of manure and urea (Kinsman et al. 1995). The average concentration of CO_2 in dairy buildings is 1900 ppm (Phillips et al. 1998).

CONCLUSIONS

1. The air temperature and the airflow velocity registered moderate divergences from the admitted limits in the majority of the investigated barns.
2. The relative humidity showed important abberance from the values recommended for dairy cows.
3. The lighting intensity was inappropriate in all of the studied barns.
4. The ammonia concentrations exceeded the treshold value in 60% of the investigated cattle houses, indicating the need for improvement in the future's housing conditions. The values determined in the morning were significantly higher than the values measured in the evening.
5. In none of the barns the carbon dioxide exceeded the admitted limit value for dairy cows.
6. The hydrogen sulphide was found in none of the investigated dairy houses.
7. Excepting the ammonia concentrations, no significant differences were found between the morning and the evenening determinations.

ACKNOWLEDGMENT

This work was supported by CNCSIS –UEFISCSU, project number 1095 PNII – IDEI 1492/2009.

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STUDIES CONCERNING HYGIENIC BODY SCORES AND THE RISK OF INTRAMAMMARY INFECTIONS IN COW

Elena ROTARU, B. TAŞBAC, Elena MITRĂNESCU, Maria CRIVINEANU, V. NICORESCU, Magda GONCIAROV

Faculty of Veterinary Medicine Bucharest, 105 Splaiul Independentei, 050097, Bucharest, Romania, elenrotaru@yahoo.com

Abstract

Intramammary infections in cows leads to major economical losses due to the decrease of milk production (the consequence of pathological process), withdrawal period (drug residues in milk) and increased percentage of reformed cows (compromised mammary gland). Many authors agree that one of the major causes of mastitis is represented by high charges of microorganisms in the environment. The aim of this study, performed in a farm with 270 dairy cows near Bucharest, was to follow the correlations between the presence of mastitis (bacteriological exams) and hygienic body score (mammary gland and posterior members - according to Cooke's evaluation key). The evaluation was made on 27 cows (10%) which showed obvious problems of mammary gland and posterior members' hygiene (score 3 and 4); these cows were chosen by clinical inspection at the entrance in the milking area. The study revealed a high percentage of 3 and 4 hygienic scores, which indicates an unacceptable hygiene in the farm and also the fact that poor hygienic scores are positively correlated with the presence of microorganisms responsible for mastitis, especially Arcanobacterium pyogenes, Streptococcus agalactiae and Staphylococcus aureus.

Key words: cow, environment, hygienic body score, mastitis.

Intramammary infections in cows are multifactorial diseases. The relationship between the environment as potential contamination source and the risk of mastitis was studied by many authors (1, 2, 3). The results demonstrated that environmental salubrity is an important factor for the decrease of mastitis prevalence and incidence in dairy cows farms.

MATERIALS AND METHODS

The study was performed on 27 dairy cows from a farm near Bucharest (10%), chosen aleatory at the entrance in the milking area. Using Cook system, there were evaluated posterior members and mammary gland and there were given scores between 1 and 4 depending on their degree of dejections dirtiness.

For posterior members it was appreciated the quantity of dejections and their area, score 1 corresponding to no identified dejections, score 2 – low quantity of dejections, score 3 – boards of dejections, but with visible pelt and score 4 – boards of dejections.

For the mammary gland, hygienic scores were: score 1 – clean mammary gland, score 2 – mammary gland a little dirty at the side of mamelons, score 3 – the existence of dejections boards in the inferior half of mammary gland and score 4 – the presence of dry dejections boards. Finally it was calculated the proportion of 3 and 4 scores and their average percentage. In order to appreciate the risk of intramammary infections, there were determined, by usual methods, the number of total germs (NTG) and the somatic cells count (SCC) in milk samples collected before morning milking. Several authors demonstrated that samples collected after milking are less susceptible to be contaminated, because the mamelon and papillary duct are washed and disinfected (2). Other authors admit that, if the personnel

are specialized and the sampling is correct, the difference between the contamination of a sample before and after milking is minimal (3).

RESULTS AND DISCUSSIONS

Visual inspection of shelters and paddocks in the studied unit demonstrated the existence of inadequate environmental conditions for appropriate body hygiene. Thus, in figs. 1-7 are presented the main deficiencies, respectively: inadequate hygiene of posterior area (fig. 1, 2) and mammary gland (fig. 3, 4) as a consequence of the contact with dirty sheet (fig. 5), and also inadequate paddocks hygiene, a risk factor that influences milk salubrity and mammary infections incidence (fig. 6, 7).

Score 3

Score 4

Fig. 1, 2 – Dirty posterior members

Score 3

Score 4

Fig. 3, 4. Dirty mammary gland

Fig. 5 – Contact of mammary gland with dirty sheet

Fig 6, 7. Inadequate paddocks favor the existence of dirty animals

The results obtained following the examinations are presented in table 1 and have the following significance:

- scores 1 and 2 indicate clean animals;
- scores 3 and 4 indicate dirty animals - a risk situation which can be improved;
- number of total germs / ml has reference values under 50000;
- somatic cells count /ml has reference values under 200000.

Table 1

The results obtained after the examination of body hygiene, number of total germs and somatic cells count in milk

Nr.	**Ident. nr.**	**Mammary gland score**				**Posterior members score**				**Number of total germs (nr./ml)**	**Somatic cells count (nr./ml)**
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**		
1	3148				x				x	560000	290000
2	3543		x				x			45000	49000
3	2902	x				x				35000	160000
4	3007			x			x			139000	450000
5	3578	x						x		42000	45000
6	2433				x				x	293226	750000
7	2652				x				x	459350	870000
8	2699			x				x		173100	250000

9	3932				x			x		683870	450000
10	3745	x					x			36600	75000
11	3312	x				x				30000	90000
12	9043	x				x				32500	85000
13	4405		x			x				49000	120000
14	3312				x				x	389000	650000
15	5217				x				x	967400	950000
16	3200			x				x		950000	870000
17	3301		x				x			27600	49000
18	4278	x					x			20000	49000
19	2315		x				x			14000	75000
20	1722		x				x			49000	85000
21	3387		x			x				37000	49000
22	9012				x				x	173100	750000
23	1545				x				x	368500	750000
24	7751			x				x		857300	550000
25	2346				x				x	1200000	980000
26	1312				x				x	748300	690000
27	2267			x				x		595400	750000

In table 1 it is noticeable that, from all 27 analyzed cows, in 12 cases (44.44%) there were given scores 1 and 2 (clean posterior members and mammary gland), while 15 cows (55.55%) obtained 3 and 4 scores (dirty posterior members and mammary gland). Between the two body regions the differences of scoring are insignificant; as a rule, dirty posterior members are correlated with dirty mammary gland, rarely dirty posterior members being correlated with clean mammary gland. This fact is explained by the contact of the two areas with dirty sheet, with consequent negative effects. The analysis of total number of germs and somatic cells count as indicators of intramammary infections risk demonstrates that for the cows with precarious posterior members and mammary gland hygiene, the values of these parameters are higher than reference values.

Present study demonstrates that:

- mammary hygiene is positively correlated with posterior members hygiene;
- there are positive correlations between mammary hygiene and total number of germs and somatic cells count as risk factors for mastitis (Fig. 8, 9).

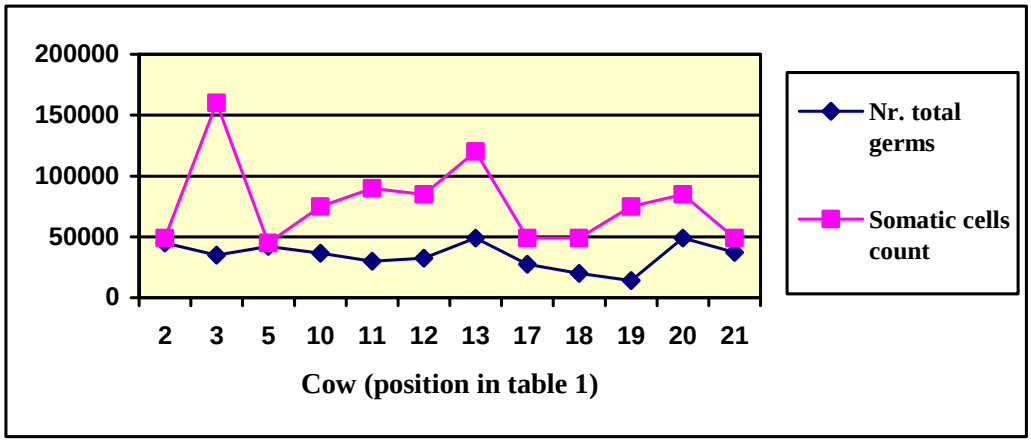

Fig. 8. The correlation between scores 1, 2 and NTG / SCC

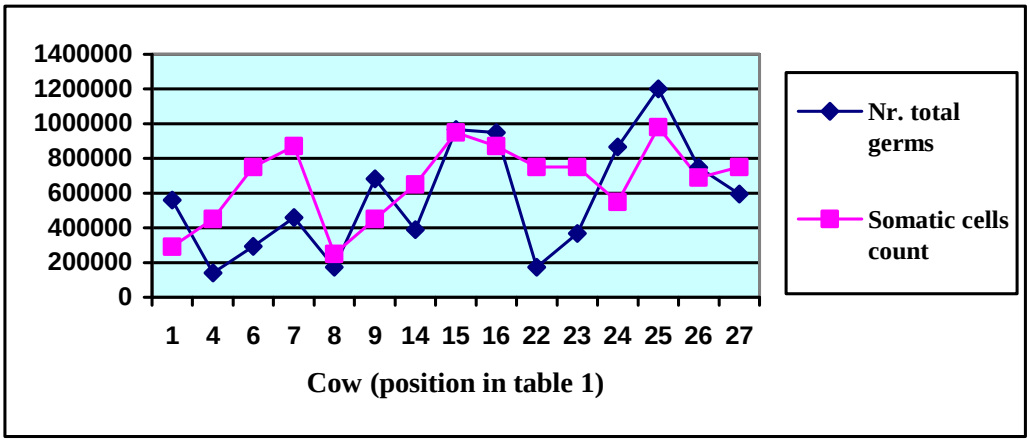

Fig. 9. The correlation between scores 3, 4 and NTG / SCC

CONCLUSIONS

1. In the studied farm there are significant differences in milk quality depending on the hygiene, especially mammary gland and posterior members' hygiene.
2. Somatic cells count had different values, from an average of 56083 in case of clean animals (scores 1 and 2) to an average of 660714 in case of dirty animals (scores 3 and 4).
3. Number of total germs also had different average values, from 34808 in case of clean animals to 568725 in case of dirty animals.
4. The study showed the fact that there are positive correlations between mammary hygiene and total number of germs and somatic cells count as risk factors for mastitis.

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KEY BIOCHEMICAL PARAMETERS CHANGES OF THE *APIS MELLIFERA CARPATHICA* BEE IN STRESSFUL CONDITIONS

Şapcaliu Agripina[1], I.Rădoi [2], Tudor Poliana[2], Tutunaru Alexandru[2], Măgdici Maria[3], Căuia Eliza[1] ,N. Tudor [2]
[1]ICDA Bucuresti
[2]Facultatea de Medicină Veterinară Bucureşti
[3]ICSA Iaşi
agripinasapcaliu@yahoo.com

The study presents changes in biochemical parameters of the bee Apis mellifera Carpathica hemolymph in stressful conditions compared with values observed in healthy bees hemolympha. Measurements were made over two years on a total of 176 families of healthy bees and 20 bee families under stress factors. Harvesting and processing of hemolymph fresh samples from both groups were followed by analysis via classical biochemistry. In stressed bees hemolymph were found significant increases in alkaline phosphatase (ALP), from 60-70 UI/l to 110-178UI/l, in aspartataminotransferaze (AST), from 336-393 UI/l to 421-587 UI/l, in gammaglutamiltranspeptidaze (GGT), from 2.56 – 3.33 UI/l to 31-262 UI/l, in creatinine from 6.1 – 6.9 UI/l to 8.1 – 11.48 UI/l, in total calcium from 3.1 – 3.28 mg/dl to 4.6 – 6.4 mg/dl, in triglycerides from 34.9 – 36.7 mg/dl to 55 – 77.7 mg/dl, and significant reduction of magnesium from 3.4 - 3.99 mg / dl to 0.34 - 0.55 mg / dl, compared with healthy bees. Monitoring the metabolic health of bees in stress conditions requires evaluation of a few biochemical parameters (ALP, AST, GGT, total Ca, Mg, Cre and TG).

Keywords: haemolymph, bee, stress, biochemical parameters

The haemolymph is the body insects' fluid comparable with the blood of the vertebrates, but unlike haemolymph, the blood carries not only the nutritive substances to the cells but also a complex protein substance, called haemoglobin, which forms the colorant material of the blood and transports the oxygen. The haemolymph has only a nutritive role, the respiratory function being negligible [4]. The haemolymph is transparent, uncoloured (or light yellowish) but the quantity represents about 25-30% from the total weight of the bee at the hatching stage and decreases as the bee becomes older. The haemolymph contains proteins, amino acids, sugars, hormones, enzymes and many other substances in different concentration as compared with vertebrate blood [1, 3, 4].

At the international level, most part of the already carried out researches have focused on biochemical analyses connected with the sugars (fructose, trehalose and glucose), proteins (vitellogen), amino acids (proline), hormones (juvenile hormone) contents and the activity of some enzymes. For example, a series of data shows that the total sugars level from honeybees` haemolymph is the highest from all the insect species, being used as the main energetic supply in the flight activity which requires a very high energetic consumption [2].

The main stress factors found in natural conditions to *Apis mellifera Carpathica* bee causing serious losses to beekeepers and country economy are traveling for long distances, tiring, in poor conditions, at high temperature and poor ventilation, low humidity and insufficient food, correlated with poor access to water supply [5, 6].

The goal of this experimental study was to determine the variability of the main biochemical parameters from healthy honeybees compared to stressed bees (exposed to high temperature, over 30°C for 36 hours, poor ventilation, after a tiring journey in FOTI cages).

MATERIALS AND METHODS

The study was conducted over a two years period (2008-2009) and included two distinct categories of subjects: healthy bee families (176) belonging to Research and Development Institute of Apiculture and bee families affected by stress (20) from different apiaries of some private beekeepers.

Stress conditions were achieved by fatiguing transport for 4-8 hours and maintaining bee families in Apiclimatron in FOTI transport cages up to 24-30 hours, in high temperature(over 30°C), low humidity, poor ventilation in the internal tube, compared to healthy bees that were kept in hives in normal microclimate conditions in the external tube (transportation stress).

Measurements were made on samples collected from active season - in April 2008 and the active season - in September 2009. Each sample contains haemolymph randomly harvested from 50 bees.

Formation (organization) of the experimental group consisting of 176 healthy honeybee colonies, sanitary-veterinary surveyed and 20 stressed honeybee colonies.

RESULTS AND DISCUSSION

The results obtained during the study are presented in Tables 1, 2, 3, 4, 5 and 6.

Table no.1 The main biochemical parameters of haemolymph in sample 1 (april 2008 – healthy bees)

No.	Parameter (UM)	Value
1	ALP (UI/l) – alkaline phosphatase	60
2	GPT/ALT (UI/l) - alanine amino transferase	311
3	GOT/ AST (UI/l) - Aspartate amino transferase	393
4	total Ca (mg/dl) – calcium	3.28
5	CPK (UI/l) – creatininphosphokinase	498
6	Cre (mg/dl) Creatinine	6.90
7	GGT (UI/l) - Gamma glutamyl transpeptidase	2.56
8	GLU (mg/dl) – glucose	340.5
9	Mg (mg/dl) – magnesium	3.99
10	T-Pro (g/d) –total protein	2.51
11	TG (mg/dl) - triglycerides	36.7
12	UA (mg/dl) –uric acid	2.88
13	BUN – urea (urea nitrogen)	42.63

Collection of the required quantity of fresh haemolymph (300 µl/determination) by means of a glass capillary introduced between 3 and 4 tergites and the collection of the haemolymph from capillary in a special syringe. Biochemical analyses on the fresh haemolymph with the Alfawassermann apparatus. Processing and interpretation of the obtained data. For haemolymph biochemical, the samples were analyzed by wet chemistry using Alfawassermann device aiming at assessing ALP (IU/L), SGPT/ALT (IU/L), SGOT/AST (IU/L) in total (mg/dl), CPK (IU/L), Cre (mg/dl) , GGT (IU/L), GLU (mg/dl), Mg (mg/dl), T-Pro (g/d), TG (mg/dl), UA (mg/dl) and urea (mg/dl).

Table no.2 The main biochemical parameters of haemolymph in sample 2 (september 2009 – healthy bees)

No.	Parameter (UM)	Value
1	ALP (UI/l) – alkaline phosphatase	70
2	GPT/ALT (UI/l) - alanine amino transferase	368
3	GOT/ AST (UI/l) -Aspartate amino transferase	336
4	Ca total (mg/dl) calcium	3.1
5	CPK (UI/l) creatininphosphokinase	506
6	Cre (mg/dl) – Creatinine	6.1
7	GGT (UI/l) - Gamma glutamyl transpeptidase	3.33
8	GLU (mg/dl) – glucose	377
9	Mg (mg/dl) - magnesium	3.4
10	T-Pro (g/d) – total protein	2.4
11	TG (mg/dl) - triglycerides	34.9
12	UA (mg/dl) – uric acid	2.62
13	BUN – urea (urea nitrogen)	41.2

Table no.3 The main biochemical parameters of haemolymph in sample 3 (april 2008 – healthy bees)

No.	Parameter (UM)	Value
1	ALP (UI/l) – alkaline phosphatase	110
2	GPT/ALT (UI/l) - alanine amino transferase	321
3	GOT/ AST (UI/l) - Aspartate amino transferase	421
4	Ca total (mg/dl) - calcium	4.6
5	CPK (UI/l) - creatininphosphokinase	5.96
6	Cre (mg/dl) – Creatinine	9.6
7	GGT (UI/l) - Gamma glutamyl transpeptidase	33.3
8	GLU (mg/dl) – glucose	308.5
9	Mg (mg/dl) - magnesium	0.55
10	T-Pro (g/d) – total protein	2.83
11	TG (mg/dl) - triglycerides	61.06
12	UA (mg/dl) – uric acid	1.86
13	BUN – urea (urea nitrogen)	43.21

Table no.4 The main biochemical parameters of haemolymph in sample 4 (april 2008 – healthy bees)

No.	Parameter (UM)	Value
1	ALP (UI/l) – alkaline phosphatase	140
2	GPT/ALT (UI/l) - alanine amino transferase	353
3	GOT/ AST (UI/l) - Aspartate amino transferase	496
4	Ca total (mg/dl) - calcium	5.91
5	CPK (UI/l) - creatininphosphokinase	526
6	Cre (mg/dl) – Creatinine	8.1
7	GGT (UI/l) - Gamma glutamyl transpeptidase	31
8	GLU (mg/dl) – glucose	346
9	Mg (mg/dl) - magnesium	0.41
10	T-Pro (g/d) – total protein	3.1
11	TG (mg/dl) - triglycerides	63
12	UA (mg/dl) – uric acid	2.8
13	BUN – urea (urea nitrogen)	49.2

Table no.5 The main biochemical parameters of haemolymph in sample 5 (september 2009 – healthy bees)

No.	Parameter (UM)	Value
1	ALP (UI/l) – alkaline phosphatase	139
2	GPT/ALT (UI/l) - alanine amino transferase	383
3	GOT/ AST (UI/l) - Aspartate amino transferase	587
4	Ca total (mg/dl) - calcium	6.40
5	CPK (UI/l) - creatininphosphokinase	400
6	Cre (mg/dl) – Creatinine	11.48
7	GGT (UI/l) - Gamma glutamyl transpeptidase	221.34
8	GLU (mg/dl) – glucose	362
9	Mg (mg/dl) - magnesium	0.34
10	T-Pro (g/d) – total protein	2.18
11	TG (mg/dl) - triglycerides	77.7
12	UA (mg/dl) – uric acid	3.56
13	BUN – urea (urea nitrogen)	40.96

The results Analysis summarized in table 1-6 shows the existence of significant increases in the values of biochemical parameters characterizing enzyme activity (ALP, AST, GGT) energy metabolism (CRE) and total calcium levels in lymph. It was found that alkaline phosphatase activity increases under stress, very significantly, from 1960 to 1970 IU/L at 110-178UI/L, AST enzyme activity significantly increased from 336-393 IU/L at 421-587 IU/L.

GGT enzyme activity increased under stress, very significantly from 2.56-3.33 IU/L, 31-262 IU/L. Creatinine values increased significantly from 6.1 to 6.9 IU/L to 8.1 to 11.48 IU/L, values are 5-6 times higher than the mammal blood. n comparison, total calcium values increased significantly from 3.1 to 3.28 mg/dl (bee sound) from 4.6 to 6.4 mg/dl (in terms of stress). Triglycerides increased very significantly from 34.9 to 36.7 mg/dl to values between 55 to 77.7 mg/dl in terms of exposure to stressful factors.

It is noted that for monitoring the health of bees in metabolic stress conditions, is necessary to evaluate a small number of biochemical parameters: ALP, AST, GGT

enzymes, creatinine, total calcium, magnesium and total triglycerides, other biochemical parameters of moving insignificant hemolymph.

Table no.6 The main biochemical parameters of haemolymph in sample 6 (september 2009 – healthy bees)

No.	Parameter (UM)	Value
1	ALP (UI/l) – alkaline phosphatase	178
2	GPT/ALT (UI/l) - alanine amino transferase	362
3	GOT/ AST (UI/l) - Aspartate amino transferase	455
4	Ca total (mg/dl) - calcium	5.98
5	CPK (UI/l) - creatininphosphokinase	671
6	Cre (mg/dl) – Creatinine	10.1
7	GGT (UI/l) - Gamma glutamyl transpeptidase	262
8	GLU (mg/dl) – glucose	312
9	Mg (mg/dl) - magnesium	0.5
10	T-Pro (g/d) – total protein	2.1
11	TG (mg/dl) - triglycerides	55
12	UA (mg/dl) – uric acid	4.5
13	BUN – urea (urea nitrogen)	42.6

Compared with previous research conducted on healthy bees [6], which were followed seasonal variations of biochemical parameters of bees hemolymph, we found low variability in most biochemical parameters studied in healthy bees in the two years of the experiment. Glucose and total protein values were comparable to those reported in literature, and activity levels of enzymes AST, ALT, GGT, CPK, and total urea showed higher values compared with those found in the blood of mammals, while calcium, magnesium, uric acid and triglycerides had values within the range in blood plasma of mammals [5, 6].

CONCLUSIONS

1. The values obtained for the main studied biochemical parameters in the haemolymph of the healthy honeybees collected from honeybee colonies kept in natural conditions show a slowly variable evolution in the two consecutive years of experiments.

2. The values related to the level of glucose and total proteins are comparable with those reported in the literature.

3. The AST, ALT, GGT, CPK enzymes, glucose and ureea levels in haemolymph registered very high values comparing with those found in the blood plasma of the mammals, the calcium, magnesium, uric acid and triglycerides values are close to those from the blood plasma and the creatinine values are 5-6 times higher than those detected in the blood plasma.

4. The parameters of the mineral metabolism of hemolymph are characterized by low detectable values for Ca and some very fluctuant values for Mg.

5. The results of wet chemistry analysis performed on the two groups of bees showed significant changes in biochemical parameters of hemolymph concentration and enzymatic activity under the influence of stress**.**

6. Metabolic profile investigation of bee families under stress compared with bees families unstressed in terms Apiclimatron, based on the assessment of major changes in biochemical parameters in lymph, shows increased enzyme activity, the amount of total

calcium, creatinine and triglycerides, while decreasing total magnesium under conditions of stress.

7. Assessment of metabolic profile of bees in stressful conditions shows the importance of monitoring enzyme activity (alkaline phosphatase, aspartataminotransferaze, gammaglutamil-transpeptidaze) and calcium and magnesium total concentration, creatinine and triglycerides, reducing the number of investigated biochemical parameters

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THE EFFECT OF $XAG_2O(1-X)[42B_2O_324CAO34P_2O_5]$ OXIDE POWDERS ON GRAM POSITIVE BACTERIA

R. STEFAN, Silvana POPESCU, Marina SPINU,
N. FIT, A. MACRI, G. NADAS
University of Agricultural Science and Veterinary Medicine-3400, Cluj-Napoca,

Glass from xAg_2O (1-x) $[42B_2O_324CaO34P_2O_5]$ system with high molar silver oxide content $0 \leq x \leq 11$ mol%, has been obtained using undercooled method. Antibacterial properties against gram positive bacteria were tested using diffusimetrical method for Staphylococcus aureus strains ATCC 6538p, Arcanobacterium pyogenes, Bacillus cereus ATCC 14579. The greater effect was observed for Arcanobacterium pyogene.
Antibacterial effect was dependent on Ag_2O concentration into the glass matrix, evaluated by measuring the diameter of inhibition area for the investigated compounds.

Keywords:silver oxide, glass, antibacterial.

INTRODUCTION

The oxidic materials that contain silver [1] and copper [2] have been used since the oldest times for the treatment of infections generated by bacteria and pathogen agents [3]. The long time in which the silver was used for the treatment against bacteria led to the idea that bacteria cannot adapt to its action, like many types of antibiotics do, developing specific resistance. The usage on a large scale of this type of materials, can be achieved depending on the interaction with the biological environment in which this will be investigated and used. The chemical durability leads to the projection of a biodegradable material that gradually releases the active substance, while a material with high chemical durability can serve in implantology, having active substance only on the surface.

Recent research [4, 5] showed the necessity to obtain glass with components that are closest to those of the organism in order to be used in bone implants, or as mechanic supports on which bone cells to be implanted to develop.

The structural study of the boron -based oxidic glass was widely investigated [6, 7] and it was showed that the structure of the boron-based oxidic glass may contain $[BO_3]$ and $[BO_4]$ units interconnected depending on its composition.

Phosphorus oxide is a component that forms bioactive structures [8, 9] and has a high dissolution rate if it is encountered in the organism. Therewith, the behavior of the vitreous components in biological liquids depends on the chemical composition and their structure.

In the present work, we studied the antibacterial effect of the oxidic boron and phosphorus based vitreous materials from the system $xAg_2O(1-x)[42B_2O_324CaO34P_2O_5]$ prepared with a high content of silver in the samples (x=11mol %).

MATERIALS AND METHODS

The oxidic glasses from the xAg_2O (1-x)$[42B_2O_324CaO34P_2O_5]$ system were obtained using as start materials P_2O_5, H_3BO_3, $CaCO_3$, Ag_2O with a chemical purity of 99+. The oxides were mixed in appropriate proportions, homogenized and melted at 1230 °C, founded and pressed between stainless steel plates.

For the fitting the experimental points the Origin v8.1. software was used. The vitreous character of the samples was tested though diffraction of X-rays using a

diffractometer. Detailed experimental descriptions should be restricted to X ray powder diffraction data were obtained with Bruker D8 Advanced Diffractometer equipped with Si monochromator.

The antibacterial efficiency of the studied compounds was tested on gram positive germs (standard germs *Staphylococcus aureus ATCC 6538p, Arcanobacterium pyogenes, Bacillus cereus ATCC 14579*) through the method of diffusion in 2% agar gel. The agar pored on a Petri plates with a diameter of 12 cm, was sown with the bacterial inoculum, prepared from the 24 hour culture on the agar Mueller Hinton, diluted in physiological serum at the density of tube 0.5 on Mc Farland scale. Immediately after sowing wells with a diameter of 4,5 mm were made, in which the compounds were placed, in a concentration of 15%, in a quantity of 50 µl/well. Each compound was double tested. The diameters of the inhibition areas were read after an incubation of 24 hours at 37°C.

