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CONTENTS

The effect of antihistamines on allergic inflammation and anxiety in dogs and cats

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Abstract

At first, the terms "allergy" and "hypersensitivity" were equivalent, but with the progress of knowledge in the field of immunology, the hypersensitivity reactions were classified into two categories: immunological and non-immunological hypersensitivity. Today, the term "allergy" is used to describe allergic-type hypersensitivity reactions. ***Anxiety*** *is a state of mental discomfort, a disorder of affectivity, manifested by anxiety, unmotivated fear, anxiety. These changes of psychological nature lead to behavioral alterations which increase over time and often result in the social exclusion of the affected person. This study looked at the efficacy of* ***antihistamine*** *medication in the pathology of dog and cat allergy reactions, as well as in the increasingly frequent anxious manifestations of these species. In order to identify and diagnose allergic reactions to dogs and cats, 5 cats and 11 dogs were examined which showed a specific symptom such as: pruritus of different intensities, depilation, skin erythema or edema. For the examination of the animals, working methods were used in the following sequence: anamnesis, clinical examination, dermatological examination. Wood examination for skin lesions was performed in a dark room after the lamp had been heated beforehand. The otoscopic examination, in case of impaired auditory conduction, was performed with the otoscope that was connected to a computer allowing a better visualization of the images