RESULTS AND DISCUSSION

The antibacterial effect of the oxidic glass in the $xAg_2O(1-x)[42B_2O_3 24CaO 34P_2O_5]$ with $0 \leq x \leq 7$ vitreous system was evaluated by measurement of the diameter of the inhibition area, noted d, of the bacterial growth in the case of *Staphylococcus aureus* ATCC 6538 p (Fig. 1). The radius of the inhibition zone grows monotonously once with the growth of the molar content of de Ag_2O in the vitreous matrix. The samples doped with Ag_2O antibacterial effect dependant of the molar concentration of the metal in the matrix effect also obtained in the case of other boron based oxidic systems doped with silver ions. [10]. Thus, the inhibition zone is contained in the interval $d \in [16;21]$ mm. The sample that does not contain silver (x=0) also induces an inhibitive effect and the dependence of the effect on the silver oxide molar composition is obvious starting with x=5mol%.

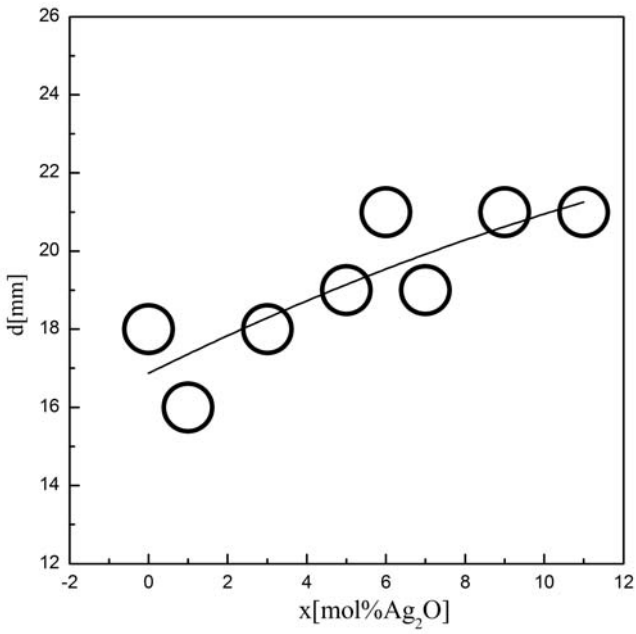

Figure 1. The dependence of the radius of the inhibition zone of the *Staphylococcus aureus* ATCC 6538 p strain on the A_2O molar content in the case of the $xAg_2O(1-x)[42B_2O_3 24CaO 34P_2O_5]$ vitreous system.

The Arcano Bacterium Pyogenes inhibition diameter (Fig. 2) depends on the molar concentration of Ag_2O of the vitreous samples of the $xAg_2O(1-x)[42B_2O_3 24CaO 34P_2O_5]$ system. For the 0≤x≤5 compositional level there are no modifications in the size of the inhibition zone, the samples with silver exibiting the same antibacterial effect as those without silver content (x=0), the effect is due more to the existence of the powder and the mechanical interaction between these and the inoculated strains.

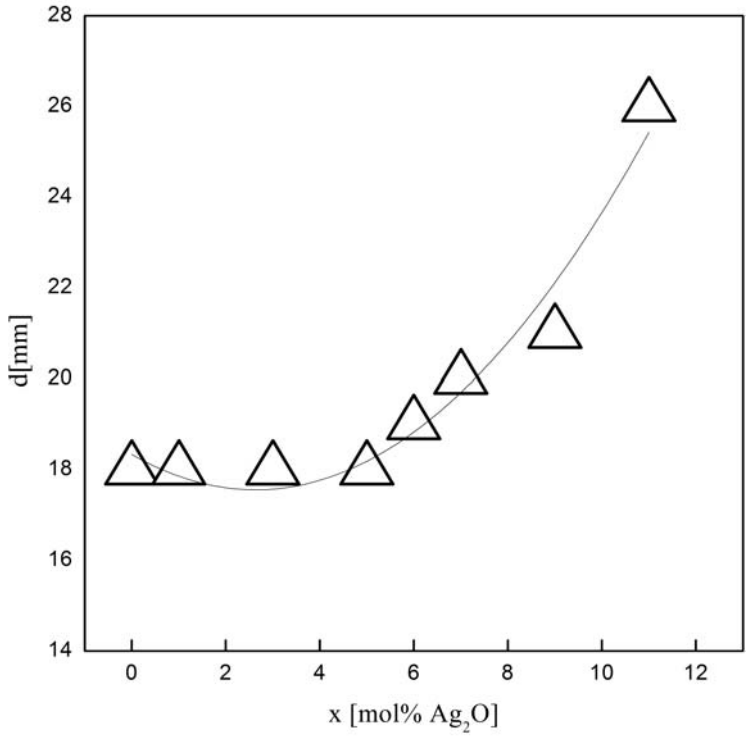

Figure 2. The diameter of the inhibition zone vs Ag_2O concentration for the Arcano Bacterium Pyogenes strain.

For x>5, the effect is exponential and the silver excess from the surface of the material propagates through the nutritive substratum, inhibiting the most intensely and rapidly the growth of this type of bacterium of all the gram positive investigated bacteria. The inhibition process is not saturated for the x=11 mol % Ag2O in vitreous matrix either.

Figure 3 illustrates the manner in which the *Bacillus cereus strain* interacts with the vitreous powder of the xAg_2O $(1-x)[42B_2O_3 24CaO 34P_2O_5]$ system.

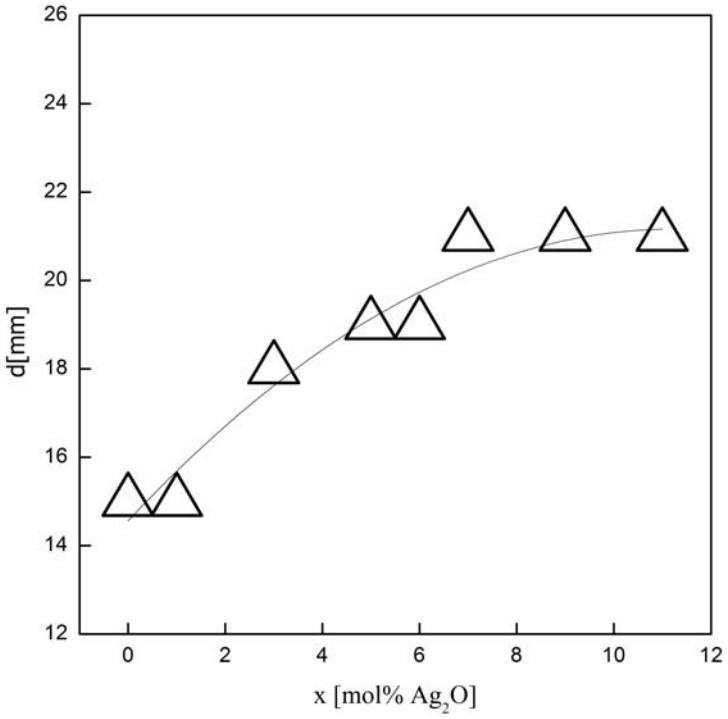

Figure 3 The modification of the inhibition diameter of the growth of *Bacillus cereus* depending on the molar composition of the xAg_2O (1-x)[$42B_2O_3 24CaO 34P_2O_5$] system.

The antibacterial effect depends on the silver oxide molar content in the $1 \leq x \leq 6$ compositional domain. For $x \geq 7$ the environment is saturated in Ag^+ ions and a more intense effect is not produced.

From the point of view of the inhibitive behavior *Bacillus cereus* behaves like *Staphiloccocus Aureus* at the interaction with oxidic vitreous powders, the diameter of saturation being dmax=21 mm. For x=7 mol% the process is saturated, the inhibition zone remaining constantly for higher molar concentrations of silver oxide.

CONCLUSIONS

1. The antibacterial effect is strong, depending on the concentration of Ag_2O in the structure of the investigated starting with $x \geq 5$ mol %.
2. The samples which does not contain silver have an inhibitive effect due especially to the mechanical interaction from these and the studied strains.
3. Arcano Bacterium Pyogenes is the most sensitive at the action of the investigated vitreous system.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, project number 1117 PNII – IDEI code 2528/2008

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THE ANTIBACTERIAL EFFECT OF SILVER CONTAINING B_2O_3-CAO-P_2O_5 GLAS MATRIX ON GRAM NEGATIVE BACTERIA

R. STEFAN, Marina SPINU, Silvana POPESCU,
N. FIT, Maria BINDEA, Flore CHIRILA
University of Agricultural Science and Veterinary Medicine-
3400-Cluj-Napoca,Calea Manastur, 3-5, Romania

Abstract
Antibacterial effect of vitreous samples from B_2O_3-CaO-P_2O_5 glass matix doped with silver ions was measured by the diffusimetrical method in agar inoculated with gram-negative species.
Escherichia coli ATCC 10536, Salmonella typhymurium, Klebsiella pneumoniae bacteria were investigated, the antibacterial effect being present on every investigated strain. The greater effect was measured for Salmonella typhymurium.
Key words: gram negative, antibacterial efect, silver glass

INTRODUCTION

The necessity to obtain oxidic materials based on phosphorus in order to use them in medical applications (implants, structures for cellular growth, etc.) appeared along with the biocompatibility tests [1, 2], which showed that as useful as a biomaterial might be, the main conditions is not to be rejected by the organism as a foreign body.

The P_2O_5 based glass was widely investigated in the last years [3, 4] and there were highlighted specific relations between the internal structure and the behavior in biological liquids. The calcium oxide was introduced in the initial composition of the glasses in order to realize the bioactivity of the material in relation with the bone tissue. The molar relation between the two oxides influences the physical properties among which the chemical durability in such a way that this can be used in implantology as well as biodegradable storage for active substances [5, 6]. In the case of the use of implantology or in external use in order to avoid the apparition of recurrent infections, it had been attempted the introduction of ions with antibacterial properties in the first case on the surface through the method of ionic change and in the second in the structure through the sol gel method of under cooling of the melting

In the present work the antibacterial effect of the B_2O_3-CaO-P_2O_5 matrix doped with Ag_2O was studied prepared through the method of under cooling of the melting, in the case of gram negative bacteria.

MATERIALS AND METHODS

The oxidic glasses from the $xAg_2O(1-x)[42B_2O_3 24CaO 34P_2O_5]$ system were obtained using as start materials P_2O_5, H_3BO_3, $CaCO_3$, Ag_2O with a chemical purity of 99+. The oxides were mixed in appropriate proportions, homogenized and melted at 1230 °C, founded and pressed between stainless steel plates.

For the fitting the experimental points the Origin v8.1. software was used. The vitreous character of the samples was tested though diffraction of X-rays using a diffractometer. Detailed experimental descriptions should be restricted to X ray powder

diffraction data were obtained with Bruker D8 Advanced Diffractometer equipped with Si monochromator.

The antibacterial efficiency of the studied compounds was tested on gram positive germs (standard germs
Escherichia coli ATCC 10536, Salmonella typhymurium, Klebsiella pneumoniae) through the method of diffusion in 2% agar gel. The agar pored on a Petri plates with a diameter of 12 cm, was sown with the bacterial inoculum, prepared from the 24 hour culture on the agar Mueller Hinton, diluted in physiological serum at the density of tube 0.5 on Mc Farland scale. Immediately after sowing wells with a diameter of 4,5 mm were made, in which the compounds were placed, in a concentration of 15%, in a quantity of 50 µl/well. Each compound was double tested. The diameters of the inhibition areas were read after an incubation of 24 hours at 37°C.

RESULTS AND DISCUSSION

In the case of Escherichia coli ATCC 10536m the diameter of the inhibition zone has the value contained in the interval d∈ [16,19] mm (Fig.1) depending on the molar content of the silver oxide in the structure of the samples. This bacterium is sensitive to the action of the silver ions in the structure of the samples. [7-9]. The samples which does not contain any Ag_2O, inhibit bacterial growth as well as the sample with a low concentration of silver (x=0,5 mol%). For 0,5 ≤ x ≤ 4 mol% the area of inhibition increases and depends on the quantity of Ag^+ available on the surface of the glass that is released in the dissolution environment. When x ≥ 5 mol% the growth environment is saturated with silver ions and no matter how much the molar saturation with silver oxide grows the process saturates. For this type of strain it can be stated that 16 mm represents the diffusion area of the proper powders.

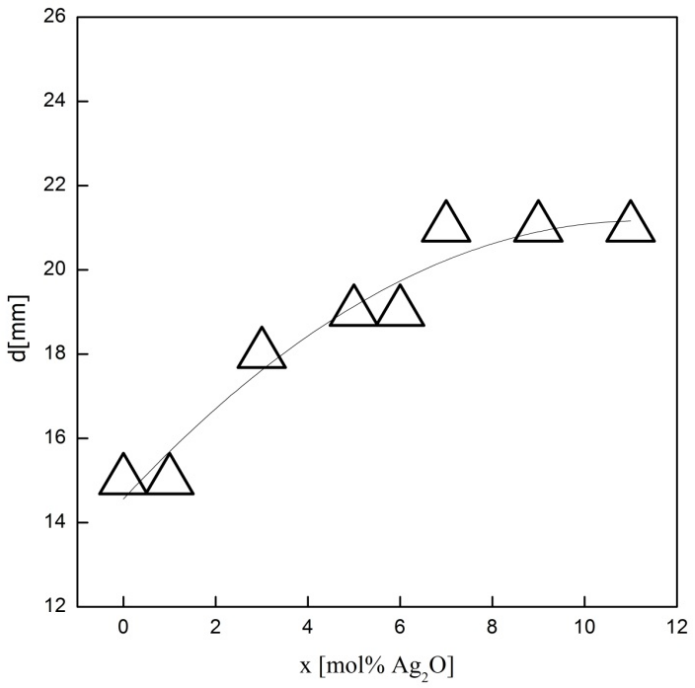

Fig. 1 The inhibition diameter (d) vs the Ag_2O molar concentration in the B_2O_3-CaO-P_2O_5 matrix.

Salmonella typhymurium is most strongly inhibited by the investigated vitreous materials (Fig. 2). In the 1≤x≤5 compositional domain here is no increase of the inhibition area, the quantity of silver being necessary but not enough in order to manifest a more extended bacteriostatic effect. For x > 5it can be observed an increase of the inhibition area along with the molar content of Ag_2O in the samples a line is drawn only in order to highlight, the points being linearly fitted . This strain is the most sensitive on the action of these samples.

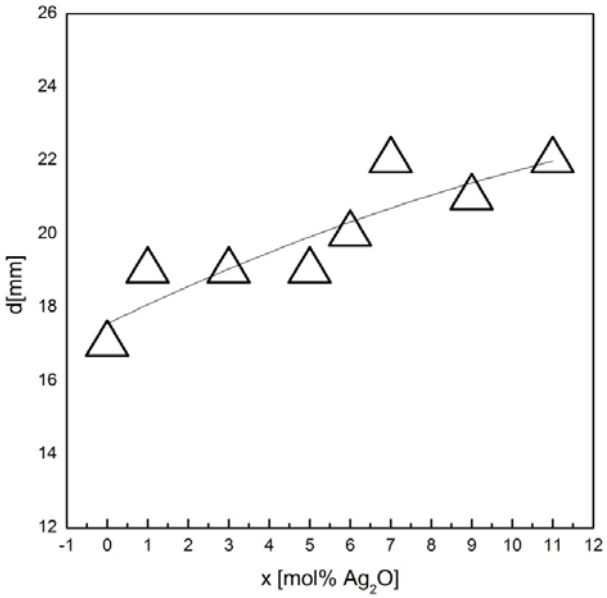

Fig. 2 The dependence of the diameter of inhibition on the molar content of silver oxide from the B_2O_3-CaO-P_2O_5 matrix.

The least sensitive gram negative strain to the action of the vitreous samples from the B_2O_3-CaO-P_2O_5 matrix doped with silver ions is Klebsiella pneumoniaae (Fig. 3), whose development is inhibited only on an area with the diameter of d_{max}=17 mm.

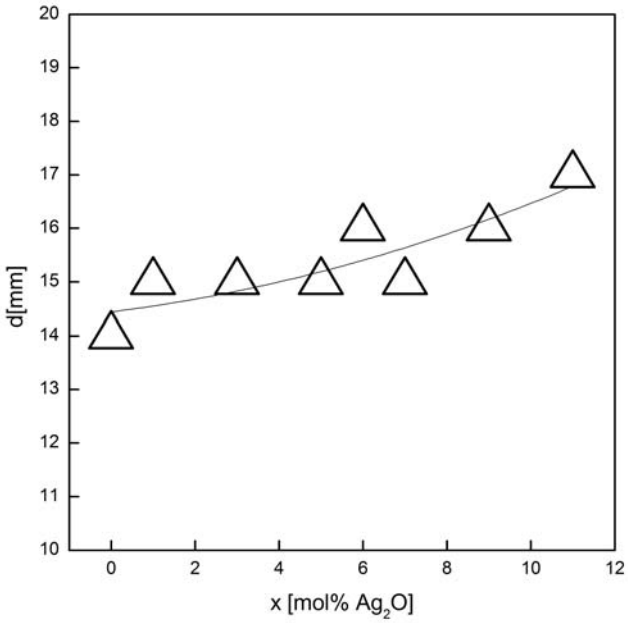

Fig. 3 The dependence between inhibition diameter and molar content of silver oxide for Klebsiella pneumoniaae strain.

The minimum rate of inhibition is given by the sample which does not contain silver and it can be supposed that this is due to the diffusion of the sample in the nutritive environment of the bacterium. For x ≤ 5ml% these is no dependence of the inhibition area on the molar Ag_2O content all the samples from this compositional area inhibiting equally the development of the bacterium culture.

CONCLUSIONS

1. There were prepared vitreous structures with high concentration of silver oxide from the Ag_2O-B_2O_3-CaO-P_2O_5 system through the method of undercooling of the melt.
2. The antibacterial effect is dependent on the silver concentration from a certain molar concentration for each gram negative strain separately.
3. The most sensitive strain is Salmonella typhymurium.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, project number 1117 PNII – IDEI code 2528/2008

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MANGANESE IMPACT ON MALE REPRODUCTIVE SYSTEM INTEGRITY AND PERFORMANCES BIOMARKERS

Nicoleta-Simona Steliac (Munteanu), Alexandra Trif
Calea Aradului nr.119 , cod: 300645, Timisoara
simonasteliac@gmail.com

Abstract
The aim of this study was to determinate manganese impact on morphological (body, genital organs, sexual accesory glands weight; arhitecture of testis, epididym, seminiferous tubuls) markers and biochemical (seric testosterone, LH, FSH level)and sperm quality biomarkers based on recent references.
The study pointed out the manganes impact on some male reproductive system integrity and performances biomarkers: controversially data regarding the impact on body weight, decreas of size and weight of sexual organs and some accesory sexual glands, severe architecture changes in sexual organs (testes degeneration of germinal epithelium characterized by loss of developing spermatozoa and spermatids, degeneration of Sertoli cells and Leidig cells), necrotic seminiferous tubules,epididymis damages in epithelial cells of epididymis, tubules with sperms loss, and sexual accesory glands (prostatis degeneration),controversially opinians regarding the imact on sexual hormones, especially testosterone decrease of sperm quality (decrease of sperm count, density, mobility, fertilizing capacity, abnormalities). Clinical observation of impotence, loss of libido in exposed workers are mentioned.

Key word: manganese, reproductive toxicity, male

INTRODUCTION

The origin of the name manganese is complex. In ancient times, two black minerals from Magnesia in what is now modern Greece were both called *magnes*, but were thought to differ in gender. In the 16th century, manganese dioxide was called manganesum (note the two n's instead of one) by glassmakers, possibly as a corruption of two words since alchemists and glassmakers eventually had to differentiate a *magnesia negra* (the black ore) from *magnesia alba* (a white ore, also from Magnesia, also useful in glassmaking). Michele Mercati called magnesia negra *Manganesa*, and finally the metal isolated from it became known as manganese (German: *Mangan*). The name *magnesia* eventually was then used to refer only to the white magnesia alba (magnesium oxide), which provided the name magnesium for that free element, when it was eventually isolated, much later.(2,19)

Manganese is a chemical element, designated by the symbol Mn. It has the atomic number 25. It is found as a free element in nature (often in combination with iron), and in many minerals. As a free element, manganese is a metal with important industrial metal alloy uses, particularly in stainless steels (10).

Manganese phosphate is used as a treatment for rust and corrosion prevention on steel. Depending on their oxidation state, manganese ions have various colors and are used industrially as pigments. The permanganates of alkali and alkaline earth metals are powerful oxidizers. Manganese dioxide is used as the cathode (electron acceptor) material in standard and alkaline disposable dry cells and batteries (10).

Manganese(II) ions function as cofactors for a number of enzymes in higher organisms, where they are essential in detoxification of superoxide free radicals. The element

is a required trace mineral for all known living organisms. In larger amounts, and apparently with far greater activity by inhalation, manganese can cause a poisoning syndrome in mammals, with neurological damage which is sometimes irreversible (16).

The most common oxidation states of manganese are +2, +3, +4, +6 and +7, though oxidation states from -3 to +7 are observed. Mn^{2+} often competes with Mg^{2+} in biological systems. Manganese compounds where manganese is in oxidation state +7, which are restricted to the oxide Mn_2O_7 and compounds of the intensely purple permanganate anion MnO_4^-, are powerful oxidizing agents. Compounds with oxidation states +5 (blue) and +6 (green) are strong oxidizing agents and are vulnerable to disproportionation (10).

The most stable oxidation state for manganese is +2, which has a pink to red color, and many manganese(II) compounds are known, such as manganese(II) sulfate ($MnSO_4$) and manganese(II) chloride ($MnCl_2$). This oxidation state is also seen in the mineral rhodochrosite, (manganese(II) carbonate). The +2 oxidation state is the state used in living organisms for essential functions; other states are toxic for human body (26).

The +3 oxidation state is known, in compounds such as manganese(III) acetate, but these are quite powerful oxidizing agents and also disproportionate in solution to Mn(II) and Mn(IV). Solid compounds of Mn(III) are characterized by its preference for distorted octahedral coordination due to the Jahn-Teller effect and its strong purple-red color (26).

The oxidation state 5+ can be obtained if manganese dioxide is dissolved in molten sodium nitrite (26). Manganate (IV) salts can also be produced by dissolving Mn compounds in alkaline melts in air (18).

Permanganate (+7 oxidation state) compounds are purple, and can give glass a violet color. Potassium permanganate, sodium permanganate and barium permanganate are all potent oxidizers. Potassium permanganate, also called Condy's crystals, is a commonly used laboratory reagent because of its oxidizing properties and finds use as a topical medicine (for example, in the treatment of fish diseases). Solutions of potassium permanganate were among the first stains and fixatives to be used in the preparation of biological cells and tissues for electron microscopy (18).

Methylcyclopentadienyl manganese tricarbonyl is used as an additive in unleaded gasoline to boost octane rating and reduce engine knocking. The manganese in this unusual organometallic compound is in the +1 oxidation state (8,27).

Manganese(IV) oxide (manganese dioxide, MnO_2) is used as a reagent in organic chemistry for the oxidation of benzylic alcohols (i.e. adjacent to an aromatic ring). Manganese dioxide has been used since antiquity to oxidatively neutralize the greenish tinge in glass caused by trace amounts of iron contamination (19). MnO_2 is also used in the manufacture of oxygen and chlorine, and in drying black paints. In some preparations it is a brown pigment that can be used to make paint and is a constituent of natural umber (6).

Manganese(IV) oxide was used in the original type of dry cell battery as an electron acceptor from zinc, and is the blackish material found when opening carbon–zinc type flashlight cells. The manganese dioxide is reduced to the manganese oxide-hydroxide MnO(OH) during discharging, preventing the formation of hydrogen at the anode of the battery (6).

MATERIALS AND METHODS

For writing this paper were used 27 references.

The aim of this study was to determinate manganese impact on reproductive system integrity and performances biomarkers. Biomarkers are tracers that give evidence of some

events in different samples or biological systems. Certain biological biomarkers were used to localize in males physiopatological modification produced by toxic substances. The interest of this study was focused on the morphological biomarkers and biochemical and sperm quality biomarkers (20).

►Manganese impact on morphological biomarkers: (body, genital organs, sexual accesory glands weight; archiotecture of testis, epididym, seminiferous tubuls).

•***Manganese impact on body weight;***

The opinions on the subject are controversially:

Administration of manganese(II) tetraaza macrocyclic complexes did not bring about any significant change in the body weights of the treated rats (25).

Inhalation exposure to $MnSO_4$ during gestation and lactation did not affect maternal body weight gain or terminal maternal body weight. Combined *in utero* and postnatal inhalation exposure to $MnSO_4$ did not affect neonatal body weight gain in either female or male pups (7).

High-dose $MnSO_4$ exposure was asociated with decreased body weight in pups (7).

Mancozeb-a fungicide of ethylenebisdithiocarbamate orally administered at doses of 500, 1.000 and 1.500 mg/kg body weight/day for 30, 90, 180 and 360 days determined in both, treated animals and control ones, increase of body weight the, percent increase beeing in order of 133>116>85>80 of rats exposed to Mancozeb (0, 500, 1.000, 1.500 mg/kg/d), respectively in control (12).

•***Manganese impact on genital organs and sexual accesory glands weight;***

Administration of manganese(II) tetraaza macrocyclic complexes caused changes in genital organs weight. The weights of testes, epididymis, seminal vesicle and ventral prostate were decreased significantly in all the experimental groups when compared with the vehicle treated controls(25).

Male mice exposed to elevated dietary manganese showed decreased size of the testicles, seminal vesicles, and preputial glands (9).

The absolute weight of testes and epididymis of animals exposed to different doses (500, 1.000 and 1.500 mg/kg/d) of Mancozeb did not show any change except for a mild decrease in epididymal weight, at 1.000 and 1.500 mg/kg/d for 360 days. The relative weight of testes and epididymis after the exposure to different doses of Mancozeb (500,1.000 and 1.500 mg/kg/d) during 30-90 days did not indicate any appreciable change. Higher doses (1.000 and 1.500 mg/kg/d) over a period of 180 and 360 days produced a slight increase in relative weight of testes and a decrease in epididymis (12).

• ***Manganese impact on testis, epididym, seminiferous tubuls arhitecture;***

Microscopic examination of testes and epididymis of rats exposed to Mancozeb at doses 500 and 1.000 mg/kg/d over a period of 360 days did not exhibited any significant pathomorphological changes. However, testes of rats dosed with Mancozeb (1.500 mg/kg/d) produced time dependent pathological changes in seminiferous tubules. The observed changes included necrotic seminiferous tubules with sloughing of germinal cells, formation of giant cells and accumulation of debris matter into the lumen. The clubing of spermatogonia and spermatocytes may be responsible for the formation of giant cells. Similarly, rats exposed to high doses of Mancozeb has also showed damaged epithelial cells in the tubules of epididymis with loss of sperms (12).

Adult rats exposed to varying doses: 306, 612, 1225, and 1838 mg Mn/kg dissolved in distilled water for 63 days, presented segmental degenerations of germinal epithelium within seminiferous tubules and also to characterize loss of developing spermatozoa and spermatids. Histopathological examination confirmed testicular degeneration in treated rats of 612, 1225,

and 1838 mg Mn/kg groups. The most prominent abnormality observed in male animals was testicular degeneration. Affected animals has mild to moderate segmental degeneration of germinal epithelium within seminiferous tubules. This change was characterized by loss of developing spermatozoa and spermatids, vacuolation and swelling of Sertoli cells, degeneration of Sertoli cells, and widening of the tubular luminal diameter. Binucleate and multinucleate cells were observed within tubular lumina and degenerate epithelium. Many degenerated tubules also had a decreased cross-sectional diameter. The basement membrane of affected tubules appeared intact, but occasionally had a wavy appearance. Often tubules had relatively normal areas adjacent to affected areas. These changes, collectively, were consistent with segmental tubular degenerations (22).

One rat in high dose group, 1838 mg Mn/kg, had a lesion consistent with a sperm granuloma. This mass consisted of a large collection of spermatozoa encased in a thick sheath of macrophages and multinucleate giant cells surrounded by several layers of dense fibrous connective tissue. No significant lesions were observed in the coagulation glands, seminal vesicles or ductus deferens (22).

Katira et al. (13) reported disturbed spermatogenesis and histopathological observations revealed degeneration of seminiferous tubules, epididymis and prostatitis.

Chandra (3) reported a cellular change induced by manganese in the rat testis. Studies on rabbits instillated with MnO_2, demonstrated that a single high dose of 158 mg/kg, as MnO_2 could cause severe degenerative changes in the seminiferous tubules and lead to sterility.

Testicular damage was produced in rats by subcutaneous administration of manganese chloride (3 mg/kg) for a period of three weeks (5).

Singh et al. (24) reported that in rats manganese sulphate injection for 25 days produced degeneration in seminiferous epithelium, depleted number of spermatids and no spermatocytes in the seminiferous tubules.

In humans, reproductive effects such as impotence and loss of libido have been noted in male workers exposed to high levels of manganese by inhalation (1).

►Manganese impact on biochemical (seric testosterone, LH, FSH level) and sperm quality biomarkers.

Prestifilippo et al. (23) described that $MnCl_2$ can cause LHRH release from the MBH (medial basal hipotalamus) in adult male rats and this peptide controls the pituitary secretion of LH and FSH. In order to determine the *in vivo* effect of $MnCl_2$ on plasma LH levels, rats were stereotaxically implanted with a cannula into the lateral cerebral ventricle and received a single intracerebroventricularly (i.c.v.) injection of $MnCl_2$ (10µg/5µl saline). Results pointed out that $MnCl_2$ increased the plasma LH levels at 30 min postinjection. The hormone remained elevated at 60 min, then declined to preinjection levels at 90 min postinjection. Control rats injected with saline did not showed a change in LH secretion at any of the time points.

The rats that were injected with saline or the 1.0µg dose of $MnCl_2$ exhibited no change in LH released as compared to their respective basal levels. The rats that were injected with the 2.5, 10 and 25 µg doses of $MnCl_2$ exhibited three-, six- and seven-fold increases in LH release, respectively, compared with their basal levels (9).

Serum gonadotropin levels were not altered at 48 or 55 days in animals that received the 10 mg/kg supplemental dose of $MnCl_2$. The 25 mg/kg dose caused non-significant elevations in LH, FSH, testosteron and spermatogenesis by 48 days. Supplementation of animals with the 25 mg/kg dose of Mn until 55 days of age produced increased levels of mean serum LH, FSH and testosterone compared with the control animals (17).

Many studies on experimental animals have reported strong evidence of harmful effects of exposure to manganese on male fertility (11). Manganese effects on fertility, serum

concentrations of follicl stimulating hormone (FSH) and testosterone, morphological indices of reproductive development, and behavior have been noted in several species (9). Experimental results in animals are consistent with the clinical observations of impotence and reduced excretion of 17-ketosteroids (as consequence of decreased testosterone production) in humans following exposure to Mn dust (5).

Laskey et al. (14) reported that there was a reduction in the serum testosterone of the animals, but no indication of hypothalamic or pituitary malfunction in Long-Evan rat pups that were dosed orally with Mn3O4 for 21 days. The decreased testosterone secretion may lead to delayed growth and maturation of testes and other reproductive tissues. These results suggest that the site of manganese damage that causes depression of sustained serum testosterone concentration is in the testicular Leydig cell.

Testosterone levels were reduced in male rats given an oral dose of 13 mg Mn/kg/day for 100-224 days (15).

In SD rats that were treated with MnCl2 at doses 7.5, 15, 30 mg/kg/d for 40 days, the serum testosterone concentration was decreased and alterations of Leydig cell mitochondria, such as mitochondrial swelling and disruption of intramitochondrial cristae were also observed (4).

A significant decline in the sperm motility in cauda epididymis was noticed in rats treated with manganese (II) tetraaza macrocyclic complexes . Sperm density in testes and cauda epididymis were also reduced. Sperm motility and fertilizing capacity of spermatozoa were affected severely, which could be due to the androgen deficiency. Manganese(II) tetraaza macrocyclic complexes cause reduction in the weight by the accessory sex organs which indicates the atrophy of glandular tissue
and also reduction in the secretion ability. Thus reflecting the decreased levels of testosterone (25).

Abnormal sperm morphology was observed in mice treated with 23-198 mg manganese/kg body weight per day as potassium permanganate or manganese sulfate by gavage in water for up to 3 weeks (21).

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ANATOMOPATHOLOGICAL AND EPIDEMIOLOGICAL STUDY OF VISCERAL AND NONVISCERAL HEMANGIOSARCOMA IN DOGS

TĂBĂRAN A.F., C. CĂTOI, A. GAL, P. BOLFĂ, M. TAULESCU, A.L. NAGY, COSMINA CUC, G. BORZA, R. MOUSSA

University of Agricultural Sciences and Veterinary Medicine, Department of Pathology, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania

email: flaviutabaran@gmail.com

Summary

From *two hundred* and *thirty* two canine tumors that were necropsic and histolopatologic examined during the 3-year period from May 2007 through April 2010 twelve cases of hemangiosarcoma were diagnosed. Visceral types of hemangiosarcoma stand for up to approximately 3% of the specimens submitted for histologic examination and the nonvisceral hemangiosarcoma for 2%. The average age at the time of the diagnosis of hemangiosarcoma was 9 years. The primary tumors were found to be developed mainly in the spleen, with frequent metastasis in liver and omentum. In 1/3 cases the tumors were widespread, with multiple "cannonball"-like metastases in liver, lungs, omentum, and mesentery, in two cases in heart and in one case in the kidney. The commonly affected breeds were the large ones, especially the Shepherd and Rottweiler breeds. The tumors were equally distributed at both sexes, the hemangiosarcoma proving to have no sex predilection in the occurrence.

Key words: Canine hemangiosarcoma; angiosarcoma, dog hemangioendothelioma

INTRODUCTION

The hemangiosarcoma (angiosarcoma, malignant hemangioendothelioma) is the malignant tumor of the endothelial cells, accounting for up to 7% of all dog tumors in published reports (Smith 2003, Baba 2007). The hemangiosarcoma occurs infrequently in cats, horses, cows, and sheep (Jubb 2007). The ontogeny of the malignant cells is the multipotential bone marrow–derived stem cells, precursors with potential for endothelial differentiation (Angela R. 2006). The hemangiosarcoma can arise as a primary tumor anywhere in the body, but the spleen, skin, right atrium, lungs and liver are more frequent primary sites for the tumoral development (Brown1985,Fun-In 2001, Robinson,1993).

The canine cutaneous hemangiosarcomas are clinically staged according to the depth of the involvement, in stage I the tumors being limited at the dermis layer, in stage II the tumors extending themselves in the subcutaneous tissue, and in stage III the tumoral process involving the underlying muscle tissue being typically large, poorly circumscribed, with an appearance which can be mistaken for a hematoma (Withrow and Macewen, 2007).

The spreading from the primary sites may occur via metastasis, local invasion and transcavitary implantation (Oksanen 1978). The average age in clinical presentation is 11 years, and at the time of the examination 80% of the subjects had metastasis in the distant organs such as liver and lung (Gabor 2006). The hemangiosarcoma tends rapidly to metastasize through the bloodstream, since the tumor cells have an easy access to the vascular channels, and it presents a markedly poor prognosis even if intensive treatment is provided (Fujita 2008, Gulbahar, 1997). When metastases are widespread; the primary tumor site may be difficult to be determined, the multicentric origin of the tumor being a possibility.

By immunohistochemical staining for factor VIII-related antigen and CD31 (marker of endothelial cells), poorly differentiated hemangiosarcomas may be differentiated from spindle cell sarcomas or other nonendothelial neoplasms (Withrow 2007).

MATERIALS AND METHODS

From the two hundred and thirty two canine tumors that were brought begging from May 2007 until April 2010 at the Department of Anatomic Pathology, Necropsy and Forensic Medicine from the Faculty of Veterinary Medicine Cluj-Napoca for examination we determined the total number of hemangiosarcoma cases. The biopsy and the necropsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5-7 µm with a microtome Leica RM 2125 RT, and stained routinely by Hematoxylin-Eosin (HE) and Masson's trichrome (TM) methods. For the cytologic examination we used the Diff Quick panoptic staining protocol. The slides were examined using the Olympus BX 51microscope and the images were taken with the Olympus SP 350 digital camera and processed by a special acquisition and image processing program, Olympus Cell B.

After histopathological diagnosis, the number of diagnosticated cases were statistically compared to the total amount of canine tumors examined during this 3 years period. Then, the age, sex, breed and location of the primary and metastatic tumors were analyzed, submitted to statistical interpretation and afterwards the numbers were correlated with the necropsy and histological findings.

RESULTS AND DISCUSSION

Pathological findings

The main gross findings of the hemangiosarcomas found secondary to the necropsic examination were: soft consistence, friable, easily removable, reddish black to black in color, resembling sometimes in a grade to the aspect and consistence of an organized hematoma. The shape of these tumors was in most cases round, with the diametric size ranging from a couple of millimeters to extreme sizes of 15-20 cm.

On the cut surface, the tumors' characteristics vary, from a sarcomatous with yellowish aspect to reddish black to black color. In some cases, especially in large tumors necrotic and hemorrhagic areas were seen.

The primary localization of the visceral hemangiosarcoma was the spleen, the hemangiosarcoma being in our cases the most frequent cause of tumoral enlargement of this organ. Metastatic tumor mass with "cannonball"-like aspects were scattered throughout the abdominal cavity, mainly in the liver, on the parietal and visceral peritoneal serosa (omentum) and diaphragm.

In the cases of widespread hemangiosarcoma the metastases were encountered in the liver, omentum, pleura, lungs and in two cases in the heart. In one case, the metastasis from the primary splenic hemangiosarcoma was found in the scapular region, with the infiltration of the subcutaneous tissue, regional muscle and scapula, followed by diffuse bone destruction.

The five cases of primary cutaneous (nonvisceral) hemangiosarcomas, were localized, in the first case in the mammary gland of a 10 year German shepherd bitch and, in the second one involving both the soft and osseous tissues in a 9 year Great Dane male. The other three localizations were in one case the prescapular region and the soft tissues from the hind limbs in the other two cases.

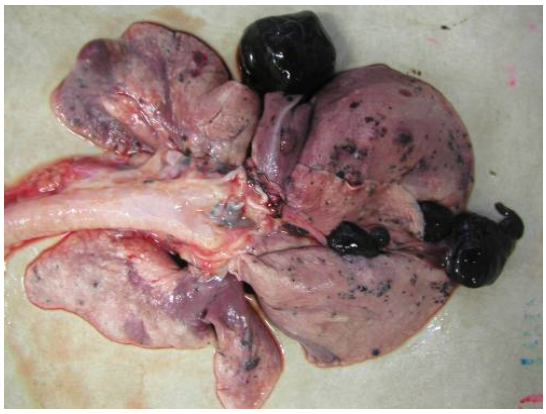

Fig.1 Multiple pulmonary hemangiosarcoma metastasis in a 6 year old Dalmatian female.

Fig 2 Cardiac metastasis in the left atrium

Fig.3 Hepatic metastasis of a primary splenic hemangiosarcoma in a 10 years, common breed male

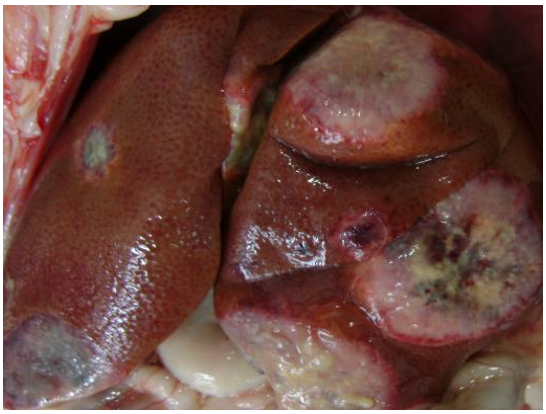

Fig. 4 Hemangiosarcoma multiple metastatic tumor in a 10 years, common breed male

These five hemangiosarcomas were clinically staged according to the depth of involvement like stage II tumors in the mammary, prescapular and hind limb localizations and stage III in the facial localization because of the involvement of the underlying muscle and bone tissue.

Microscopic examination

Histological, analyzed hemangiosarcomas consist in vascular spaces delimitated by anaplastic, elongated, endothelial cells. The vascular spaces were delimitated by single or multiple layers of cells and containing erythrocytes in variable numbers or thrombi. In some cases, endothelial cells formed solid areas or vascular spaces and cavernous channels supported by discrete collagen fibers that were detected through Masson's trichrome staining. The neoplastic endothelial cells were pleomorphic with variation in the nuclear diameter, chromatin characteristics, and mitotic index. Hemorrhage and lymphocytic infiltration were found in some cases.

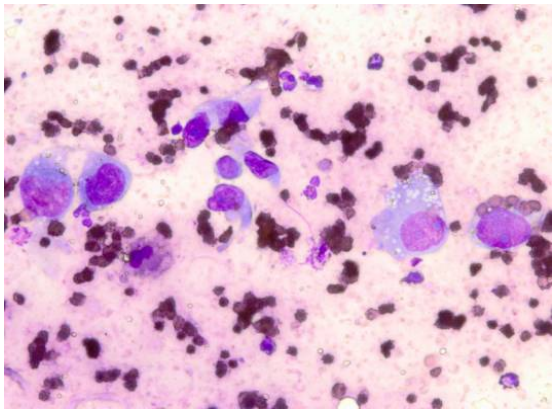

Fig.5, Cytologic examination of an hemangiosarcoma; spindle and plump shaped cells; ob x100, Diff Quick panoptic stain

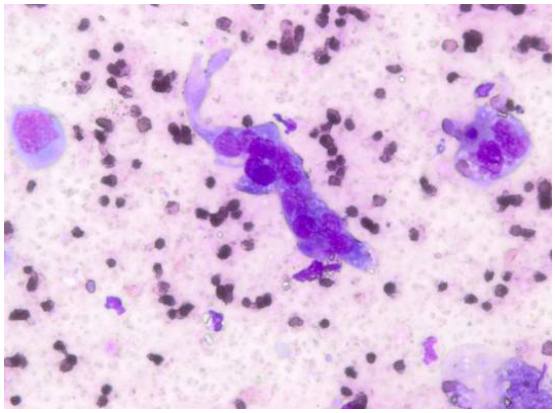

Fig.6. Cytology of hemangiosarcoma; the smear contains pleomorphic neoplastic cells; ob x100, Diff Quick panoptic

The solid areas of some tumors showed small sizes, vascular spaces lined by a single layer of pleomorphic plump, shaped young endothelial cells with hyperchromatic nuclei and pale, abundant cytoplasm that formed irregular vascular spaces. Tumors were considered poorly differentiated, when the cells were pleomorphic forming solid sheets rather than channels.

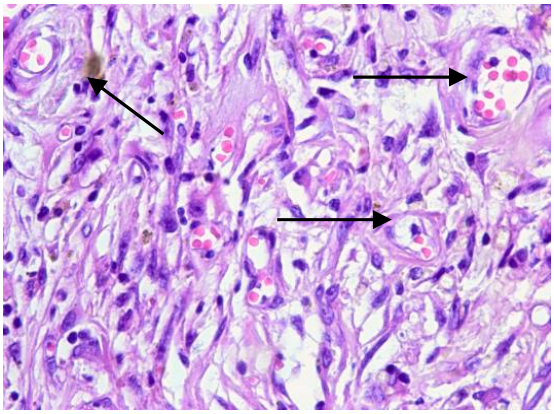

Fig.7. Hemangiosarcoma in a 10 year Cocker Spaniel female; vascular spaces delimitated by anaplastic, elongated, endothelial cells HE, ob x40

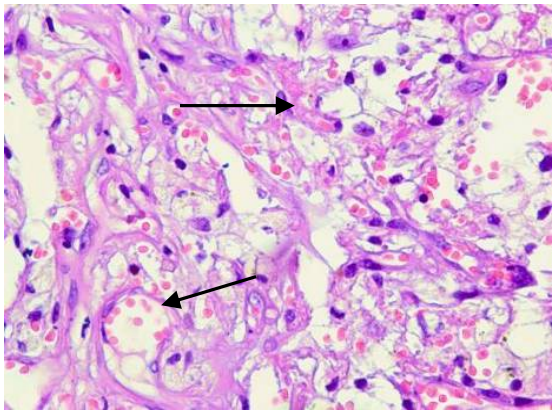

Fig.8 Hemangiosarcoma-vascular spaces delimitated by pleomorphic, elongated and plump endothelial cellsHE, ob x40

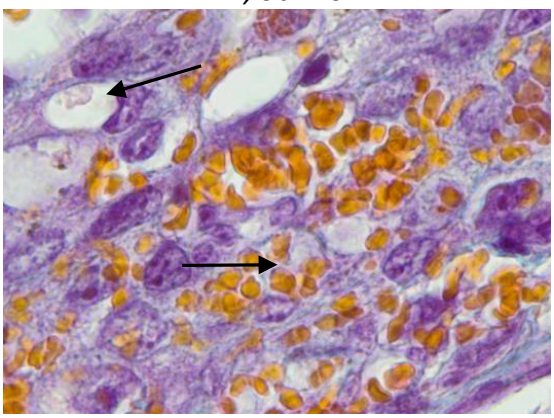

Fig.11 Hemangiosarcoma, proliferation of vascular structures delimitated by pleomorphic, plump tumoral endothelial cells; TM ob x100

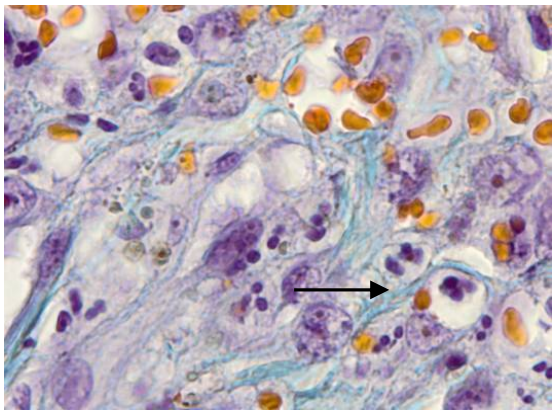

Fig.12 Hemangiosarcoma; tumoral structures delimitated by polymorphic, elongated and plump endothelial cells; TM ob x100

Cytological examination of tumoral samples reported spindle shaped cells, sometimes quite plump. The nuclei usually contain prominent, often multiple nucleoli. The cytoplasm is light, basophilic and sometimes vacuolated.

STATISTICAL ANALYZE

The age at the time of the hemangiosarcoma diagnosis, ranged from 6 to 15 years with an ăvisceral type of hemangiosarcoma which was diagnosticated in 3% of all tumors, and a reduced incidence of nonvisceral hemangiomas represented in approximately 2% of the examined tumors. Three from ten hemangiosarcomas were highly metastatic, with multiple metastases in liver, lungs, omentum, heart (in two cases) and kidney in one case.

CONCLUSIONS

1. The occurrence of hemangiosarcoma is age related, affecting especially the middle aged and old aged animals.
2. In dogs, the visceral types of hemangiosarcoma are more frequent than nonvisceral (coetaneous) ones.
3. The hemangiosarcoma is the most frequent cause of tumoral enlargement of the spleen.
4. The hemangiosarcoma has no sex predilection in the occurrence.
5. The frequently affected breeds are the large ones, especially the Shepherd and Rottweiler breeds.
6. The majority of hemangiosarcomas produce metastasis, the most frequent areas being the liver, mesentery, omentum and lungs.

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ARGULUS SPP. (FISH LOUSE) INFECTION IN A COMMON CARP (CYPRINUS CARPIO) FROM A FISH FARM IN CLUJ COUNTY

M. TAULESCU, C. CĂTOI, G. BORZA, P. BOLFĂ,
A. NAGY, A. GAL, F. TABARAN, COSMINA CUC, R. MOUSSA, A. BARBU
E-mail: taulescumarian@yahoo.com
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Summary
Argulus species or the ,,fish louse,, parasitizes the skin of most fish species, frequently in cyprinids. It causes skin lesions due to direct tissue damage and secondary infections. For this study, twenty Cyprinus carpio, taken from a local private fish farm with symptoms such as abnormal swimming and death, were examined for ectoparasites and other bacterial or viral disease. The skin lesions were represented by multiple hemorrhages, necrosis of the skin and scales loss. The parasites present on the skin were identified as Argulus species. The eradication has been achieved with copper (II) sulfate, 2 doses at ten days intervals. After administration, all fish were checked for parasite. No parasite was observed on the fish.
Keywords: Cyprinus carpio, Argulus, ectoparasites, fish louse, copper (II) sulfate.

INTRODUCTION

Argulids are obligate fish ectoparasites, commonly called *fish louse*. In wild populations where the parasite's burden is low, it usually does not cause death. *Argulus sp.* were reported from different fish species worldwide (**Molnar *et. al*.**, 1995). *Argulus foliaceus* prefers to parasitize cyprinids. The species is widely spread in temperate zones and frequently found in shipments of ornamental tropical fish. *Argulus spp.* are found nearly worldwide with about 150 species known at present. Three species documented in Europe are *Argulus foliaceus, Argulus japonicus* and *Argulus coregoni (***Oge,** 2002).

The general body-form of *Argulus* is a dorso-ventrally flattened body covered by a large chitinous carapace (**Soulsby,** 1982). The body can be divided into 3 distinct regions: cephalothorax, thorax and abdomen. The cephalothorax is so named because in *Argulus* the head is fused with the first segment of the thorax. One of the most obvious features of this group of parasites is the large ventral suckers. These are modified maxillae and are the main attachment organs in adult argulids. Various other spines and hooked/clawed appendages are also believed to play a major role in aiding the attachment of the animal to its host. In fact larval stages must depend on these numerous spines etc to keep them fixed to their hosts (**Walker *et al*.**, 2004). Once attached on the fish body, the parasite pierces the flesh using stinger mouthparts and will suck the blood of the fish. Argulus inject a toxin that will kill smaller fish and leaves reddened, inflamed lesions on larger fish. This lesion often becomes infected. Clinical signs in infected fish include erratic swimming, poor growth and flashing. The intense irritation brought on by the Argulus parasite causes fish to rub or scrape against objects from the water. Fish that have been infected with *Argulus spp*. often show several small haemorrhagic regions. In all fish, mucous and club cells will be absent from the epidermal tissue within the crater but mucous cells are often abundant in the tissue observed around the wounds margins. However, the infection increases susceptibility of the fish to bacterial pathogens such as *Flavobacterium columnare, Aeromonas*, *Pseudomonas* and spring viremia (*Rabdovirus carpio*) of carp which can lower survival rates (**Yildiz *et al.***, 2002). *Argulus*

- *Saprolegnia* infections significantly suppress carp growth and indicate that comparative growth rates can be used as indicators of parasite stress (**Singhal *et al*.** 1990). Deaths will occur if the number of parasite is too high. The most successful and effective treatments against lice are organophosphates and other pesticides or potassium permanganate in water.

One study showed good results from the use a treatment with DDVP (dichlorvos, 0,0-dimeethyl-0-2,2-dichloro vinyl phosphate) against to A. foliaceus. Trials were carried out at water temperature of 26 °C. After administration, all fish were checked for parasite. No parasite was observed on the fish. The symptoms observed before, disappeared after treatment (**Erol Tokşen,** 2006).

In this study, a case of infection with *Argulus spp. in Cyprinus carpio from* from fish farm in Cluj County is discussed.

MATERIALS AND METHODS

The biological material consisted of 20 fish *(Cyprinus carpio*) taken from a local private fish farm (3300 m^2 with a total of 8000 fish) in Cluj County (Dej location) with symptoms such as abnormal swimming and death. The fish were weighed, measured and body surface, body cavity and internal organs were examined for other infections. The parasites were identified morphologically following keys characteristics given by Bykhovskaya-Pavlovskaya. **The treatment** has been achieved with copper (II) sulfate, one milligram per cubic meter of water (1mg / m^3), using 2 doses at ten days intervals.

RESULTS AND DISCUSSION

The fish weighed 110-170 g and were 15 - 20 cm in size. On the skin were observed multiple foci with hemorrhages, necrosis of the skin and scales loss. In these regions a small quantity of mucus was identified also. The lesion appeared due to parasitic irritation and tissue damage. The mean number of parasites per fish was 1-4. The ectoparasites were 0,5-0,7 cm in lenght. Under the light microscope, these parasites were identified as *Argulus* spp. by the looking morphologic key characteristic.

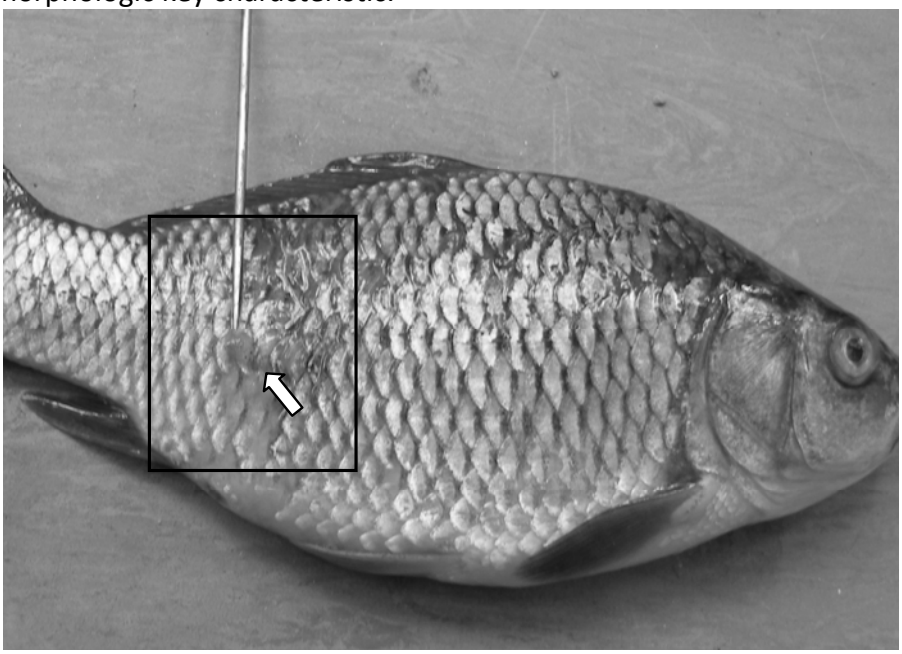

Fig.1. *Cyprinus carpio* – skin with necrosis and hemorrhages and loss of the scales (arrow); parasite - *Argulus* spp. on the skin (see needle tip). Affected region is bounded with black color.

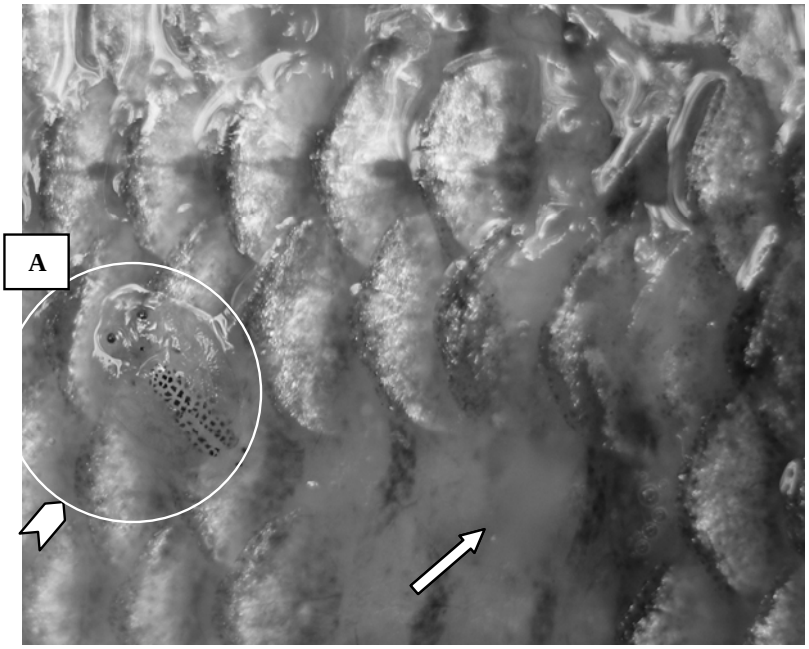

Fig.2. *Argulus* spp. (arrow head and delimited) on the skin of the carp, necrosis and hemorrhages of the skin and loss of the scales (arrow). A – anterior body of the parasite.

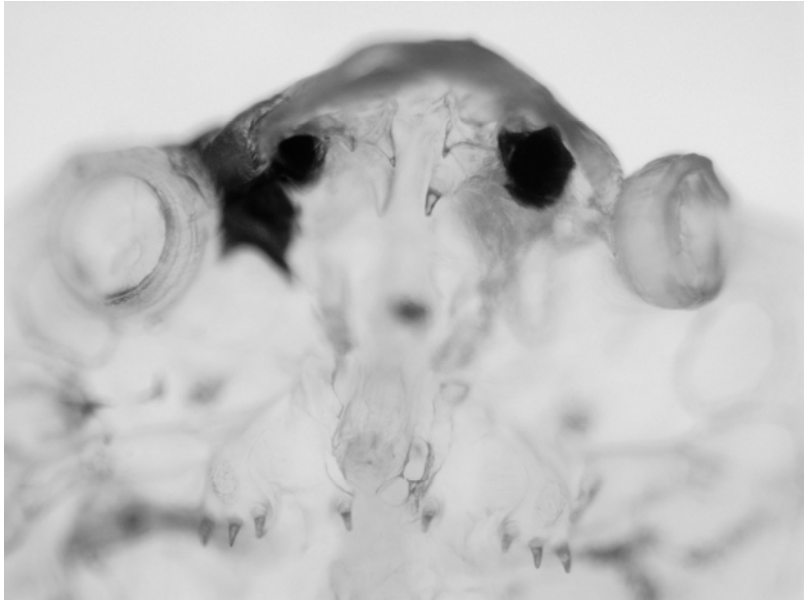

Fig.3. Anterior region of body of the *Argulus* spp. Note the two compound eyes - dark spots (ventral view)

During treatment, the adverse reactions in fish were not observed. After tratment, all fish were checked for parasite. No parasite was observed on the fish. The skin lesions disappeared after two weeks later. The eggs destroyed by flaming after were collected on wood planks placed in water.

Argulus spp. were reported from different fish species worldwide (**Molnar *et. al*.**, 1995). *Argulus foliaceus* prefers to parasitize cyprinids *(***Oge,** 2002). In this study, *Argulus* spp. was reported on carp (Cyprinus *carpio*). Three species documented in Europe are *Argulus foliaceus, Argulus japonicus* and *Argulus coregoni (***Oge,** 2002). Based on the morphological aspects in this case the fish were parasitized by *Argulus foliaceus*, it is commonly found in Europe.

Fish that have been infected with *Argulus* spp. often show several small hemorrhagic regions and mucous are often abundant in the tissue observed around the wounds margins (**Yildiz *et al*.**, 2002). In the present study the main symptoms were represented by abnormal swimming, scrape against objects from the water and death of the fish.

It is known that *Argulus* spp. infections lead to secondary parasitic infestation of the skin or secondary systemic infection with *Flavobacterium columnare, Aeromonas*, *Pseudomonas* and spring viremia (*Rabdovirus carpio*) of carp which can lower survival rates (**Soulsby,** 1982; **Yildiz *et al*.**, 2002). In this research, in all fish examined, we have not found other associated or secondary disease.

The most successful and effective treatments against lice are organophosphates and other pesticides or potassium permanganate in water (**Erol Tokşen,** 2006). The treatment has been achieved with copper (II) sulfate, one milligram per cubic meter of water (1mg / m^3), using 2 doses at ten days intervals. . After tratment, all fish were checked for parasite. No parasite was observed on the fish. The skin lesions disappeared after two weeks later.

CONCLUSIONS

Argulus folliaceus parasites the carp (Cyprinus *carpio*) in fish farms from Cluj Couty region. The infected fish with *Argulus* spp. presented lack of appetite, poor growth, abnormal swimming, scrape against objects from the water and death.

The skin lesions were represented by multiple hemorrhages, necrosis of the skin and scales loss.The treatment has been achieved with copper (II) sulfate.

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LOSS OF BODY WEIGHT – AS AN INDICATOR FOR EVALUATING LAMBS WELFARE DURING TRANSPORTATION

Mariana Țerbea [(1)], Elena Mitrănescu [(2)]
[1] National Sanitary Veterinary and Food Safety Authority
[2]) Faculty of Veterinary Medicine Bucharest

Abstract
Evaluation of animal welfare during transportation relates to the determination of the way in which are assured the conditions which to minimise the accomodation efforts of the animals at physiological and psychological stress factors. As significant indicators of animal welfare during transportation, easy to register, are the morbidity percent, mortality, the compulsory slaughtering following to accidents, bones fractures, lesions. Might be also utilized as indicators of animal welfare during transportation the behaviour and physiolological indicators.
Amongst the physiological indicators most utilized are: body temperature, heart and respiratory rate. For their signification in evaluation of the travel impact upon welfare, are determined in laboratory, certain hormones, enzimes and blood indicators.
One of the most important consequences of the animal transportation is losing of body weight.
This study was realized on batches of lambs colected from small backyard holdings intended for intercommunity trading in Greece. The consignment was realized in different environmental conditions related to teperature and watering. The duration of the transportation were of 11 hours, and the travelling distance of aproximatelly 300 km.
For lambs transported at small temperature values, without watering, the weight loss was of 4,5%, comparing with those transported in the same temperature values, but with watering during transport, where the weight loss was only of 2,6%.
At animals transported in condition of high temperature values, the percent of loss were of 5,8% in those without watering, and of 3,1%, at those watered.
The loss in body weight in lambs as an indicator of appreciation of animals welfare during transport is affected by environment temperature and by watering of sheep.

Key words: welfare, transportation, lambs, environment teperature, water deprivation, loss in body weight

INTRODUCTION

The extension of the intensive systems of rearing animals, both in Romania and worldwide, has determined the appropriate development of animal transportation. Animals have to be moved from a place to another when a holding is populated with animals, when they are replaced and sold to sloughterhouses, when they are presented in fair, markets and expositions, trade or export etc.

The conditions in which is realized the transportation has a great importance under epidemiological aspect and strongly influences the quality of products intended for human consumption, therefore the monitoring of animal circulation and of the transfortation conditions were imposed for all the Member States of the European Union.

The scope of this work is to determine the loss in body weight of transported lambs, loses determined by factors as: middle of transport used, duration of transportation and conditions for transport, watering and environmental temperature during transport, the preparing of the transport.

MATERIAL AND METHODS

The study was realized during the period July 2008 – November 2009 on lambs batches transported from backyards holdings from the North-Weast of the country in a collecting centre for sheep intended for intercommunity trade in Greece, from Prahova County.

The animal consignments were realized in different environmental temperature conditions, with two type of vehicles: ones having functional watering system, in accordance with the provisons of the Commission Regulation no. 2005/1/CE, and other without having functional watering systems. Also, some groups of lambs were watered during travel, others were not.

All lambs batches were weighted before and after embarkment.

The travel distance was of 300 km, and duration of the transportation was of aproximately 11 hours, the time of the travell being registered from the first animal embarked at origin, to the last animal landing in the collecting centre at destination.

RESULTS AND DISCUSIONS

In table no. 1 are presented, comparatively, the average values percent of the body weight loss of lambs transported at low temperature conditions (5-6 ^{0}C) in both category of animals: with watering, and in absence of watering during travel.

Making an analyse of the obtained values, may be seen that, in batch of lambs transported in absence of watering, the average percent of body weight loss was of 4,5%, and for the batch where the watering was realized, the average of body weight loss was of 2,6%.

We have to underline that the duration of transportation and the environmental temperature for both batches were the same.

Table no. 1

The average values of body weight loss during transportation in low temperature environmental conditons

Without watering during transportation	No. of animals	Environmental temperature	Duration of transportation (hours)	Body weight of the lamb batches at embarkment (kg)	Body weight of the lamb batches at landing (kg)	Average of loss in body weight (%)
	342	6^{0}C	11	9234	8818,47	4,5
Watering during transportation	339	5^{0}C	11	9153	8915, 03	2,6

The average values of the body weight loss in lambs batches transported in conditions of raised environmental temperature conditions (24-25^{0}C) are presented in the Table no. 2.

From the analysis realized, may be observed that the average percent of body weight loss in lambs transported withour watering was of 5,8%, and in those transported being watered, was of 3,1%.

Table no. 2

The average values of body weight loss during transport in raised environmental temperature conditions

Without watering during transport	No. Of animals	Environmental temperature	Duration of transportation (hours)	Body weight of the batches at embarkment (kg)	Body weight of the batches at landing (kg)	Average percent of body weight loss (%)
	381	25^0C	11	8382	7895,85	5,8
With watering during tranportation	392	24^0C	11	9016	9836,51	3,1

Animas transported in rised conditions of temperature, with a vehicle which does not coresponding from the point of view of the functional watering system during transportation, and which were not watered before embarkmenthave had an average of the body weiht loss higher with 2,7%, comparing with animals which were transported at the same temperature, but were watered before embarkment and during travel.

A similar situation has been registered in case of animals transported in low environmental temperature conditions, with a vehicle without a functional system of watering during transport, and which were not watered before embarking, which registered an average body weight loss with 1,9% higher, comparing with animals which have been transported at a similar temperature, but had been watered before transportation and during travel.

In accordance with the data found in the literature, in which is accepted a body weight loss of about to 3%, from the obtained results may be concluded that, for the batches of lambs which were watered during travel, no matter the value of the environmental temperature in which the travel took place, the average loss in body weight is joining in the accepted limits, respective 2,6% at a temperature of 5^0C and 3,1% at a temperature of 24^0C.

Overtakings of the percent of 3% body weight loss were registered at batches which were transported in conditions without watering, respective 4,5% at a temperature of 6^0C and 5,8 % at 25^0C.

The loss in body weight, as an indicator of animal welfare during transportation has registered adequate values with data in literature.

CONCLUSIONS

1. The verage body weight loss for animals without watering during transportation has been with 1,9% higher than fore those watered, at low temperatures.
2. At higher temperatures, the average body weight loss for animals which were not watered during the transportation has been with 4,7% higher than in those watered.
3. The watering of animals and the environmental temperature during transportation influence the loss in body weight of lambs and this is an indicator in appreciation of animal welfare during transportation.

RECOMANDATIONS

In oder to register a level in average of body weight as small as posible, we reccomend that, before embarking, animals to be watered, and the system for watering to be functional during travel.

During the seasons with high environmental temperatures, animals need to be embarked and transported in the very early morning in order to avoid the highest temperatures of the day which might influence dehidration and, implicitelly, the body weight loss of the animals in an important percent.

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COMPARATION OF TWO METHODS FOR DETERMINING *SALMONELLA* spp.

I. ȚIBRU,SORINA MARIA IRIMIE
Faculty of Veterinary Medicine Timișoara
Calea Aradului No. 119, 300645, Timișoara, Romania
E-mail: tibruioan@yahoo.com

Abstract
This work present analysis results of two methods for detecting bacteria of the genus Salmonella on the surface of pig carcass and environmental samples from the production of foods and moving them. The first method of detection is horizontal (ISO 6579:2002) and the second is an indirect method that analyzes impedance device using µ-Trac 4200.

Key words: Salmonella spp. detection, analysis methods.

The determination of total germ counts and germ counts from selected micro-organisms on the basis of their indicators and/or index characters in, respectively, raw materials, interim and end products as well as on surface that come into contact whit those products, including whitin the contex of the HACCP programs, has become increasingly significant as an indicator of good manufacturing practice in the food or drinks industry (1).

The most effective of these are controls of the product during manufacture, treatment and introduction into the supply chain (process controls) (1, 11).

Especially microbiological process controls require instrumentation which delivers results quickly, so that the process can be adjusted where necessary (1, 8, 11).

Genus Salmonella belongs to the family *Enterobacteriaceae* and include species *S. Bongor, S. Enteridis, S. Typhi, S. typhimurium* and *S. choleraesuis*, with subspecies *choleraesuis, arizonae, diarizonae, hautenae,* show and *salamae* (3).

Salmonella spp. is an important cause of food-borne illness in humans. Farm animals and foods of animal origin form an important source of human Salmonella infections (2, 3, 11).

The most common species involved in salmonelossis food-borne in humans are *S. typhimurium, S. enteridis* and *S. infantis* (11).

From statistics recently acquired salmonelossis is placed first among zoonoses. Salmonellosis in 2007 was second zoonosis after camphilobacteriossis, accounding for 151,995 confirmed human cause in E.U. (11) .

Horizontal method for the detection of *Salmonella* spp (7).

Sampling and their preparation for analysis will be specifically dealing with international standard product. If there is not an international standard specifically recommended than parties can conclude an agreement regarding this subject.

Detection of *Salmonella* spp. is in compliance with ISO 6579:2002 and has four successive stages:

The first step is pre-enrichment in non-selective liquid medium, peptone-buffered water, but if the samples are the cocoa or cocoa products as environment pre-enrichment will use water-buffered peptone casein or milk powder and Green Diamond . If acid or acidified foods using whole-buffered peptone water, but the pH of the sample with pre-enrichment environment should not exceed pH 4.5 (4, 5, 6).

The second step is selective enrichment in liquid medium. Selective liquid media are recommended: Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann broth tetraionat / novobiocyn (MKTTn) (7, 9).

Isolation and identification is the third stage. This stage involves the use of solid culture media xylose-lysine-dezoxycolat agar (XLD) and Brilliant Green agar (BGA). Confirmation of identity is the final step and is the biochemical and serological tests (7, 9).

The media used in this stage are: agar TSI (Triple sugar / iron agar), urea agar (Christensen), the average L-lysine decarboxylation (9).

Also used for identity confirmation reagents for detection - galactosydase, Reagents Voges-Proskauer reaction (VP), reagents for indole reaction and a mono or polyvalent sera against Salmonella (9).

As we see issue of the analysis time required is five days of sample taking.

In economic terms this method has the following price/ sample. The price of each media is shown in the Table 1 (10). For each sample it was considered that will be used 10 ml of liquid medium and 12ml of solid medium.

Table 1.

The price of each media and of one sample

Medium	Medium price (RON)	Sample price (RON)
Peptone-buffered water	145,77	0,05
MKTTn	535,85	1,43
RVS	171,36	0,18
XLD	145,77	0,22
BGA	245,61	0,41
Api 20 E	800	40
Nutritive agar	428,28	0,24
Total	2472,64	42,53

Microbiological analyzer device using µ-Trac 4200, which has the following principle. The µ-Trac 4200 is based on the principles of impedance analysis.

Impedance analysis is a dynamic process, which determines the metabolic capacities of proliferating micro-organisms.

Microbial metabolism utilises nutrient substrates which for the most part consist of higher molecular compounds, producing lower molecular, charged descomposition products. These newly produced, charged compounds and/or their dissociation products alter the conductivity of liquid nutrient and lower their resistence. This reduction can be techniclly measured using a minimum of 2 electrodes in the nutrient solution. If an electrical AC voltage is applied to the electrodes then according to definition the reduction of this so-called impedance in the AC current field can be measured (8).

The typical µ-Trac measurement curve can be divided into 3 charecteristic ranges: initial or adaptation phase (the micro-organism adapt their metabolism to the substrate present; the result ions cannot be mesured tehnically), exponential phase and stationary phase (8).

The higher the contamination rate of the sample, the shorten the length of time before the formation of the characteristic measuring curve.

Qualitative methods-presence/absence of Salmonella

Sample preparation is made like this: 25g of sample are pre-enriched in 225 ml Buffered Peptone Water at 37° for 16- 18 hours; for red meat pre-enrichement time can be reduced to 6-8 hours but then pre- warmed (40°C) Buffered Peptone Water should be used (4 ,5 ,6).In 9,9 ml of steril BiMedia 205A are inoculeted 0,1 ml of the enriched culture.
µ- Trac 4200 analyser must be setting: temperature at 37°C, duration 22,5 hours, warm up time 1 hour, threshold 10%, scale -1% to 25% M, classification red 22,5 hours, name of analysis Salmonella- PA .Positive samples (red colour) are indicating the presence of Salmonella in the sample and must be confirmed by subculture on selective agars or by immunological or molecular – biological methods(1, 8).Such analysis report is issued within 3 days. The cost of a pack of 120 measuring cell for determining *Salmonella* spp. is 2277.66 RON and a package measuring 120 cells of *Salmonella* spp and 12 confirmatory tests is 2466.16 RON (10) .

Advantages of impedance microbiology

Using dynamic detection of metabolic processes to measure micro-organisms levels eliminates the need for dilution or separation of micro-organisms, which considerably reduces the sample preparation time.The liquid media used in impedance analysis is far better suited to the proliferation demands of mocro-organisms then the semi-solid agar media in the traditional process. The proliferation rate is accelerated and the analytical times are reduced. A further reduction of the analysis time is achieved via the higer detection sensitivity.

As a signal changes can be determineted via electrical measurement techinques once the micro-organisms have increased to 10^6-10^7 CFU/ml in comparation to the plate method (10^8-10^9 CFU for a visible colony), the detection sensitivity exceeds that of the plate method by a factor of 1000 (8).

In all, the total analytical time for impedance analysis rarely exceeds 24 hours. In most cases the results are available within only a few hours whit the great advantage that the results are generally avaible erlier, the more contamineted a sample is.

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THE DETERMINATION OF COLISTIN RESIDUES FROM PORK MEAT AND ORGANS USING MICROBIOLOGICAL AND HPLC ANALYSIS

V. TRIFAN[1], Maria CRIVINEANU[2], A.i OANCEA[1], C. LUPESCU[3], V. NICORESCU[2]

[1]Crida Pharm S.R.L.Bucharest, Romania, trifanavasile@yahoo.com,

[2]Faculty of Veterinary Medicine Bucharest, Romania,

[3]ANSVSA, Bucharest, Romania

Abstract

The consequences of the drug residues presence in animal organism and in animal origin food products represent a controversial subject and also an actual one in the conditions of a large diversity of the substances used in veterinary therapy.

The aim of this study was to determine colistin residues in pork meat and organs after an oral treatment in pigs with a commercial product based on colistin. There were collected samples both from control and experimental lots. The determination of colistin residues were comparatively effectuated, using microbiological and HPLC analysis. HPLC determinations completed the microbiological study, in order to confirm the colistin levels in the days when microbiological analysis did not reveal the presence of colistin. Using HPLC method, the results were more accurate and precise.

The results obtained using the two methods allowed to establish the withdrawal period for colistin in pork meat and organs.

Keywords: colistin, pork, microbiological analysis, HPLC.

The consequences of drugs residues presence in animal organism and in animal origin food products represent a controversial and actual subject in the conditions of a large diversity of the substances used in veterinary therapy (2).

International organizations and governments are involved in adapting the necessary methods for reducing and eliminating the potential risk over the consumer health represented by the presence of these residues (5).

Colistin (colimycin, polymixin E) is an antibiotic produced by cultures of *Bacillus polymixa* var. *colistinus*; it belongs to polymyxin therapeutic class. Colistin is used for the prevention and treatment of diseases caused by sensitive bacteria in rabbits, pigs, poultry, cattle, sheep and goats (6). After the oral administration, the absorption is low (1). The elimination is made by renal way, realizing high urinary concentrations (4). For the oral way is used colistin-sulphate, especially in gastro-enteritis produced by sensible germs (3).

The aim of this study was to establish the withdrawal period for pork meat and organs after oral treatment with colistin.

MATERIALS AND METHODS

The experiment was made on 3 month-aged pigs. It was administered commercial product Colicrid 50%, buvable solution in a dose of 50 mg/kg/day, for 5 days.

After this period, 2 pigs were slaughtered in the first, second, third and fourth day after the treatment interruption and there were collected muscle, liver, kidney and fat

samples.

In order to detect colistin residues, all samples were analyzed using the microbiological method based on the measurement of the inhibition area produced by colistin residues in a culture of *Bordetella bronchiseptica*.

The determination of the colistin residues value in the analyzed samples (µg residue / kg sample) was realized by comparing the inhibition area with standard curve values.

In the same time, there were collected samples in order to detect colistin residues by high performance liquid chromatography (HPLC).

The HPLC study was intended to complete the microbiological analysis in order to detect the values of colistin residues in the days in which the values obtained by microbiological analysis were under the limit of detection, respectively in the 4th day after treatment interruption.

RESULTS AND DISCUSSIONS

Colistin residues concentrations in muscle, liver, kidney and pork fat are presented in tables 1-4.

Having in view the interpretations of the obtained results, it was taken into consideration the fact that maximum admitted limits of colistin residues according to Council Regulation (CEE) Nr. 2377/90, are: 150 µg/kg in pork muscle, 150 µg/kg in liver, 200 µg/kg in kidney, 150 µg/kg in skin and fat.

Table 1

The levels of colistin residues in pork muscle (µg/kg)

	Slaughtered at 1 day after the treatment	Slaughtered at 2 days after the treatment	Slaughtered at 3 days after the treatment	Slaughtered at 4 days after the treatment	
	Microbiol. method	*Microbiol. method*	*Microbiol. method*	*Microbiol. method*	*HPLC*
Pig 1	2200	870	313	<LOD	23.20
Pig 2	2020	754	253	<LOD	32.58
Average	2110	812	283	<LOD	27.89

LOD= Limit of detection

From table 1 it can be noticed that the highest decrease of the residues concentration was produced between days 1-2 after the treatment was stopped. It could also be noticed that there aren't big differences between the residues concentrations in the 2 pigs' muscle. In the 4th day, although microbiological method didn't detect residues, by HPLC there were determined quantities of colistin residues, but under European MRLs (Maximum Residues Limits).

Table 2

The levels of colistin residues in pork liver (µg/kg)

	Slaughtered at 1 day after the treatment	Slaughtered at 2 days after the treatment	Slaughtered at 3 days after the treatment	Slaughtered at 4 days after the treatment	
	Microbiol. method	*Microbiol. method*	*Microbiol. method*	*Microbiol. method*	*HPLC*
Pig 1	604	321	170	<LOD	22.39
Pig 2	572	313	<LOD	<LOD	19.11
Average	588	317	170	<LOD	20.75

LOD= Limit of detection

In the 2nd table it is shown the fact that, in liver, the residues decrease significantly in the first three days after the treatment and there aren't major differences between the concentration decreasing dynamic of the two pigs. In the 4th day, although by microbiological method there were not detected residues, by HPLC there were determined low quantities of colistin residues, also under European MRLs (Maximum Residues Limits).

Table 3

The levels of colistin residues in pork kidney (µg/kg)

	Slaughtered at 1 day after the treatment	Slaughtered at 2 days after the treatment	Slaughtered at 3 days after the treatment	Slaughtered at 4 days after the treatment	
	Microbiol. method	*Microbiol. method*	*Microbiol. method*	*Microbiol. method*	*HPLC*
Pig 1	2145	798	335	<LOD	44.24
Pig 2	2233	854	<LOD	<LOD	47.04
Average	2139	826	335	<LOD	45.64

LOD= Limit of detection

In table 3 it can be noticed the dynamic of residues concentration decreasing in kidney in the two pigs, this being almost the same with the one in the liver, starting obviously from different levels. In the 4th day, although microbiological method didn't detect residues, by HPLC there were determined quantities of colistin residues, higher than in muscle and liver, but under European MRLs (Maximum Residues Limits). This fact is due to the renal elimination of colistin.

Table 4

The levels of colistin residues in pork fat (µg/kg)

	Slaughtered at 1 day after the treatment	Slaughtered at 2 days after the treatment	Slaughtered at 3 days after the treatment	Slaughtered at 4 days after the treatment	
	Microbiol. method	*Microbiol. method*	*Microbiol. method*	*Microbiol. method*	*HPLC*
Pig 1	133	<LOD	<LOD	<LOD	<LOD
Pig 2	145	<LOD	<LOD	<LOD	<LOD
Average	139	<LOD	<LOD	<LOD	<LOD

LOD= Limit of detection

In table 4 it can be noticed that the residues level in fat is much lower in the first day in comparison with other organs, but starting the second day, it decreases under the quantity detectable by microbiological method. HPLC analysis performed in the 4th day did not reveal quantifiable values of colistin residues.

Because it is known that colistin absorption is low after oral administration, it can be noticed that in some tissues (liver, fat) the levels of the colistin residues are low, even in the first days; in the fat, colistin residues are under MRLs from the first day after the treatment's interruption.

CONCLUSIONS

1. Consecutive the determinations using microbiological method, the values of colistin residues in pork meat and organs after the administration of Colicrid 50% product decrease under maximum residues limits starting the 4th day after treatment's interruption.
2. HPLC method detects colistin residues in pork meat and organs more precise and accurate than microbiological method.
3. HPLC study completes microbiological analysis, providing valuable data in the days in which the values obtained by microbiological analysis were under the limit of detection.
4. Withdrawal period for pork meat and organs after the treatment with Colicrid 50% product was established at 4 days.

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RESEARCH CONCERNING THE EXTRACTION METHODS FOR PATHOGENIC YERSINIA ENTEROCOLITICA FROM PORK MEAT

La. TUDOR[1], I. ȚOGOE,[1] I.L. ILIE[1],
ANCA MARIA GALIȘ[1], ANETA LAURA TUDOR[2]
[1]Faculty of Veterinary Medicine Bucharest
[2] Faculty of Veterinary Medicine "Spiru-Haret"

Abstract
At the moment, there are a lot of methods for the DNA extraction from meat, and most of them do not succeed in removing the inhibitors for the PCR reaction. In this study, we compared 2 methods of DNA extraction in order to determine which one removes better and in a higher quantity the PCR specific inhibitors. The amplification of the specific yadA gene from Y. enterocolitica pure cultures can be carried out with both of the protocols that we investigated during this study. Both protocols were able to highly detect a lower limit of 0,4 fg/µl of DNA extracted from Y. enterocolitica pure culture. The main conclusion was that both protocols can be used for the detection of Y. enterocolitica in meat, where the background microflora is usually very rich.

Keywords: DNA extraction, PCR reaction, meat samples.

Causing a great variety of intestinal and extraintestinal symptoms, Y. enterocolitica is a very well-known human pathogen (1, 5, 7, 8, 15). This most frequent serotypes that are associated with human pathology are: 4/O:3, 2/O:9, 1B/O:8 and 2/O:5,27(6, 9, 10, 11). From all these 4/O:3 is the most spread and it's frequently isolated from living pigs. Healthy pigs were demonstrated to be the main reservoir of this disease all around the world (2, 3), but the pork meat was not considered to be a vector of this bacterial species, especially for this serotype (4, 12, 13). Of course, not all the serotypes are pathogenic, and the pathogenic strains for this species contain a virulence plasmid which was called pYV, with great importance in the clinical sciences. Among the virulence factors which this plasmid encodes, there can be mentioned: Yersinia puter proteins (Yops) and the adhesine A (YadA). The latter promotes the adhesion to the intestine epithelial cells and among the bacteria themselves, at the same time producing autoagglutination and interference with the bactericidal action of serum (14). In most cases there are a lot of difficulties concerning the capacity of PCR reaction to detect microorganisms, depending upon the purity of the template, which is used as target and the number of target molecules. In some types of samples, meat for example, a lot of PCR inhibitors may be present, in different forms and compositions.

The objective of this work was to compare two different techniques used for DNA extraction in order to determine which one removed a higher quantity of PCR inhibitors.

MATERIALS AND METHODS

In this study, we used as inoculum, two strains of Y. enterocolitica, one from the university collection and the other isolated directly from pigs, raised on industrial conditions in our country. These strains were kept in Luria broth, supplemented with 20% glycerol at 20°C. A colony of Y.e. obtained on MacConkey agar was inoculated in 100 ml of TSB and incubated for

18 h at 25 °C to obtain a concentration of 1 x 10^9 CFU/ml. One milliliter of this concentration was used to perform the DNA extraction when the extraction was done directly from the pure culture or to inoculate the samples.

We used two types of meat samples: pork salami and minced meat. From each sample, consisting of one of the two mentioned types, we obtained a 25 g aliquot and we inoculated it with 1 ml of the prepared inoculum, and afterwards we placed it in a 225 ml of TSB. The inoculated sample went to a stomacher, where it was kept for 90 s at 2000 rpm. All the samples were incubated at 25°C, and the DNA was afterwards extracted.

RESULTS AND DISCUSSIONS

The DNA extraction protocols maintained the DNA intact, and the amplification of YadA gene was successful in both cases. Both of the sample types could be used successfully for the Y.e. detection, even when the concentration of this bacteria was not higher than 1 CFU/ml. As well, both protocols detected up to the lowest concentration for the examined samples, that of 0,4 fg/µl. Since the concentration was in some cases very low, the PCR reaction increased the sensitivity of the assay, due to its specific parameters.

In the next stage, we selected two methods of DNA extraction, for comparison. The first one included the next steps: 100 µl of the enriched sample were centrifuged at 16,000 g for 10 minutes at 4°C, the pellet was resuspended in 50 µl of PCR buffer solution, with 0,2 mg proteinase K/ each ml. After incubation at 37 °C for 1 h, the suspension was boiled for 10 minutes and centrifuged again at 16, 000 g for 5 minutes, at 4°C. The supernatant was used to perform the PCR.

The second protocol consisted of: 100 ml of the enriched sample was washed with 100 µl of ammonia hydroxide, 150 µl of ethanol, 200 µl of petrol ether and 10 µl of SDS (15 %). The sample was centrifuged at 14,000g for 10 min at 4 °C, and the pellet was resuspended in a solution containing 100 µl of 5M urea, 90 µl of absolute ethanol, 150 µl of petrol ether, 25 µl of SDS (10%) and 10 µl of 3M sodium acetate. A second centrifugation for 5 min at 12,000g at 4 °C was performed, and the pellet was resuspended with 300 µl of TE buffer (Tris–EDTA) pH 7.5, 15 µl of SDS (10%) and 5 µl of DNase-free RNase (10mg/ml). The tubes were incubated at 37 °C for 30min before the addition of 15 µl of Proteinase K (20mg/ml). This processed sample was incubated at 37 °C for 30 min. Finally, 100 µl of 5M sodium perchlorate and 300 µl of phenol chloroform isoamyl alcohol were added for DNA extraction. The sample tubes were subjected to centrifugation at 10,000 g for 7 minutes, and the aqueous phase was collected. The nucleic acids were precipitated with alcohol and the DNA was dissolved in 15 µl of deionized water.

For the DNA integrity test, 2 µl of each solution, containing the extracted DNA obtained by the techniques described above were introduced and tested in 0,9 % agarose gel, after electrophoresis at 85 V for 30 min. The gels were stained with ethidium bromide and the DNA bands were visualized using a transluminator with UV light source.

The PCR reaction included a quantity of 5 µl of templated for the first PCR step, and 2 µl for the second step. The reaction mixture contained: 1 U Taq DNA-polymerase, buffer solution, 100 µM for each dNTP, and 0,1 µM for each primer. The amplicon size was 747 bp and the product size 529 bp. These products were determined by using electrophoresis in 1 % agarose gel.

The PCR sensitivity was determined by using serial dilutions from 1 x 10^4 CFU/ml of Y.e., with virulence plasmid on TSB. From each concentration, 1 ml was inoculated on 25 g samples. Each sample was introduced in TSB (225 ml) and homogenized in a stomacher for 90

s. After incubation at 25°C, 1 ml of each concentration was taken and the DNA extraction and PCR reaction were carried out.

The detection of Y.e. strains in the meat samples simplified enormously, yadA was identified in 18 samples (25,7 %) from pork salami and 52 (74,3 %) from minced meat.

CONCLUSIONS

1. The presence of Y. enterocolitica in meat and meat products is considered debatable. Culture techniques are very precise, but time consuming, while molecular biology techniques are rapid, but the PCR inhibitors can lead to wrong results. In this work two methods of DNA extraction were studied, both of them applied successfully considering the DNA extraction and the removal of PCR inhibitors. The two methods that we chose for comparison were used before by different researchers and most of them were also able to remove any PCR inhibiting agents, considering the samples that we chose for the assay: pork salami and minced meat.
2. When a direct detection of the searched pathogen is carried out, false-positive results can be observed due to the presence of dead cells in the samples studied. This could lead to an erroneous conclusion in relation to the potential risk for the consumer. Therefore, an enrichment step was included in this work in order to promote the growth of the target organism and dilute any dead bacterium or exogenous DNA present in the sample. The differences observed between the results obtained by
3. PCR and by the culture methods may be due to the lower sensitivity of the selective culture media. It is also important to emphasize that the PCR is a rapid method for the detection of virulent strains. The possible existence of a small number of damaged or stressed cells could be the cause of long incubation times needed so that these cells may reach the detection limit of the culture methods.
4. The results of this work allow us to conclude that the studied protocols were able to eliminate satisfactorily the PCR inhibitors present in the studied foods.

Acknowledgments. This study has been financed and is a of ID-153 grant CNCSIS, the contract IDEI 289 / 2007.

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RESEARCH CONCERNING THE IDENTIFICATION OF SEROTYPE O:9 OF YERSINIA ENTEROCOLITICA FROM MEAT

L. TUDOR[1], I. ȚOGOE[1], I.L. ILIE[1],
ANETA LAURA TUDOR[2], ANCA MARIA GALIŞ[1]
1 Faculty of Veterinary Medicine Bucharest
[2] Faculty of Veterinary Medicine "Spiru-Haret"

Abstract
Based on a 181-bp fragment originating from a cloned per gene, a real time PCR was used in order to detect Yersinia enterocolitica O:9 (Ye O:9). The validation comprised 50 Ye O:9, 25 Ye non-O:9 and 40 other closely related strains, which contained homologies of the same gene. The PCR assay was used for specific Ye O:9. The limits of detection were from 1 to 20 genome copies of DNA and the amplification indicated a linear regression.

Yersinia enterocolitica species comprises a series of human pathogen serotypes, O:9 being one of them (2, 3, 9, 12). It presents an identical O-antigen structure to the ones from Brucella spp., consisting of a homopolymer of N-formylperosamine, encoded by per gene (1, 5, 7). Until this moment, there have been developed different types of PCR tests in order to detect the two genera mentioned above, from various types of samples (4, 10, 11). With a successful application in this field of work, identification methods like the slide agglutination and biochemical tests were used. In comparison to these kinds of tests, PCR assay is a much more specific and reliable method to use in this direction of research (6, 8).

MATERIAL AND METHODS

Fifty strains of Ye O:9, 25 Ye non-O:9 strains and 40 other closely related strains were included in a selectivity test. The strains were collected from meat samples, coming from species like swine, cattle and poultry, from all over the southern part of Romania. In this study there has been used the MasterMix 2X (a diagnosis kit with premixed substances) and the optimal primers, and the concentration of the samples was 200 nM, respectively 80 nM. The reading was performed on the Light Cycler thermo-cycler 2.0 (Roche). A 2 µl quantity was added over 23 µl of primary mix. The optimal temperature for 54°C normalization was determined using the gradient characteristic included in the thermo-cycler software. The specificity of the test considering the pure cultures. The specificity studies were performed using 32 bacterial strains. Yersinia spp. pure cultures were maintained in BHI broth at 30°C and for other bacterial species at 37°C, for 16-18 hours. The bacterial DNA was extracted from 1 µl quantity, from an enrichment medium left overnight, which was transferred in a 100 µl quantity of sterile distilled water and successively boiled for 10 minutes, for the bacterial lysis to take place. A 2µl quantity from this bacterial suspension, subjected to the thermal treatment through boiling was used as a pattern for the PCR technique.

The assay reagent was mixed with a uracil-N-glycosydase enzyme and deoxythimidine (replacing deoxyuridine). The final reaction comprised the following:

- Ye O:9 :
  - 250 nmol forward primer 5VTGTGCTGAAGCTTTTGGATCT3V;
  - 230 nmol of reverse primer 5VGAGGCCGATACACCTTGATT3V;
  - 80 nmol probe (5VFAMTGGACGACATGTAGGTACCTTTGGTGA TAMRA3V).
- IAC reaction:

- 75 nmol of forward primer 5VCTGCTTAACACAAGTTGAGTAG- 3V;
- 60 nmol of reverse primer 5VTTCCTTAGGTACCGTCAGAA- 3V;
- 45 nmol of probe 5VVICRTTCATGAGGACACCTGAGTTGATAMRA3V.

All these quantities were added to 0,5 mM of each deoxynucleoside triphosphate mixture (dNTPs), the reaction buffer, 0,8 U of DNAzyme II recombinant polymerase, 7,5 mM $MgCl_2$, 2,5 % v / v glycerol, and 0.7 mg/ml swine serum albumin.

The PCR reactions were performed in a LightCycler 2.0 (Roche Applied Biosystems), using the next profile: initial denaturation at 96°C , 10 min, followed by 40 cycles of 96 ^{o}C, 20 s; 60 ^{o}C, 30s and 74°C C, 30s, with the fluorescence measuring after the annealing step.

The DNA from 3 Ye O:9 was serially diluted in buffer solution, with 0.15 mM EDTA over a range of 10 logs corresponding to 150 genome copies for each reaction.

RESULTS AND DISCUSSIONS

The DNA was isolated using a continuous heating for cell disruption. So, the strains were taken from refrigerated stocks and introduced in Yersinia Selective Medium and blood agar plates. From each of them, a colony was suspended in 100 ml double distilled water, and placed at 80°C for 20 minutes, before being centrifuged for 10 minutes at 15,000 x g.

The sequences of the per gene of the microorganisms were used to design the primers for amplification of different parts of the gene, while the amplicons were subjected to sequencing in both direction. Afterwards, we sequenced a PCR amplicon of per gene (541 bp) which contained a fragment of interest in seven Ye O:9 strains already isolated, in order to asses the degree of genetic variation within the per gene fragment which was amplified. In the end of this stage, the sequences we obtained were 100% identical.

The next step comprised the hydrolysis of the samples, using TaqMan R, labeling with 6- carboxyfluorescein (FAM) and suppression with 6- carboxytetramethylrhodamine (TAMRA). Subsequently, the internal amplification control (IAC) included a second set of PCR primers, a second TaqMan probe and an artificial 124 bp amplicon. This step was performed in order to minimize the competition and also the influence on the limits of detection.

The RT-PCR test, developed in order to detect the presence of Y. enterocolitica *ail*-positive strains, is a specific one for all the strains with pathogenic potential. Y. enterocolitica strains (approx. 10^4 bacteria for a single PCR test), that belong to the pathogenic serotype have showed a positive reaction at C_t values comprised between 25 and 26, meanwhile the nonpathogenic strains of Y. enterocolitica, Y. pseudotuberculosis and other Yersinia species, concluded in obtaining of negative results. The specificity of the primers used in this study was tested by Nakajima and col. (14). These obtained similar results for the 14 pathogenic strains of Y. enterocolitica, 13 nonpathogenic strains of Y. enterocolitica, 31 for Y. pseudotuberculosis, 11 for Y. frederiksenii, 17 for Y. intermedia and other 12 bacterial strains were tested through conventional PCR technique. The lowest detection limit of this PCR test in order to identify Y. enterocolitica *ail* gene in pure cultures was of 10^3-10^4 CFU/ml, because the DNA was extracted from a 100 µl quantity of pure culture and a 2 µl quantity was used as a pattern. However, the detection limit of the PCR reaction was of 1-10 CFU for a PCR test. These data are correlated with the latest studies, performed on the same subject.

After the enrichment step through incubation for 16-18 hours at 25°C, the PCR method was able to detect 1-10 CFU/ml of liquid extract from biological product sample. The former enrichment step, before PCR technique application is necessary usually in order to raise the sensitivity when studying the naturally contaminated samples. Moreover, the enrichment step lowers the risk of obtaining false positive results, in case of PCR technique,

due to the presence of dead cells. The detection rates for *ail*-positive Y. enterocolitica strains, especially considering the biological products or the exterior sources, naturally contaminated, were especially low by using both work methods. A reason for the high number of negative results can be the low number of pathogenic strains present in these samples. The 2 µl-pattern used in this study had low values. An alternative for raising the sensitivity could be the when using a 5 µl pattern, which can be afterwards added to a 45 µl quantity of primary mix. Another reason for obtaining negative results can be the high number of microorganisms which compose the microbial flora of the medium, considering the samples which are studied. The nonselective enrichment step used in this study allows the development of all bacteria and this fact can determine a decrease in the sensitivity of this test, if the microbial flora of the medium is present in a high concentration. It had been demonstrated that a high concentration of external DNA can represent the cause of a negative effect on the amplification efficiency. This fact could exist due to the different interactions between the bacterial DNA and the PCR reagents. In order to overcome this problem, it can be used a selective enrichment step, that doesn't allow the inhibition of the necessary factors for PCR technique.

An explanation for obtaining low detection rates in case of using PCR technique could be the presence of the enzymes with a bactericide and bacteriostatic effect, as well as other inhibiting substances naturally present in biological products, fact which leads to obtaining some false negative results. The DNA extraction procedure used in case of this study was a quick and easy one, but not necessarily the most efficient for discarding the inhibiting agents from the biological products. A positive internal control has to be elaborated as a step of this test, in order to monitor the possible false negative results.

CONCLUSIONS

1. All the Ye O:9 strains included in the study produced FAM cycle threshold values (Ct) in the range of 9.5 to 32.5 with 97 % of these being between 10.5 and 15.5. Five strains showed no signal for IAC, due to inhibition of FAM signal. A second PCR reaction of these strains confirmed the results.
2. All the Ye non-O:9 presented values of FAM Ct above 55, and the Ct values for IAC were not inhibited, considering the negative results. The PCR assay detected 5 to 15 genomic copies of Ye O:9 DNA. The R-values were above 1,16 in all stages, with a optimum correlation between FAM Ct-value and DNA content.
3. The IAC is mandatory for standardization of RT-PCR assay, especially for Ye O:9 reactions. This is the way to differentiate true negative results from false-negative ones.
4. In what concerns the appliance of PCR reaction to this species and serotype, it is one of the many trials and variants considering this subject, and in the future, we hope that this technique will improve, in order to offer us a standard method for this sector of microbiology testing.

Acknowledgments. This study has been financed and is a of ID-153 grant CNCSIS, the contract IDEI 289 / 2007.

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DIROFILARIA IMMITIS INFECTION – A NEW CHALLENGE FOR VETERINARY PRACTITIONERS IN IASI COUNTY

D. ACATRINEI, L. MIRON, SIMONA DIMITRIU,
ANA MUSTEA, LARISA PARASCA, RAMONA ȘORIC
Faculty of Veterinary Medicine Iași

Abstract
The first case report of canine heartworm disease in Iași was diagnosed in march 2009. Since than, 27 new cases were diagnosed in dogs owned by Iași citizens and 41 in vagrant dogs from shelters and public kennels by microscopic examination of blood smears and specific rapid tests (SNAP Heartworm RT and Dirochek). The increase of the frequency of new cases, the zoonotic character of the disease as well as the presence of microfilaria in clinically healthy dogs can be a serious hazard to both human and animal health.

Key words : *Dirofilaria immitis*, heartworm, dog

Dirofilaria immitis (Leidy, 1856) is a dixenic spirurid nematode from the Family *Onchocercidae*.The adult stage is usually hosted by the dog, although, there are also reported infestation of the felids (*Felis catus*- Liu *et al*., 2005, *Panthera pardus* – panther - Mazzariol *et al*., 2010, *Panthera tigris* - tiger, *Panthera leo* - lion, *Panthera onca* - jar, *Uncia uncial* - snow leopard - Murata *et al*., 2003), canids other than *Canis familiaris* (*Canis familiaris dingo* - dingo dog - Starr *et al*., 1988, *Canis lupus* – wolf - Dulceanu *et al.,* 1994, *Canis latrans* - coyote - Kazacos *et al*., 1979, *Canis aureus* - golden jackal - Kesdangsakonwut *et al.*, 2006, *Vulpes sp*. – fox - Kazacos *et al.,* 1979, *Nyctereutes procynoides* - raccoon dog - Sato *et al*., 2009), Mustelidae (*Mustela putoriuus* furo - weasel), Hominidae (*Homo sapiens sapiens* - human - Dulceanu *et al*., 1994, Kim *et al.*, 2001, Genchi *et al.*, 2005).

The larvae stages 1 to 3 are hosted by the mosquitoes of the Family *Culicidae* and the ticks of *Ixodidae* which ingest them together with the blood taken from the definitive hosts. The L_3 larvae are inoculated in the definitive host during a new feeding. The prepatent period is of approximately 6 months; meanwhile the larvae migrate through the blood, then stop in the pulmonary artery and become adult. The increase of the parasitic density in the pulmonary artery leads to the retrograde migration of the parasites in the right atrium, the cave vein and the portal vein. The symptoms of the canine dirofilariosis are generally represented by cardiac and respiratory insufficiency, the clinical expression being directly related to the host body weight. Sometimes, these disorders are complicated with hepatic and renal insufficiency due to the microfilaria and their metabolites and also to the endosimbionts.

If in the dog can be found massive infestations (Acatrinei *et al.,* 2008), in the cat, only two adult worms can cause the heart dilatation and serious clinical signs (Liu *et al.,* 2005).

The disease has a zoonotic character, the infestations lacking the adult stages, but with the presence of the larvae in the blood vessels, the pulmonary tissue (Dulceanu *et al.,* 1994) or liver (Kim *et al*., 2001). The most frequent localization is the pulmonary one in the shape of eosynophilic granulomas limited by connective tissue, radio-opaque, that resemble the pulmonary neoplasms. The histological damages caused by the cardiovasculary parasitism of filaria consist of MPZA accumulation, aneurisms, the hyalination of the muscle fibers from the arterial walls and the collagenation and the erosion of the endothelium due to the mechanical action of the luminal parasites and the subendothelial migration of the myoblasts (Acatrinei, 2008). In the lungs, the lesions are more diverse, being caused by the congestive

cardiac insufficiency, by the intrapulmonary localization of the microfilaria and by the reactivity of the organism to the aggression of the parasites and their endosimbionts (Genchi M. et al, 2005). In the intrapulmonary bronchi was observed an intraluminal hemorrhagic infiltrate caused by the parasitic migrations, the proliferation of the ciliary pseudolayered epithelium and a peribronchial hyperplasia of the connective tissue. The subpleural pulmonary emphysema and the fibrosis of some surface areas of the pleura were also present. In the pulmonary tissue there was congestion and subacute-chronic interstitial pneumonia, followed by the interstitial vessels (Acatrinei *et al.,* 2005).

The longevity of adult filaria is of approximately 5 years in the cardiovascular system of the dog (figure no.1) and the longevity of microfilaria is of approximately 2 years. In other hosts, the longevity is shorter (Liu, 2001). So, a dog who gets infested today and remains untreated, will be a source of infestation for the intermediary hosts, for the next 5 years.

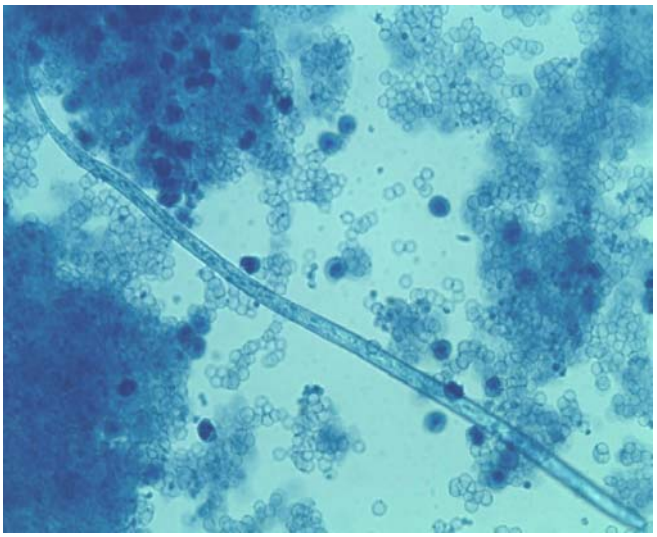

Fig.1 : Microfilaria of *D.immitis*, blood smear. Modified Knott technique.

Considering the 2 cases of cardiovascular dirofilariosis diagnosed in native dogs from Iași County in March 2009, our goal is to establish whether these are only isolated cases or we can acknowledge a new pathological hazard for the animal and public health.

Our objectives are :

1. Further parasitological investigations for the diagnosis of the *D.immitis* infestations in dogs with cardio-respiratory distress presented for examination in the clinics of FMV Iași.
2. The testing of stray dogs from the shelters administered by the local administration and the Animal Protection Associations, because these dogs are the most exposed to the attack of the intermediary hosts and can represent the referal population for this disease.

MATERIAL AND METHODS

Biological material:

- Stray dogs from the shelters administered by the local administration and the Animal Protection Associations.
- Dogs with cardiac distress presented for examination at the clinics of FMV Iași.

The sampling of the dogs from the shelters was made according to the following criteria :

- age: only the adult dogs, preferably older than 2 years because of the long prepatence of parasites
- health status: only dogs with cardiac disorders
- representativity : from each shelter, 10% of the dogs new sampled, along 6 months.

The tests used:

- SNAP Heartworm RT – IDEOX Laboratories, USA
- Dirocheck-Synbiotics Corporation USA

The positive diagnostic identifies a cuticle antigen expressed by the living females, by the ones that died following specific treatment and by the live males, antigen that binds to the imobilised antibody on the test support.

The positive response is represented by a colored area less or more intense than the positive standard. The tests alow the identification of the infestation before the microfilaria production and also the identification of the low unisex infestations which do not produce microfilaria.

Protocol:

1. The sampling of the resident canine population, the identification, the clinical examination and the individual registration of cases. From the testing lot, the dogs were selected following an effort test and the separation of the ones with cardio-respiratory distress clinically expressed.
2. The harvesting and identification of the blood samples using gel clot activator tubes (for Dirocheck) and EDTA vacutainers (for blood smears and for the SNAP heartworm RT).
3. The examination of the blood smears using whole blood or hemolysed blood enriched by the Knott modified method.
4. The negative samples have been submitted to serological rapid tests according to the producer's recommendations.
5. The centralization and the interpretation of the results.

RESULTS AND DISCUSSIONS

The results from the microscopic examination of the blood smears and the serological testing are presented in table 1.

42, 85% of the samples harvested from dogs with owners were positive. The percentage is higher than the one described by the screenings of the traditional endemic areas, situation explained by the fact that we tested only dogs suspected of dirofilariosis. Our study is not a screening, but is a proof that dirofilariosis is present in dogs from Iaşi and soon will be a real hazard for the public health and a professional chalenge for the veterinarians.

The existence of 16 serologically positive samples (5,28% of the tested samples or 21,64% of the positive samples) that were initially negative at the microscopic examination, indicate infestations with parasites in the prepatenic stage, unisex infestations or strong reactivity of the host which inhibited the reproduction of parasites. We called the 3 situations “ocult dirofilariosis”. The hidden dirofilariosis justifies the annual or twice a year serological screening using the rapid tests that take only 8-15 minutes for a result with high sensibility (98, 7%) and 100% specificity.

Table 1: Prevalence of dirofilariosis at dogs from Iași

Location	No. of analysed samples	Positive		Negative	% positive samples from the analysed samples
		Direct	Test		
Shelter 1	120	14	9	97	23.33
Shelter 2	120	11	7	102	15,00
Clinic	63	27	0	36	42.85
Total	303	52	16	235	22.44

CONCLUSIONS

1. There were 68 positive cases for *Dirofilaria.immitis* among stray dogs and dogs with owners from Iași County, fact that proves that the disease is evolving and spreading among dogs.

2. In the investigated lots there are 5,28% infestations with *Dirofilaria.immitis.*

3. The annual or biannual testing for the identification of hidden infestations with *Dirofilaria.immitis* is a must in order to protect the animal and public health.

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STUDIES CONCERNING THE HUMORAL IMMUNE RESPONSE AT GOATS AFTER VACCINATION AGAINST GANGRENOUS MASTITIS

A. Tudose, Turcu D., T. Perianu[2], Mariana Oporanu[1], P. Grigorescu[1], D. Condur[1], T. Petruț[1] [1]
Faculty of Veterinary Medicine, *Spiru Haret* University
[2] Faculty of Veterinary Medicine, Iasi University
drturcudan@yahoo.com

Abstract
The humoral immune response at goats was studied using the following dynamics of some effectors (immunological protein serum fractions IgG, total protein, lysozyme) 2 groups of goats were studied: group A that didn't get the vaccine was used as the counted group and group B that was inoculated. The second dose of vaccine was given 21 days after the first dose. The experiment lasted 65 days, during which 4 blood samples were taken. The data was statistically processed using the Student-Fisher method. Using the computer analysis of the electrophoresis, a slight increase was determined in average values of α1-globulin, α2-globulin, β-globulin and especially of γ-globulin in group B in comparison with group A after the second dose of vaccine. After the second sample, it come out that the average values of IgG and of the total protein increased by a highly significantly value ($p< 0,001$) in group B in comparison with group A. After the third and the fourth sample the lysozyme concentration presented an important increase, statistically spealing ($p< 0,001$), in group B in comparison with group A.

Key words: proteic serum fractions, IgG, lysozyme

INTRODUCTION

The animal immunological profile consists of the all the data that characterizes the cellular and humoral effectors of immunity. The changes that can occur in one or more segments of the immune mechanism establish the body's ability to recognize and to neutralize the harmful factors and to give a suitable answer when prophylactic biological products are inoculated. The main methods that are used for the immunological profile definition can be grouped according to multiple criteria, from which the most important is the one which refers to the researched effectors. Thus the effectors involved in the immune response are divided in cellular effectors (lymphocyte) and humoral effectors (immunoglobulins, complement, lysozyme, cytokine). Gjessing and all (1978) showed that goats have serum wounds, present some changes in the serum protein fractions concentration and also in the lipids one. The values of the total protein and the electrophoretic model in goats where also studied depending on age and sex (Castro, 1997). The albumin and the A/G ratio presented important differences depending on sex. The value of the total protein in goats of different ages was significantly varied ($p< 0,005$). Quiles and all (2009) studied the content in total proteins from the goats lactoserum during the lactation. The protein fractions increased significantly ($p<0,01$) during thus period. The proteins of acute phase represents another important diagnosis indicator in animals' disorders. Gonzales and all (2008) studied the changes produced in the proteins concentrations of the acute phase after they were subdued to some internal and external factors, that is the concentration in haptoglobin and C-reactive protein. Mauser (1986) and Vassiliadou (2009) determined the prevalence and the causes of subclinical

mastitis in 170 samples of goat milk and found out that 80% of *Staphylococcus* are negative-coagulase, 16% are positive-coagulase and 2% are haemolytics. Therefore after *Staphylococcus* infection, the parameter of the cellular and humoral immune response undergo changes.

In this paper the research's results are presented regarding the humoral immune response at goats after gangrenous mastitis vaccination.

MATERIAL AND METHODS

Animals. The experiment was done on 2 groups of goats: group A (n=10) that didn't get the vaccine was used as the counted group and group B got the vaccine against gangrenous mastitis. The second dose of vaccine was given 21 days after the first dose. Blood samples were taken from these animals, in day 0 (sample I), day 21 (sample II), day 47 (sample III) and day 64 (sample IV).

The 1% agarose gel electrophoresis was done in Tris-barbital swab, pH=8,6, using the horizontal electrophoresis machine, Line 1.1. The migration time was 30 minutes at 100V and 37 mA. After the slide dried out, they were coloured with 1% 10 B Amidoblack solution. The electrophoregrams were obtained through computer integration and relative (%) and absolute (g/dl) values of serum protein fractions were showed.

Simple radial immunodiffusion test (IDSR-Mancini) for IgG dosage was done using a plate 5 cm in diameter. For making this test possible, we used a reagents set prepared in Faculty of Veterinay Medicine Spiru Haret labs which contains: rabbit serum goat anti-IgG and goat serum as a control serum with a known concentration of IgG. The IgG (g/dl) concentration values from the samples were read on a standard curve depending on the precipitation ring diameters (mm).

The lysozyme measurement using the method of plate lysis determination was done using plates 9 cm diameter in which we moulded 2% agar gel, prepared in phosphate swab pH=6,2, in which the *Micrococcus lysodeicticus* culture was comprised. The serums were put in gel pitting. The reaction reading was done after 24 hours by measurement of lysis diameters (mm) and the values of lysozyme concentration (µg/ml) were read on a standard curve. As a control lysozyme we used 100 µg/ml solution of purified lysozyme (Merck).

The biuret method, for the protein concentration measurement we used the colorimetry (Spekol) at wave length of 545 nm.

The statistic analysis was done using the average calculation (x) the standard deviation (cv%) and the significance coefficient tests were calculated using the Student-Fisher method (t-test).

RESULTS AND DISCUSSION

The computer integration of electrophoresis showed 5 different protein fractions, sorted out depending on their electric charge: albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulins. The relative (%) and absolute (g/dl) average values of protein fractions in goats from group A and group B are synthesized in table 1 and 2.

Table 1. The relative (%) and absolute (g/dl) average values (x±ds) and the variability coefficient (cv%) of the proteic fractions in goats from group A.

Fractions		(x±ds)			
		Samples			
		I	II	III	IV
Albumina	%	58,74± 0,77	78,07±0,61	77,62±0,59	73,15±0,64
	g/dl	5,32±0,28	5,72±0,03	5,62±0,27	5,60±1,25
	cv (%)	5,61	4,49	4,80	4,58
α_1-globulina	%	4,94±0,44	4,75±0,40	4,74±0,45	1,93±0,08
	g/dl	0,42±0,05	0,37±0,06	0,34±0,02	0,14±0,02
	cv (%)	11,90	16,21	15,88	14,28
α_2-globulina	%	8,19±0,17	4,39±0,16	6,64±0,95	6,00±0,87
	g/dl	0,69±0,05	0,34±0,05	0,47±0,06	0,43±0,09
	cv (%)	7,24	14,70	12,5	11,62
β-globulina	%	1,43±0,40	0,77±0,03	1,26±0,21	4,68±0,44
	g/dl	0,12±0,04	0,06±0,01	0,09±0,02	0,30±0,01
	cv (%)	33,33	16,66	22,22	22,94
γ-globuline	%	26,69±1,81	12,02±0,24	12,75±1,37	14,24±1,31
	g/dl	1,70±0,06	1,73±0,04	1,79±0,07	1,86±0,28
	cv (%)	2,65	4,30	9,85	7,18

Table 2. The relative (%) and absolute (g/dl) average values (x±ds) and the variability coefficient (cv%) of the protein fractions in goats from group B.

Fractions		(x±ds)			
		Samples			
		I	II	III	IV
Albumina	%	66,38± 4,37	73,96±0,51	72,47±1,42	70,23±4,49
	g/dl	5,39±0,15	5,35±0,30	5,20±0,18	5,01±0,51
	cv (%)	2,78	5,24	3,11	8,76
α_1-globulina	%	7,74±3,99	5,21±0,27	4,90±1,43	5,34±1,73
	g/dl	0,63±0,34	0,40±0,07	0,37±0,03	0,40±0,10
	cv (%)	23,96	17,5	18,1	25,00
α_2-globulina	%	9,34±0,17	7,30±1,05	6,31±0,60	5,91±1,71
	g/dl	0,76±0,04	0,56±0,02	0,48±0,05	0,44±0,10
	cv (%)	5,26	3,57	10,46	22,72
β-globulina	%	2,31±0,36	2,14±0,25	1,60±0,53	1,96±0,65
	g/dl	0,19±0,36	0,16±0,02	0,12±0,01	0,15±0,05
	cv (%)	30,47	12,5	18,3	33,33
γ-globuline	%	16,40±0,59	13,37±1,36	13,71±2,56	14,20±3,37
	g/dl	1,72±0,09	1,82±0,09	1,82±0,21	2,26±0,22
	cv (%)	7,75	10,3	23,33	27,5

The electrophoretic analysis of albumin concentration after we took the first sample of blood from goats in group A and B showed close values (group A: 5,32g/dl and group B: 5,39g/dl) (figure 1).

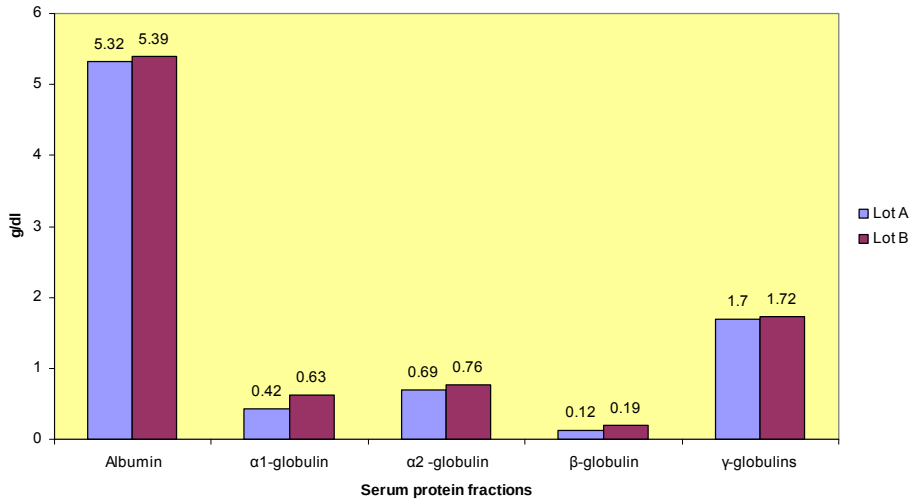

Figure 1. The absolute (g/dl) average values of serous protein fractions in goats from group A and B (sample I)

The variation rate of the relative (%) average values (x±ds) of α_1-globulin was between 4,94±0,44% and 7,74±3,99% meaning 0,42g/dl in group A and 0,63g/dl in group B. The α_2-globulin concentration showed average values (g/dl) 0f 0,69 g/dl for group A (8,19% ±0,1) and of 0,76g/dl for group B (9,34 ±0,17). The β-globulin level recorded homogenous concentrations between the 2 groups (group A 0,12 g/dl ± 0,04 meaning 1,43% ± 0,40) and group B 0,19g/dl±0,36 meaning 2,31±0,36).

In group A the γ-globulins concentration was 1,70g/dl±0,06 (26,69±1,81) and in group B 1,72±0,09 (26,40±0,59).

In figure 2 we showed the absolute average values of the protein fractions from the serum at goats from group A not vaccinated and from group B 21 days after the first dose of vaccine (sample II).

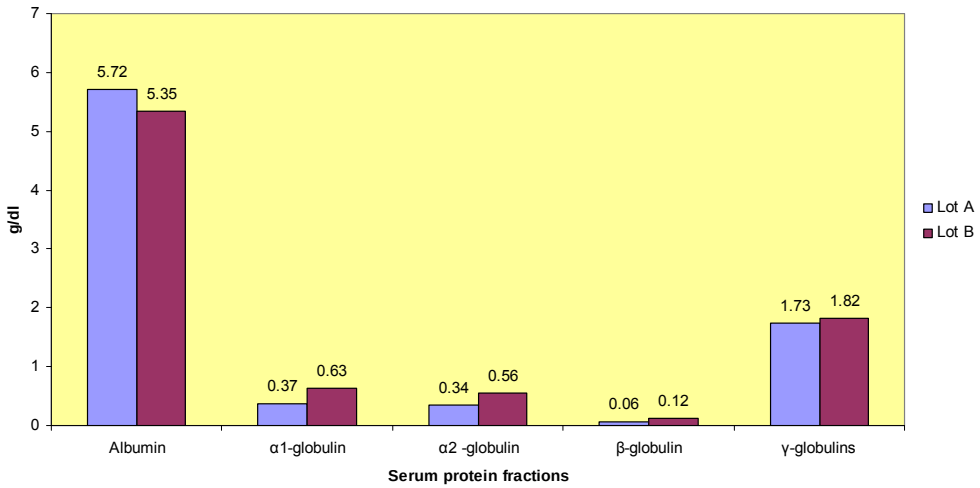

Figure 2. The absolute (g/dl) average values of serous protein fractions in goats from group A and B (sample II)

A slight decrease of albumin concentration can be see in group A 5,72g/dl (78,07±0,61) in comparison with group B 5,35g/dl (73,06±0,51). The α_1-globulin values from the second sample remained constant in comparison with the first sample in group A and B.

The γ-globulins level was higher in group B 1,82g/dl (13,37%±1,36) than in group A 1,73g/dl±0,04 (12%±0,24) table 1 and 2.

From the data analysis in table 1 and 2 and figure 3 a decrease of albumin concentration can be seen after the third sample was taken in group B 5,20g/dl±0,18 (72,42%±1,42) in comparison with group A 5,62 g/dl±0,27 (77,62±0,59). Slightly increased values of α_1-globulin, α_2-globulin and β-globulin can be seen, also in group B in comparison with group A. The γ-globulins concentration had increased values 1,79g/dl±0,07 in group A and 1,82 g/dl±0,21 in group B in the second sample (figure 3).

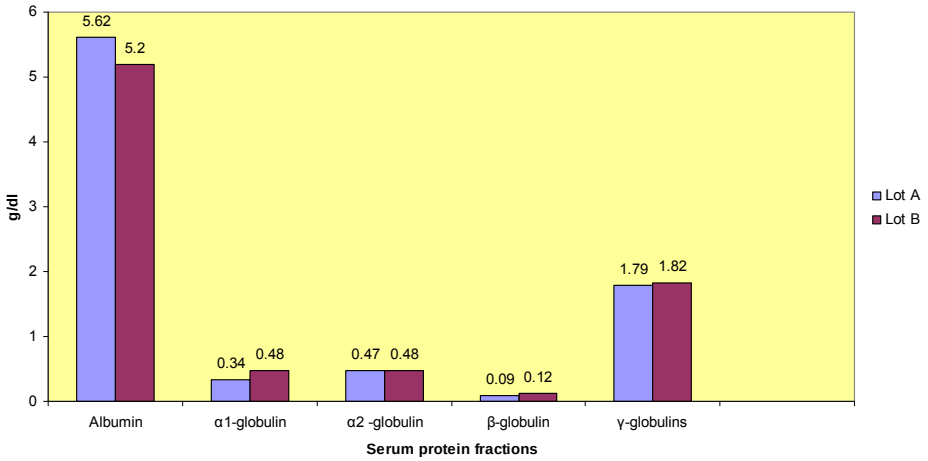

Figure 3. The absolute (g/dl) average values of serous protein fractions in goats from group A and B (sample III)

In figure 4 there are shown the absolute (g/dl) average values of serous protein fractions in the group studied in the fourth sample. In the group B the decreased level of albumin (5,01g/dl±1,25) if we analyse the data a slight increase of the average values in α_1-globulin, α_2-globulin, β-globulin and especially in γ-globulins can be seen in group B (2,26g/dl±0,22 meaning 14,20%±3,37) in comparison with group A (1,86g/dl±0,28 meaning 14,24%±1,31). The variability coefficient (cv%) was calculated in the analysed group. This experiment proves that the tests results are constant, ensuring same data acquirement which is in accordance with homogeneous value populations (table 1 and 2).

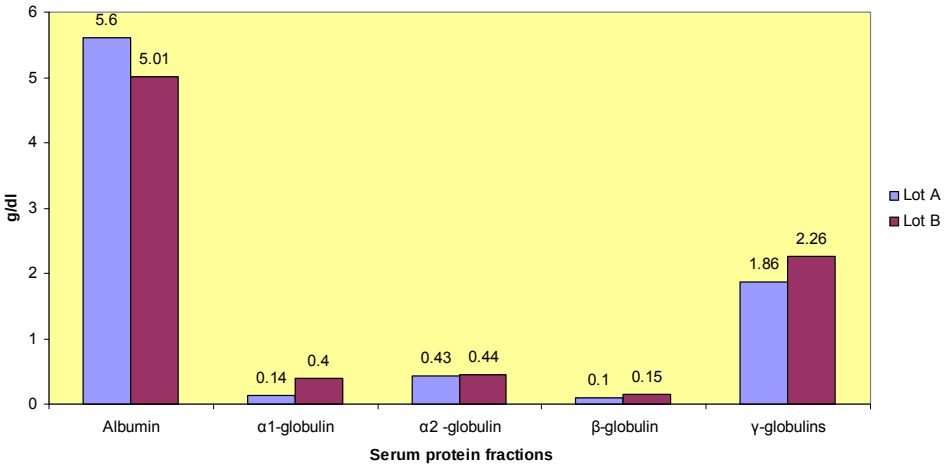

Figure 4. The absolute (g/dl) average values of serous protein fractions in goats from group A and B (sample IV)

In table 3 and figure 5, 5, 7 the results are presented in dynamics in comparison with each other concerning the average values of IgG (g/dl) and lysozyme (µg/ml) in group A and B, and the statistic meaning of the difference between them.

Table 3. The comparative results concerning the average values (x±ds) of IgG, total protein and lysozym in group A and B and the statistic meaning of the difference between the 2 groups

Parameter	**Samples**							
	I		II		III		IV	
IgG (g/dl)	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
	1,69±0,04	1,71±0,05	1,69±0,05	1,77±0,13	1,70±0,22	1,89±0,25	1,71±1,72	1,89±0,08
	p<0,5*		p<0,05**		p<0,001***		p<0,025***	
Total protein (g/dl)	7,70±0,25	7,73±0,35	7,73±0,13	7,89±0,10	7,73±0,23	8,49±0,27	7,74±0,24	8,36±0,36
	p<0,5*		p<0,05**		p<0,001***		p<0,001***	
Lyso zym (µg/ml)	5,6±0,95	5,6±0,90	5,6±0,79	7,8±1,25	5,7±0,26	10,86±0,05	5,7±0,38	8,6±0,05
	p<0,5*		p<0,05**		p<0,001***		p<0,001***	
* = unsignifiant difference ** = distinct signifiantly difference *** signifiantly high difference								

The IgG concentration in group A was 1,69±0,04 and in group B 1,21g/dl±0,05, the difference between the 2 groups was unsignificant (p<0,5) in sample I. In sample II the IgG concentration in group B (1,77g/dl±0,05) increased significantly (p<0,05) in comparison with group A (1,69g/dl±0,05). The IgG concentration in group B increased significantly high (p<0,001) in comparison with group A (1,70±0,22) in the third sample. In the fourth sample a significantly high difference was seen between group B (1,89g/dl±0,08) and group A (1,71g/dl±1,72) (figure 5).

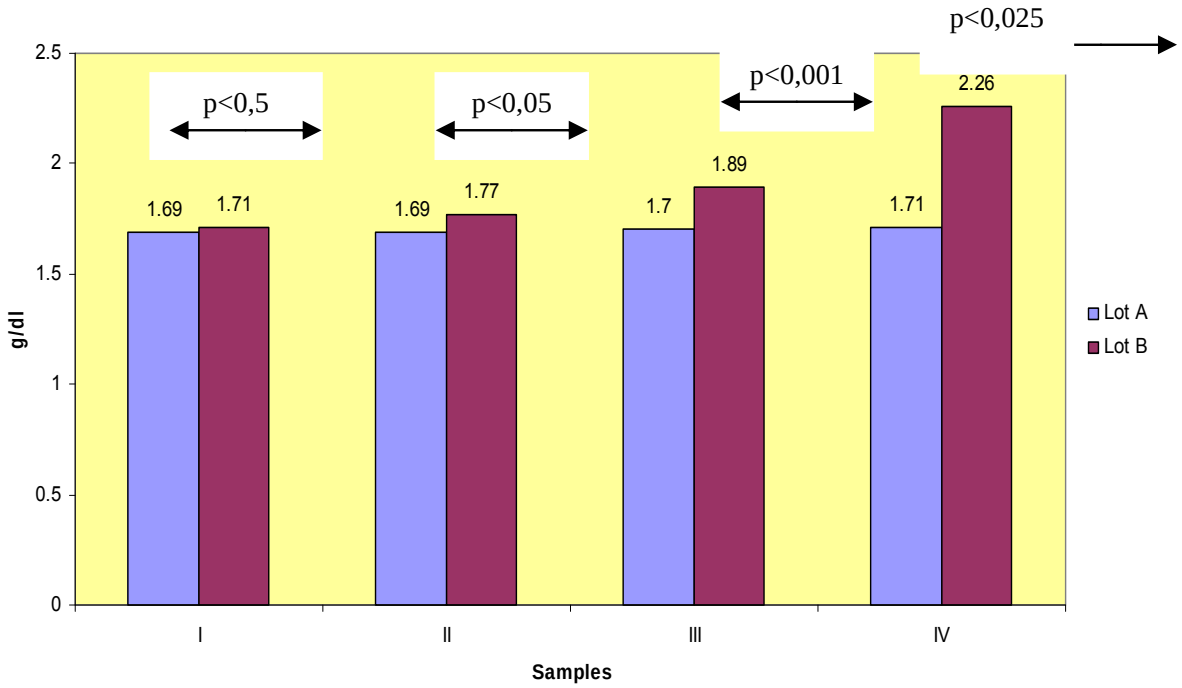

Figure 5. The IgG (g/dl) values dynamics goats from group A and B for those 4 samples

The total protein concentration in group B (3,49g/dl±0,27) increased significantly high (p<0,001) in comparison with group A (7,79g/dl ± 0,13) in the third sample, the difference between group B (8,36g/dl±1,36) and group A (7,74g/dl±0,23) stays significantly high (p<0,001) figure 6.

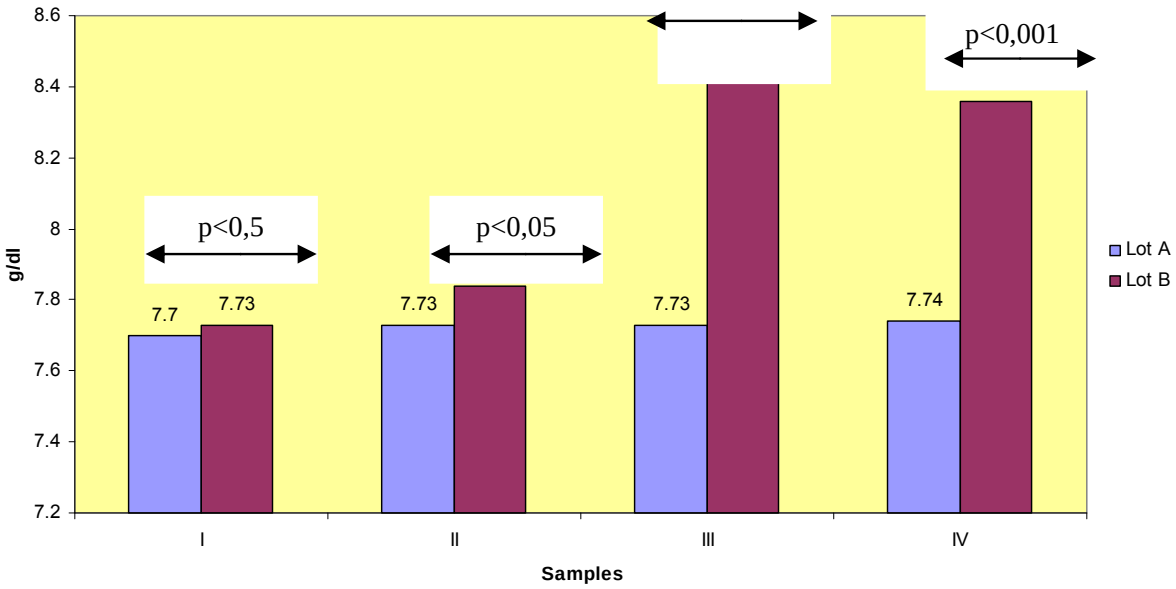

Figure 6. The total protein concentration (g/dl) for goats from group A and B for those 4 samples

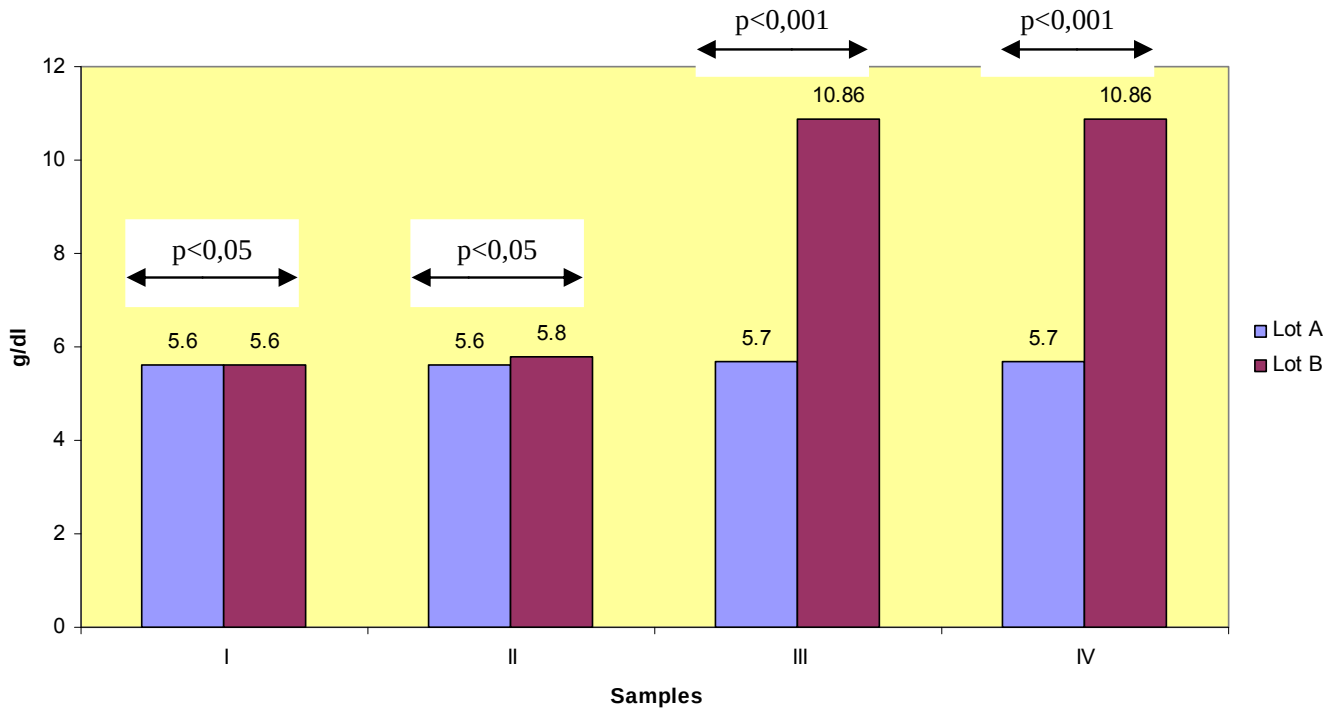

Figure 7. The lysozyme concentration (µg/ml) for goats from group A and B

In the third and fourth sample the lysozyme concentration showed an important increase statistically speaking (p<0,001) in group B (sample III and IV) (10,86 µg/ml in group B in comparison with 5,7µg/ml in group A).

The results herby presented prove that the vaccination against gangrenous mastitis has determined a meaningful increase of some humoral immunologic parameter at a significant high statistical level in comparison with the critical values.

CONCLUSIONS

The study of the dynamics of serous protein fractions at the goats from the inoculated group (B) against the gangrenous mastitis and the uninoculated group (A) showed the increase of γ-globulins concentration and the decrease of albumin level. The vaccination induced humoral immune high significant statistic response by the increase of IgG concentration, total protein and lysozym. Studied immunologic parameter revealed a significant increase after the second dose of vaccine. The study of the humoral immune effectors can reveal correct evaluation criteria of the efficiency of inoculation against gangrenous mastitis.

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THE EVOLUTION OF RABIES IN THE NEIGHBORING COUNTRIES OF ROMANIA IN THE PERIOD 2005 - 2008

Iulia Adelina TURIAC [1], F. MAZDRAG[1], C. VASIU[2].
[1] National Sanitary Veterinary and Food Safety Authority
[2] Faculty of Veterinary Medicine, Cluj-Napoca

The evolution of rabies was followed in the neighboring countries of Romania (Ukraine, Moldova Republic, Bulgaria, Serbia and Montenegro and Hungary) during the period of time 2005 – 2008. For this period of time there were recorded an total number of 10201 cases of rabies, distribution whose suffering only minor differences from year to year.
Ukraine represent the country with the largest number of cases, 9229, with a higher proportion of cases in domestic animals (55.4% from the total number of cases), the cat being placed in first place with 2167 cases (23.48% of the total number). In wild animals, most cases have been recorded in foxes (respectively 3668 cases, which represent 39.74% of the total number of cases recorded).
The highest incidence was found in year 2007 (35,02%) and the lowest in 2006 (23.76%).
Hungary and Bulgaria had the lowest number of recorded cases, which are due to the implementation of a sustained program to eradicate rabies by oral vaccination of foxes.

Key words: rabies, neighboring countries, distribution, cases, fox

In the late nineteenth century, the rabies virus reservoir was represented by dog (Canis canis) in most European countries and the main wildlife reservoir the wolf (Canis lupus). In these areas, rabies control programs in which parenteral mass vaccination campaigns and reduction of the population of stray dogs have been shown to be effective.

During the second half of the twentieth century, rabies among the canine population has disappeared gradually in most European countries and in eastern Poland occurred rabies in foxes which was spread rapidly in all directions with an 30-60 km annual prevalence rate. Except the British Isles (UK, Ireland) and Scandinavia, most European countries were infected.

The fox (Vulpes vulpes) is the main species involved in the epidemiology of rabies in western and central Europe and is the origin of rabies transmission to other species of wild and domestic animals, including, pets, cattle, sheep and goats. Raccoon dog (Nyctereutes procyonoides) is an important factor in rabies epidemiology and epizootology in eastern and northern Europe.

Despite the substantial progress in the twentieth century that have been made in reducing the burden of costs is to combat rabies, particularly in Central and Eastern Europe, the disease remains endemic in many countries.

MATERIALS AND METHOD

Analysis on the evolution of rabies in animals in the neighboring countries of Romania was based on data supplied by them to "Rabies Information System of the WHO Centre for Rabies Surveillance and Collaboration Research" data published on the website of the Rabies Bulletin of Europe.

Collected data were statistically processed by comparing the number of cases reported by each country, for the main domestic and wild species of animals, every year, using

graphs and charts to highlight the cases frequence of rabies in animal species, their share and temporal evolution.

Because the number of cases of rabies in wild animals other than foxes are small, these cases were grouped under "other wildlife" thus in graphic representations are presented three main categories namely: "Fox", "Other wild animals" and "Domestic animals."

RESULTS AND DISCUSSION

The temporal evolution and share of rabies cases during the period of time 2005 – 2008 in Ukraine are represented in graph and chart 1.

In 2005 in Ukraine were diagnosed positive a number of 2113 cases of rabies out of which 959 (45.38%) in wild animals, 1152 in domestic animals (54.51%) and were also diagnosed two cases of rabies in bats. Among domestic animals, the most numerous cases of rabies were diagnosed in dogs, 358 cases, 470 cats and bovine 283. In terms of wild animals, foxes were 91.03% of the cases i.e. 873 out of 959 cases and only 41.31% of all cases.

It is noted that the number of rabies cases in domestic animals is higher than that recorded in wild animals.

In 2006 in Ukraine were diagnosed a total of 2020 cases thereof 982 in wild animals (48.61%) and 1038 in domestic animals (51.39%). As in the previous year we see a large number of diagnosed cases of rabies in domestic animals, 400 cases in cats, dog 376 and cattle 220 cases. The 869 cases reported in foxes represented 88.49% of the 982 cases diagnosed in wild animals and only 43.01% of the total number of cases registered this year.

Although the total number of cases decreased from the previous year, there is a slight increase of rabies cases in wild animals, from 959 to 982.

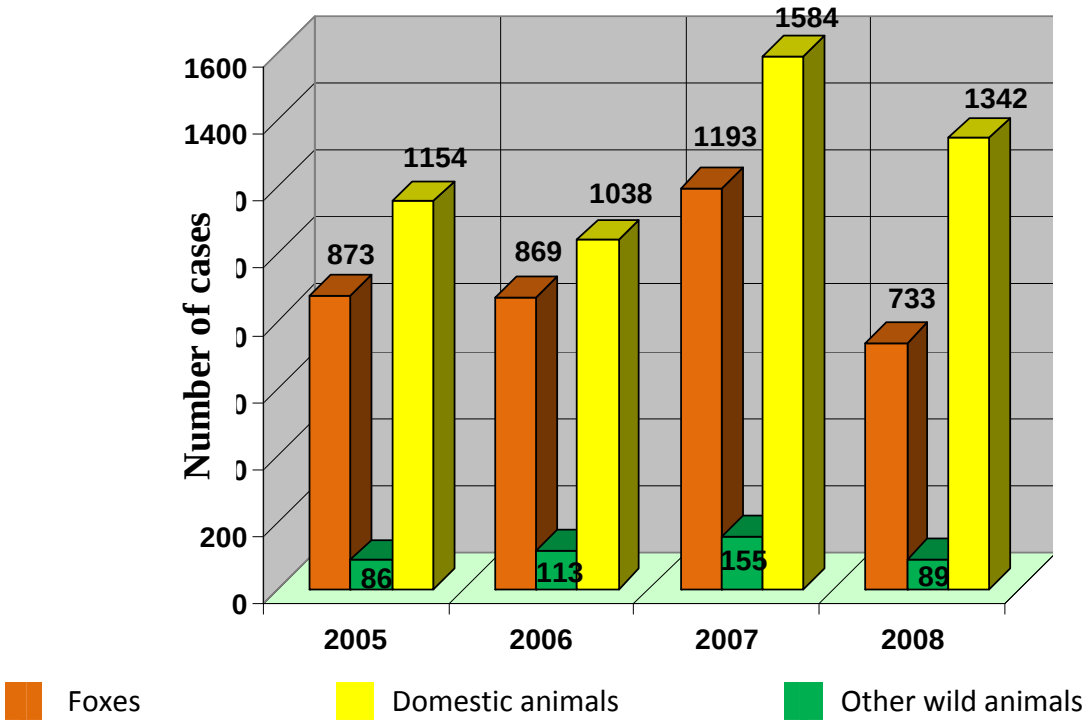
In 2007, the number of rabies cases increased by over 900 cases compared to previous years, reaching 2932. Of these, 1348 cases were in wild animals (45.97%) and 1581 in domestic animals (53.92%) and one case in bats. Foxes re-resented 88.50% i.e. 1193 cases of the 1348 cases and only 40.68% of all cases.

This year is remarkable the appearance of two cases of rabies in humans as well as an increase in cases of rabies in foxes, compared to 2006.

In 2008 there were a number of 2164 cases of which 822 in wild animals (37.98%) and 1339 in domestic animals (61.87%) distinguishing itself a high number of cases to domestic carnivores (628 cases in cat and 545 in dog) and a case in bats.

Foxes represented 89.17% i.e. 733 out of the 822 cases in wild animals, and only 33.87% of the total number of rabies cases. Other cases of rabies in wild animals were reported in raccoon dog (21 cases), marten (18 cases), 14 cases in raccoon, wolf (13 cases) and nine cases in badger.

Compared to the previous year we see a decrease in the number of cases, with decreased number of cases of rabies in wild animals. As in the previous year it is observed the evolution of rabies in humans, being reported in this year, two cases.



Graph 1. *Temporal evolution of rabies cases during the period of time 2005 – 2008 in Ukraine*

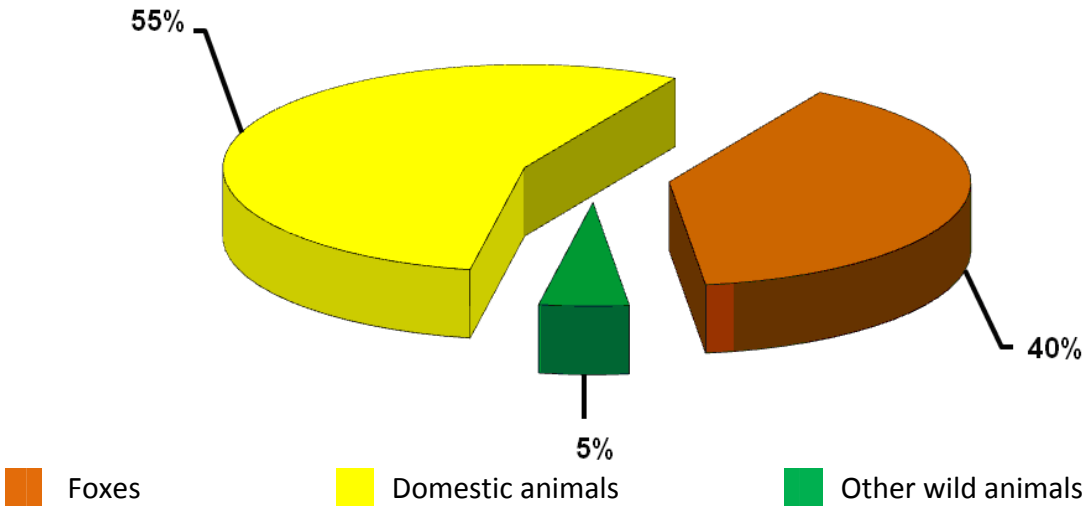


Chart 1. *Share of rabies cases during the period of time 2005 – 2008 in Ukraine*

The temporal evolution and share of rabies cases during the period of time 2005 – 2008 in Moldova Republic are represented in graph and chart 2.

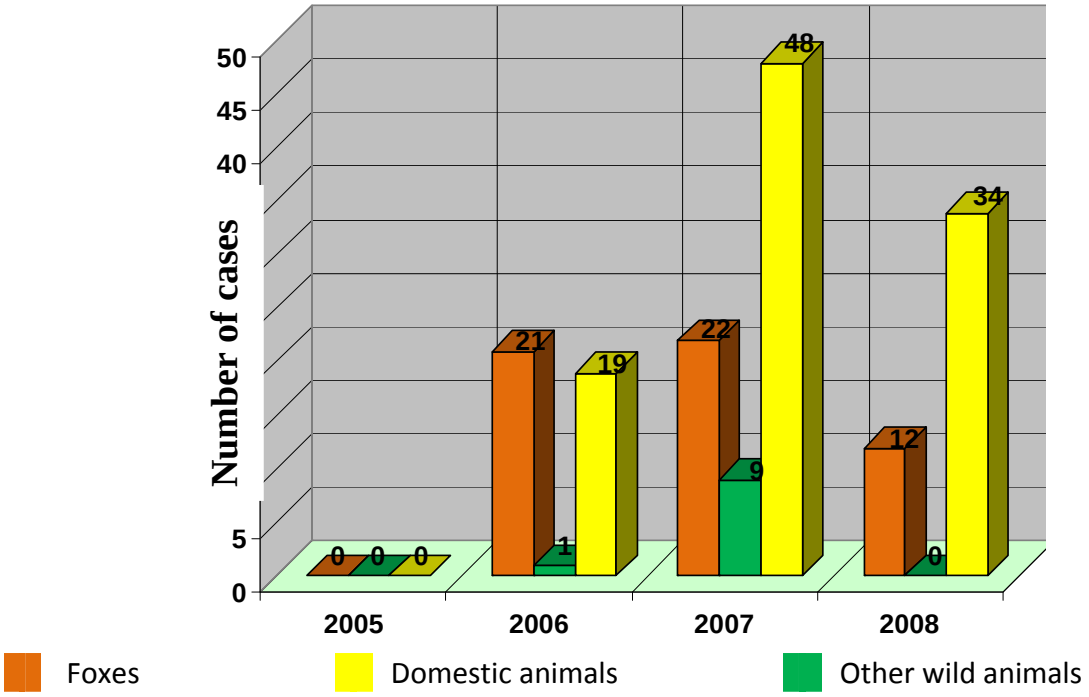
In 2005, in Moldova Republic has not been registered any case of rabies.

In 2006, were reported 41 rabies cases of which 22 in wild animals, foxes representing 51.21% of all cases, and 19 cases in domestic animals (46.34%) 12 cases in dogs and cats in equal proportions, five bovine cases and equine and goats, one case per each.

In 2007 from the 79 recorded cases, 48 (60.75%) were reported in domestic animals (20 cases in dogs, 10 cats, 16 cattle and 2 pigs) and 31 (39.24%) cases in wild animals of which 22 (27.84%) were diagnosed in foxes.

Compared with 2006, in 2007, the number of rabies cases has almost doubled.

In 2008 there is a decrease in rabies cases to 46 cases, compared to 2007. From these, 34 cases (73.91%) were reported in domestic animals, represented by 14 cases in dogs, 9 cats, 10 cattle and one case in small ruminants. The rest of rabies cases diagnosed this year were reported in wild animals (26.08%), respectively foxes.



Graph 2. *Temporal evolution of rabies cases during the period of time 2005 – 2008 in Moldova Republic*

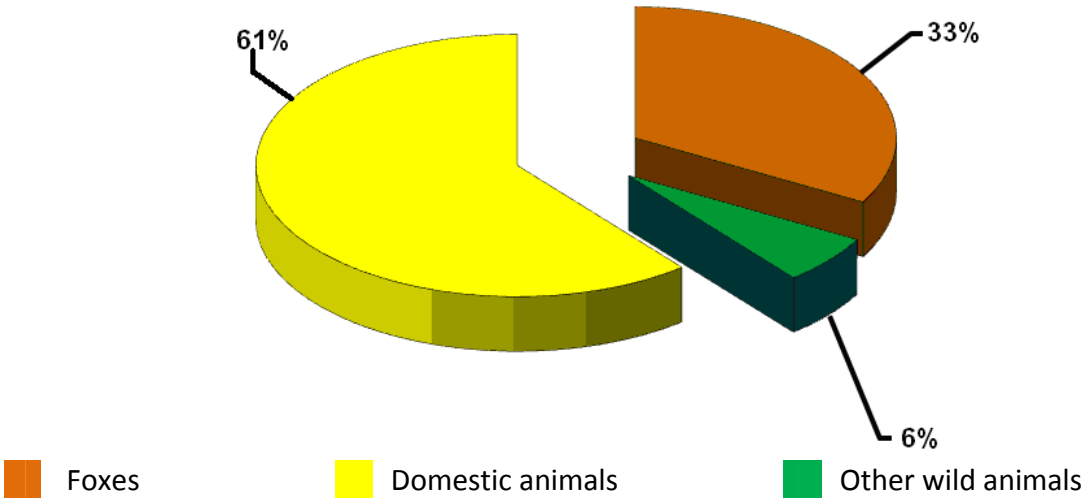


Chart 2. *Share of rabies cases during the period of time 2005 – 2008 in Moldova Republic*

The temporal evolution of rabies cases during 2005-2008 and their share in Bulgaria are presented in chart and diagram 3.

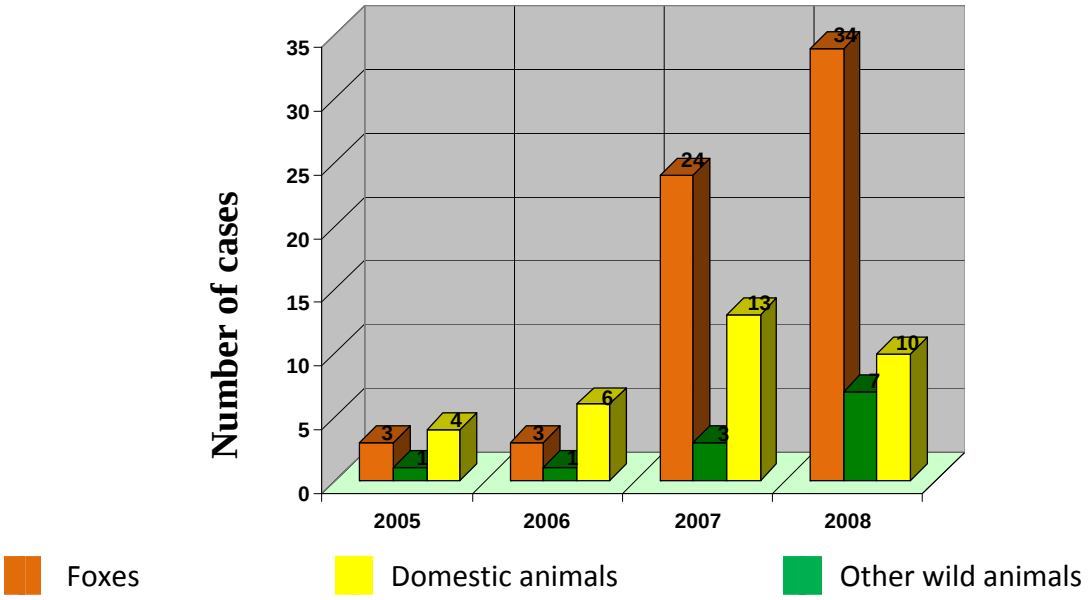
In 2005 Bulgaria had recorded a total of eight cases of rabies. The number of rabies cases in wild animals was equal to that of domestic animals. If wild animals, the fox is the main wild animal affected.

In 2006 there is a moderate increase in the number of cases from 8 to 10. Of the 10 cases only 40% is represented by wildlife cases, foxes representing 30% of all cases, and 60% in domestic animals, in cats being reported four cases which represent 40% of all cases in 2006.

In 2007 it is noted an increase by four times in cases of rabies compared to the previous year.

Of the 40 cases recorded, 27 cases (67.5%) were diagnosed in wild animals and 13 cases (32.5%) in domestic animals represented by domestic carnivores. Foxes accounted 88.89% i.e. 24 out of 27 reported cases in wild animals and 60% of all cases.

Compared with 2007, the number of rabies cases in 2008 rose by 21%. Of the 51 cases of rabies, 41 were diagnosed in wild animals and only 10 in domestic animals. The 34 cases reported in foxes is 82.9% of registered cases in wild animals



Grapf 3. *Temporal evolution of rabies cases during the period of time 2005 – 2008 in Bulgaria*

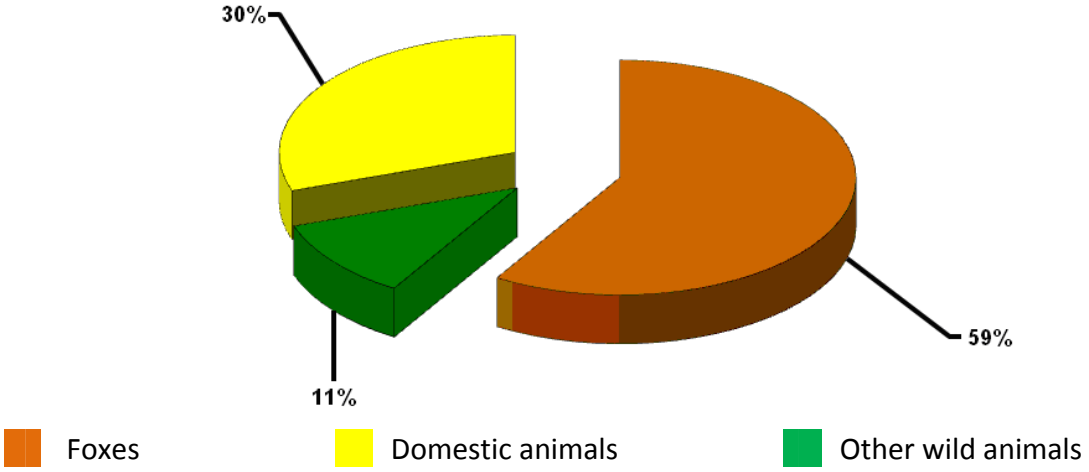


Chart 3. *Share of rabies cases during the period of time 2005 – 2008 in Bulgaria*

The temporal evolution of rabies cases and their share in the period 2005-2008, in Serbia and Montenegro are presented in the graph and chart 4.

In 2005, in Serbia and Montenegro were diagnosed a total of 101 cases. Of these, 84 were wild animals (83.2%) with 83 foxes cases (82.2%) and 17 cases in domestic animals (16.80%) of which eight cases in dogs seven in cats, and cattle and horses one case for each .

It is noted that the number of rabies cases is almost 5 times bigger in wild animals that in domestic animals.

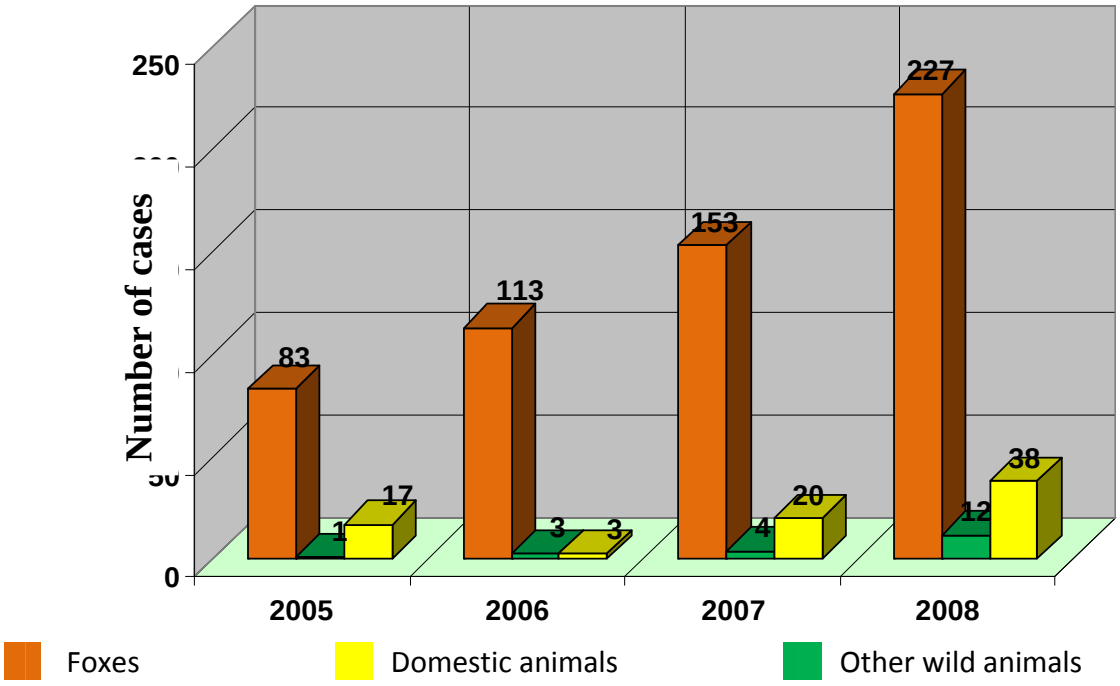
In 2006, Serbia and Montenegro had recorded a total of 119 cases. Of these, 116 were wild animals (97.47%), with 113 cases in foxes (94.95%) and three cases in domestic animals (2.53%) all in dogs. We can see that it is an increase in the total number of cases of rabies from the previous year, with the decrease almost six times of the number of cases in domestic animals.

In Serbia, in 2007 there were 160 cases of rabies of which 143, i.e. 89.37%, in wild animals with 141 fox cases (88.12%) and 17 cases in domestic animals (10.63%) of which 16 cases were in domestic carnivores (dogs and cats).

In 2007, in Montenegro was diagnosed a total of 17 cases. Of these, 14 were in wild animals (82.35%) with 12 cases in foxes (70.58%), 2 cases in wolf (11.75%) and three cases in domestic animals (17.65%) all in cattle.

During 2008, in Serbia is noted an increase in rabies cases from 160 to 234 cases, with the increase of cases in wild animals, respectively fox. Thus, of the 234 cases, 201 were diagnosed in wild animals (85.89%) with 192 cases in foxes (82.05%) and 33 cases in domestic animals (14.11%) of which 30 cases in domestic carnivores.

In 2008, in Montenegro the number of rabies cases has increased from 17 to 43 cases. Of these, 38 were in wild animals (88.37%), with 35 cases in foxes (81.39%), 2 in badger, one case in marten, and five cases to domestic animals (11.62%).



Graph 4. *Temporal evolution of rabies cases during the period of time 2005 – 2008 in Serbia and Montenegro*

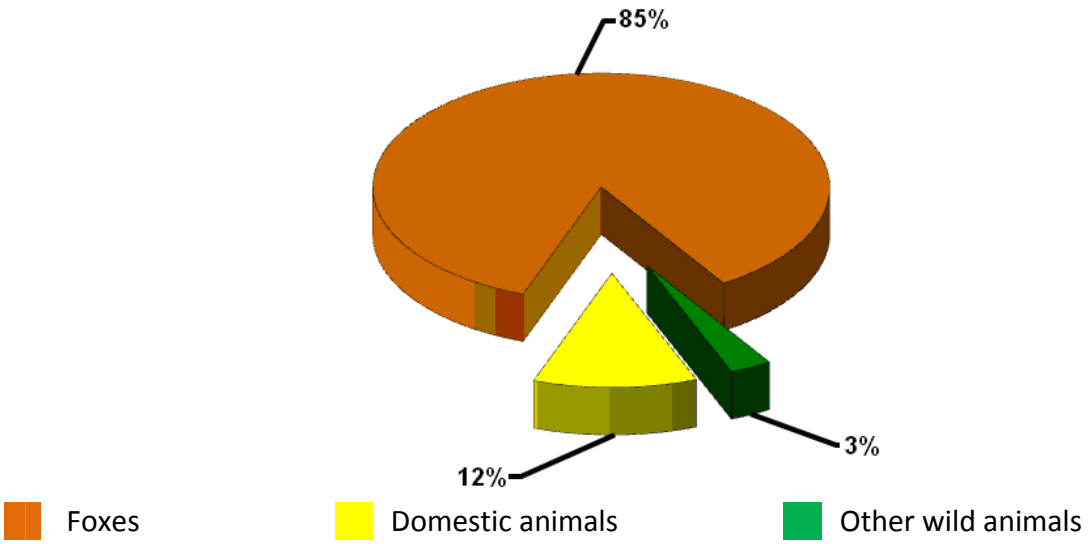


Chart 4. *Share of rabies cases during the period of time 2005 – 2008 in Serbia and Montenegro*

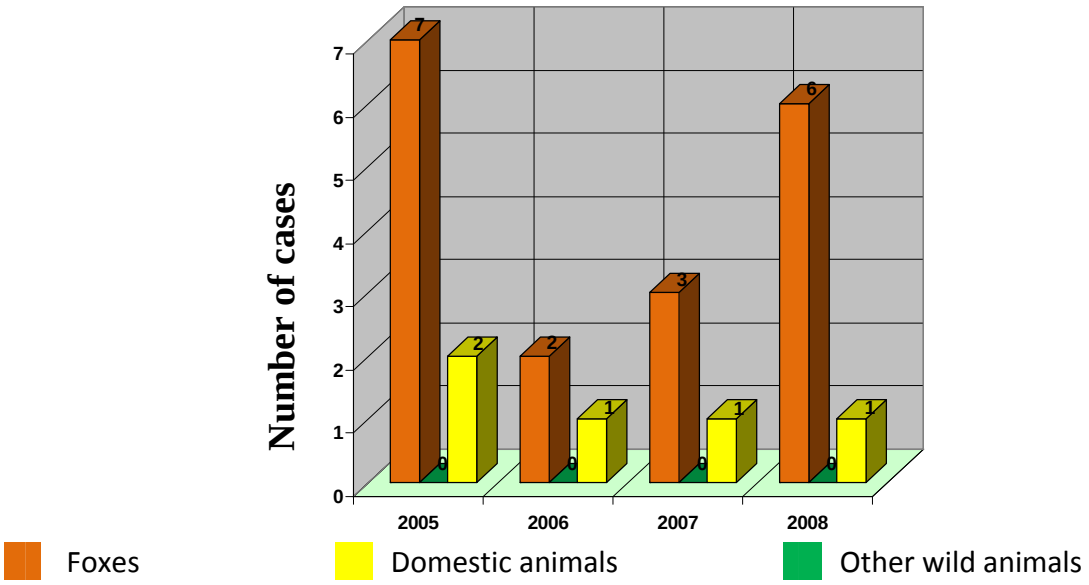
The temporal evolution of rabies cases and their share in the period 2005-2008 in Hungary are presented in the graph and chart 5.

In 2005, in Hungary the total number of rabies cases was relatively low, 9 cases of which 7 in fox (77.77%) and 2 in cats (22.23%).

In 2006 we notice a decrease by three times in the total number of rabies cases, compared to the previous year. Thus from the three cases of rabies, 2 have been present in fox (66.67%) and one in cattle (33.33%).

In 2007 there is a continuing low number of cases of rabies. So out of four cases, three were reported in foxes (75%) and one in cat (25%).

In 2008 there is a moderate increase in the number of cases of rabies. So out of seven cases, six were reported in foxes (85.71%) and one in dog (14.30%).



Graph 5. *Temporal evolution of rabies cases during the period of time 2005 – 2008 in Ungaria*

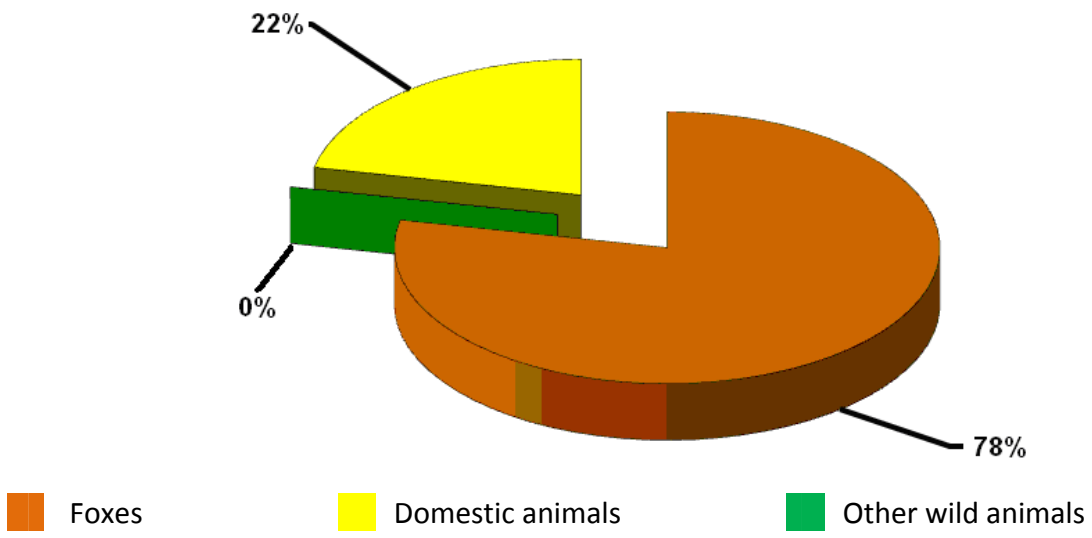


Chart 5. *Share of rabies cases during the period of time 2005 – 2008 in Ungaria*

CONCLUSIONS

1. Amongst neighboring countries of Romania, Bulgaria and Hungary (both EU member countries), had a low number of rabies cases during the period of time 2005-2008, due to the implementation of programs to eradicate rabies by oral vaccination of wild animals especially the foxes which is the main wild reservoir of rabies.

2. The high percentage of registered cases of rabies in foxes in all neighboring countries of Romania, confirms that it is the main wild animal species affected by rabies and is the natural wild reservoir.

3. In Ukraine, there were found four cases of illness, both in humans and in bats.

4. 2007 stands as the year with most cases of rabies diagnosed in Bulgaria, Moldova and Ukraine.

5. Ukraine is the country with most cases of rabies reported in the period analyzed, 9229, but also the country with most cases of rabies diagnosed in domestic animals

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RESEARCH ON RABIES EPIDEMIOLOGY OF ANIMALS IN MUNTENIA, DURING 2005 - 2009

Iulia Adelina TURIAC [1], C. VASIU [2], F. MAZDRAG [1]
[1] National Sanitary Veterinary and Food Safety Authority
[2]Faculty of Veterinary Medicine, Cluj-Napoca

The evolution of rabies in the 15 counties of the region Muntenia (Arges, Braila, Buzau, Calarasi, Dambovita, Dolj, Giurgiu, Gorj, Ialomita, Ilfov, Mehedinti, Olt, Prahova Teleorman and Valcea) was examined during the time between 2005 and 2009.
For this period of time, there were recorded in total 905 cases of rabies, of which 664 (73.37%) in wild animals and 241 (26.62%) in domestic animals.
Most cases were diagnosed in 2008, when it was recorded 259 cases (28.61% of total number of cases in the period of time mentioned), of which 218 (84.16%) in wild animals and 41 cases (15.83%) in domestic animals, and fewest cases in 2006 when there were 123 cases (13.59%) of which 95 (77.23%) in wild animald and 28 (22.76%) in the domestic animals species.
During the period of time analyzed, there were no recorded cases of rabies in Valcea county, in 2006, Mehedinti in 2007 and Giurgiu in 2008.
The distribution of rabies cases between counties analyzed, differ from year to year and during the same year, it is noted a growing number of cases in quarters I and IV, which correlated with the period of breeding in foxes.
In terms of distribution of rabies cases per animal species, from 2005 to 2009 in Muntenia, 70.83% of the total number of cases have been reported in foxes, 26.63% in domestic animals and 2.54% in other wildlife speciees, the high percentage of cases in foxes, pointing out that this species is the natural reservoir of the disease.

Key words: rabies, disease, species of animals, fox, Muntenia.

.Rabies is a viral disease that affects the central nervous system of warm-blooded animals, constituting a serious zoonosis. Once symptoms occur, the disease is always fatal in animals.

Fight against any disease, irrespective of changing population (human, animal or plant) needs to be effective, a good knowledge of its frequency and geographical distribution. Any decision involving the health of a population involves information of descriptive epidemiology. It can drain a longer or shorter time between the moment in which appears a given epidemiological situation and until it becomes known. Rapidly need to know this situation is directly related to the risk of spreading disease.

The information necessary to knowledge the epidemiological situation of a disease may be provided by descriptive investigation. By definition, any action for epidemiological surveillance, no matter what disease or any risk factor is studied, involves the collection of data on disease or risk factor, the transmission of data to processing center, processing and dissemination of results. Practical arrangements of each phase may be very different depending on disease from one country to another.

MATERIALS AND METHODS

In the epidemiologic study of disease were used retrospective investigations, analyzing data submitted through the epizootic situation, by the County Sanitary Veterinary and Food Safety Directorates to the National Sanitary Veterinary and Food Safety Authority, and after processing the data, the latter, sends it to Rabies Bulletin of Europe, an organization which focuses and process information received from the 40 cooperating countries.

Because the number of cases of rabies in wild animals other than foxes are small, these cases were grouped under "other wildlife" as that presented in graphic representations are three main categories namely: "Foxes", "Other Wild Animals "and" Domestic animals "

RESULTS AND DISCUSSION

Evolution of rabies cases in 2005

The evolution of rabies cases in 2005 is shown in Chart 1. In 2005, there were a total of 248 reported cases of rabies, of which 164 cases (66.13%) in wild animals and 84 (33.87%) in domestic animals. 155 of the 164 total number of wild animals cases, have evolved in fox, which represent a percentage of 94.51% of registered cases in wild animals and 62.5% of the total number of rabies cases that occurred in 2005.

The county with most cases reported in 2005 was Buzau, with a total of 115 cases, of which 68 (59.13%) in wild animals and 47 (40.87%) in domestics. Moreover, Buzau County is the only district where there have been cases of rabies and other wildlife then foxes, in 2005. Of the 68 cases of wild animals, 59 of cases (86.76%) occurred in foxes and other cases were diagnosed at the wolf, marten, and other wild carnivore - one case for each species listed and each three cases in wild boar and other wildlife.

Counties with fewest cases of rabies in 2005 were Giurgiu, with a case in wild and 3 in domestic animals, Ilfov with a case in wild animals and Teleorman with a case of domestic animals.

In 2005, in Muntenia the quarters with most cases of rabies have been I and IV when there were 89 and respectively 112 cases (Chart 1).

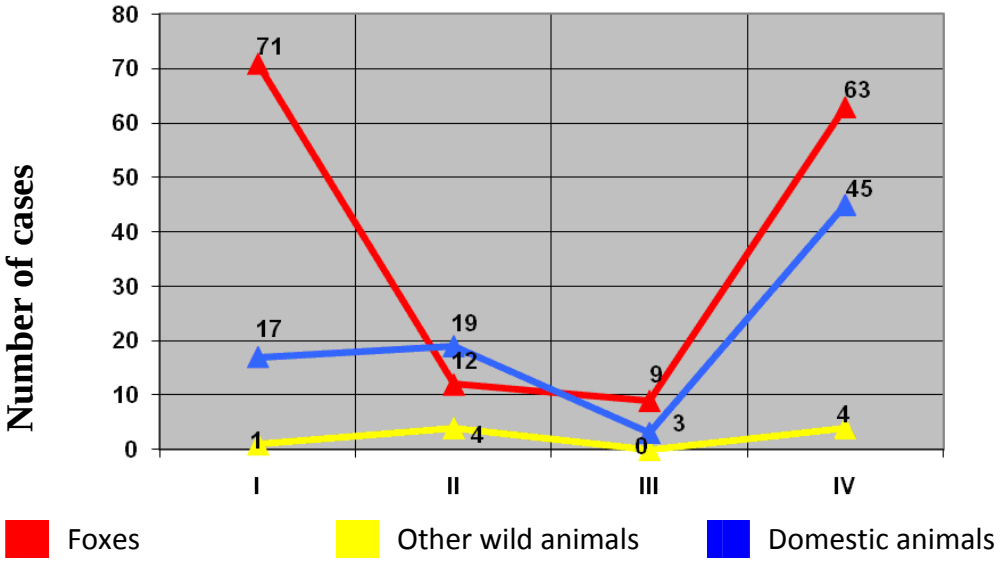


Chart 1. *Graphic representation of the evolution of rabies cases in 2005 in Muntenia.*

In terms of distribution of rabies cases per animal species in 2005 in Muntenia, 62.50% of the total numbers of cases have been reported in foxes, 33.87% in domestic animals and 3.63% in other wild animals (Figure 1).

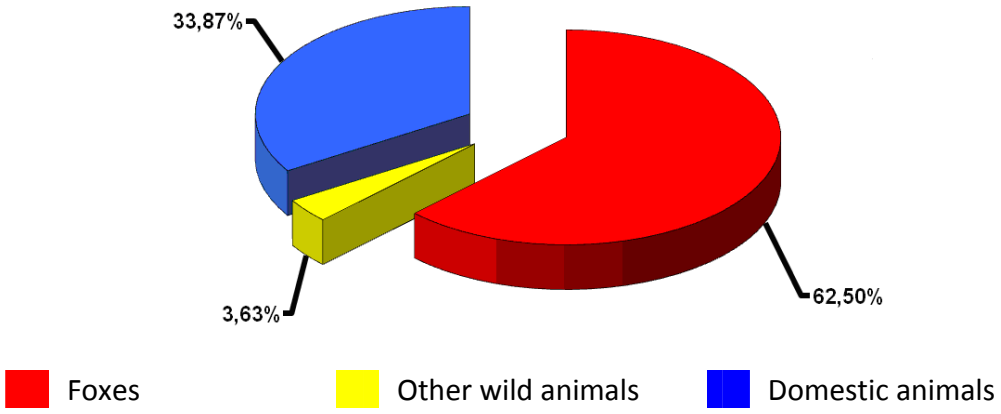


Figure 1. *Rabies cases share in Muntenia in 2005*

Evolution of rabies cases in 2006

In 2006, there were a total of 123 diagnosed cases of rabies in Muntenia, of which 95 cases (78.51%) were in wild animals and 28 (21.49%) in domestic animals.

This notes a number of 91 (95.79%) cases in foxes of all wild animals, one case per each, in badger and other mustelides and two cases in other wildlife.

The county with most cases in this year was Ialomita with a total of 38 cases, of which 34 (89.47%) in foxes, one case (2.63%) in other wildlife and three (7 89%) cases in domestic animals.

The second county in number of cases was Braila with a total of 14 cases, of which 13 (92.86%) cases in foxes and a case in domestic animals.

Calarasi county was the third county in number of cases in 2006, with a total of 11 cases; however, the particularity was the large number of cases in domestic animals, seven cases compared with four cases in wild animals namely foxes.

As a general observation is remarkable the decrease by half of the number of rabies cases diagnosed this year than last year, from 248 at 123.

The only county in which was not diagnosed any cases of rabies this year was Valcea county.

In terms of distribution of rabies cases per animal species in 2006 in Muntenia, 73.98% of the total numbers of cases have been reported in foxes, 22.76% in domestic animals and 3.26% from other wild animals. (Figure 2).

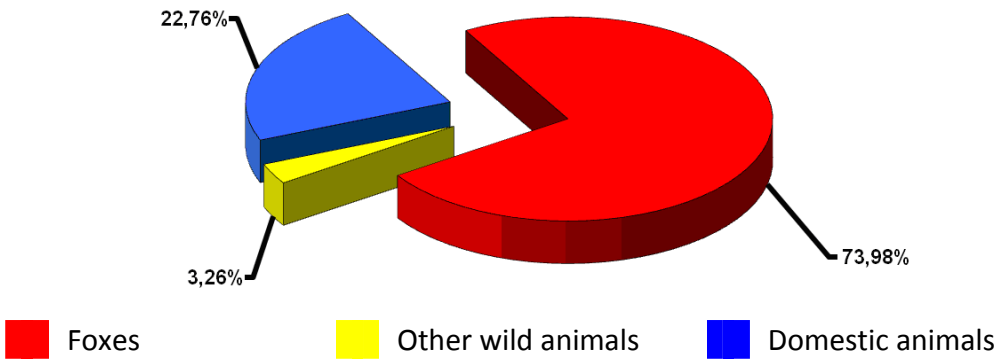


Figure 2. *Rabies cases share in Muntenia in 2006*

Evolution of rabies cases in 2007

Evolution of rabies cases in 2007 is shown in Chart 2.

In Muntenia in 2007 there were a total of 129 cases, of which 75 (58.14%) in wild animals and 54 (41.86%) cases in domestic animals. Thus, of the 75 cases in wild animals, 71 (94.67%) cases occurred in foxes, one case per each in marten and other mustelides and two cases in other carnivores.

The county that had the maniest cases in this year was Buzau where were 28 cases registered, of which 13 (46.43%) cases in wild animals and 15 (53.57%) in domestic animals. Cases in wild animals were distributed as follows: 12 cases in foxes and a case in other carnivores.

The second county in number of cases was Calarasi, with a total of 17 cases, of which 7 (41.18%) cases in domestic animals and 10 (58.82%) cases in the wild - 9 cases in foxes and one case in other carnivores.

Arges County, with a total of 12 cases recorded, was the third county in 2007. Of these cases, nine were wild animals, all at foxes and three in domestic animals.

This year in Ilfov County, has been a single case of rabies in domestic animals.

In 2007, in Muntenia, the quarters with the most cases of rabies were III and IV quarter, when there were a number of 37 respectively 67 cases of rabies (Chart 2).

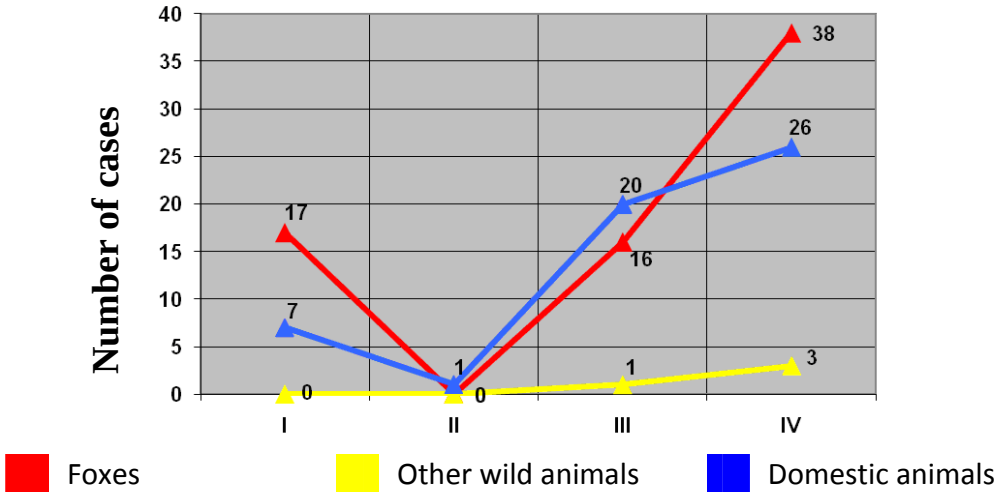


Chart 2. *Graphic representation of the evolution of rabies cases in 2007 in Muntenia*

In terms of distribution of rabies cases per animal species in 2007 in Muntenia, 55.04% of the total number of cases have been reported in foxes, 41.86% and 3.10% of domestic animals other wild animals (Figure 3).

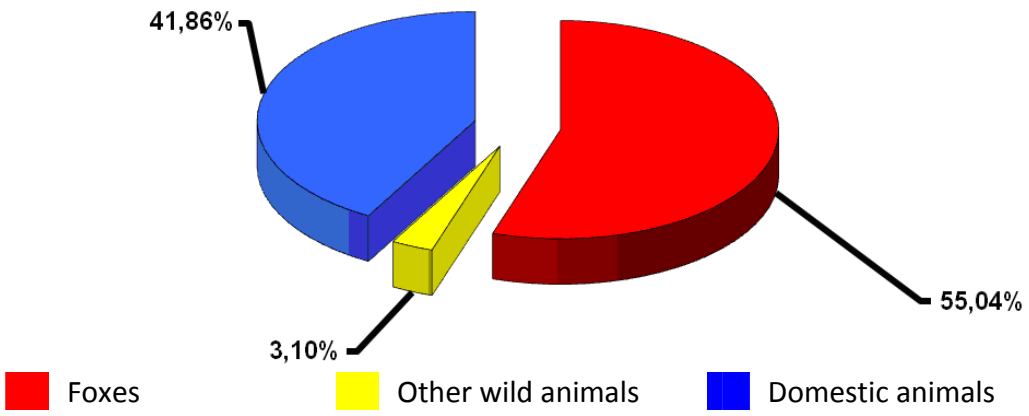


Figure 3. *Rabies cases share in Muntenia in 2007*

Evolution of rabies cases in 2008

In 2008, in Muntenia were a total of 259 reported cases of which 218 (84.17%) in wild animals and 41 (15.83%) cases in the domestic animals.

Of all cases in wild animals, 213 (97.71%) were in foxes, one case in marten and in other mustelides and three cases in other carnivores.

Most cases occurred this year have been in the Gorj county where from the total number of 65 cases, 64 occurred in wild animals namely foxes and a case in domestic animals.

The second county as the number of cases was Olt county, where 30 cases were registered, of which 23 in wild animals (76.67%), namely foxes and seven cases in domestic animals (23.33 %).

Dambovita County occupied the third place with 27 cases of which 21 in wild animals (77.78%), all in foxes and six cases (22.22%) in domestic animals.

In terms of distribution of rabies cases per animal species in 2008 in Muntenia, 82.24% of the total numbers of cases have been reported in foxes, 15.83% in domestic animals and 1.93% in other wild animals (Figure 4).

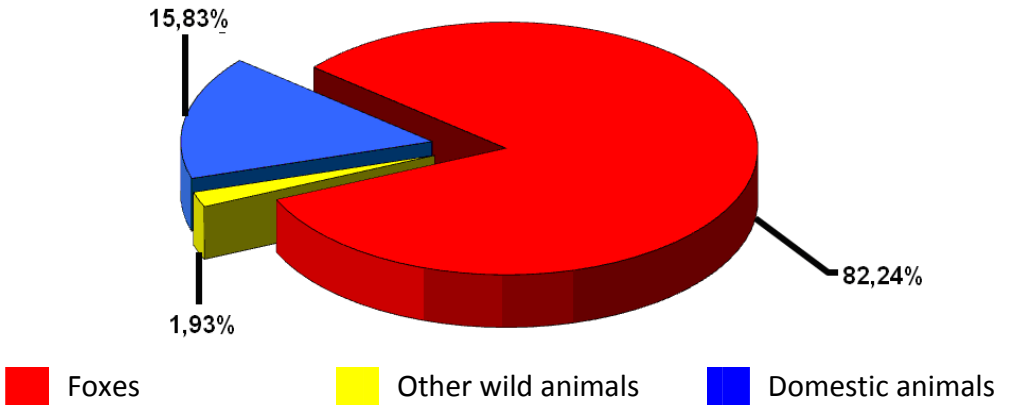


Figure 4. *Rabies cases share in Muntenia in 2008*

Evolution of rabies cases in 2009

In 2009, there is a decrease in the number of rabies cases compared with the previous year, registering a total of 146 cases, of which 112 (76.71%) in wild animals and 34 (23.29%) in the domestic animals.

Of the 112 cases recorded in wild animals, 111 were diagnosed in foxes and one single case in badger, in Valcea County.

The county with the most cases of rabies during the year 2009 in Muntenia was Buzau, with a total of 22 cases, of which 13 in wild animals and 9 cases in domestic animals.

The second county in number of cases was Olt, where there were a total of 20 cases of rabies. Particular for this county was the fact that it had the largest share of domestic animal cases, 12, compared with 8 cases that have been reported in wild animals namely foxes. It can be said that in Muntenia in 2009, most cases of rabies in domestic animals were diagnosed in this county.

The third district by the number of cases was Gorj, with a total of 18 cases, all in foxes.

County in which there were fewest cases of rabies was Braila with two cases diagnosed in foxes.

In terms of distribution of rabies cases in 2009, on animal species, 76.03% of these were foxes, 23.29% in the domestic animals and the remaining 0.68% in addition to other wildlife other than foxes. (Figure 5).

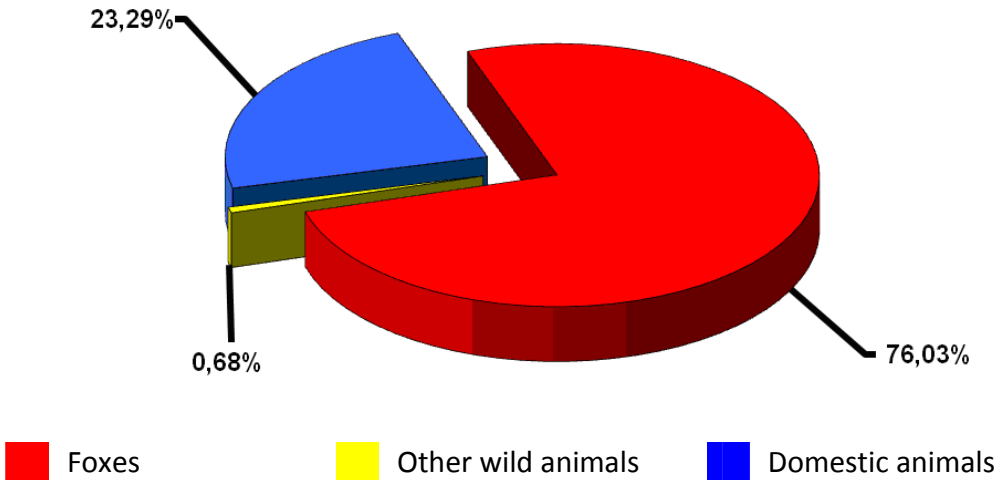


Figure 5. *Rabies cases share in Muntenia in 2009*

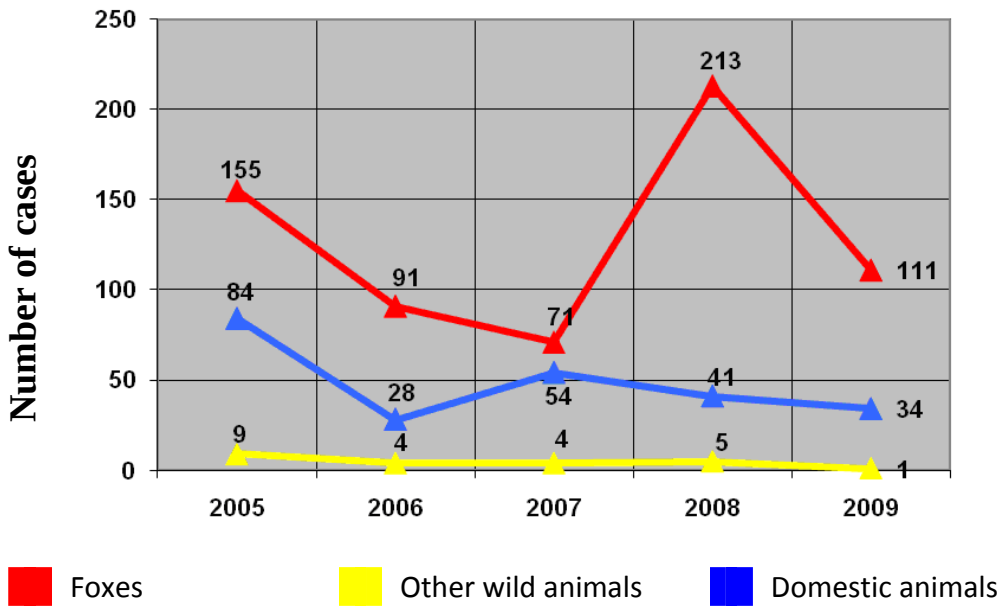
In Table 1 is detailed the distribution of cases of rabies, on affected animal species during the period analyzed, separately for each county and in Chart no. 3, the representation of the evolution of rabies cases in this period.

It is observed that all counties were affected by the disease, the highest number of illnesses being reported in wild animals, with a total of approximately three times higher than in the domestic and from the wild animals, the most affected species was the fox. Even if the number of rabies cases in foxes was highest in Gorj County, because of the high number of cases in domestic animals, Buzau county has the highest number of rabies cases diagnosed during the time of analyzed period.

Table 1.

Rabies cases distribution during the period 2005 – 2009 within the analyzed counties

Rabies cases distribution during the period 2005 - 2009											
County	Fox	Wolf	Badger	Marten	Other mustelides	Other carnivores	Wild boar	Other wild animals	Total of wild animals	Domestic animals	Total cases of rabies
Argeş	44	0	0	0	0	0	0	0	44	9	53
Brăila	26	0	0	0	0	1	0	0	27	14	41
Buzău	97	1	0	1	1	2	3	3	108	82	190
Călăraşi	59	0	0	0	0	1	0	0	60	19	79
Dâmboviţa	58	0	0	1	0	0	0	0	59	18	77
Dolj	21	0	0	0	0	0	0	0	21	8	29
Giurgiu	12	0	0	0	1	0	0	0	13	6	19
Gorj	105	0	0	0	0	0	0	0	105	11	116
Ialomiţa	70	0	0	0	0	2	0	1	73	16	89
Ilfov	11	0	0	0	0	0	0	0	11	3	14
Mehedinţi	15	0	0	0	0	0	0	0	15	1	16
Olt	44	0	0	0	0	0	0	0	44	26	70
Prahova	28	0	1	1	1	0	0	1	32	13	45
Teleorman	28	0	0	0	0	0	0	0	28	6	34
Vâlcea	23	0	1	0	0	0	0	0	24	9	33
TOTAL cases	641	1	2	3	3	6	3	5	664	241	905



Grafic 3. *Graphical representation of the evolution of rabies cases during 2005 – 2009 in Muntenia*

CONCLUSIONS

1. In 2005 has been recorded a total of 248 cases of rabies, followed a downward trend in their numbers over the next two years, then in 2008 they increased again, reaching 259 cases and in 2009, their number decreases again, reaching 146.

2. Most cases were reported in Buzau County where 190 cases were registered of which 108 in wild animals and 82 in domestic ones.

3. In Buzau county were recorded also the most cases of rabies in domestic animals in 2009.

4. Gorj County ranks second in number of registered cases where 116 cases were diagnosed of which 105 in wild animals and 11 in domestic animals.

5. During 2005 - 2009, in Muntenia 70.83% of the total number of cases have been reported in foxes, 26.63% in domestic animals and 2.54% in other wildlife.

6. The fox is the natural reservoir of wild rabies in view of the high share of cases in this species.

7. The highest number of rabies cases was reported during the quarters I and IV, which coincides with the breeding period of foxes.

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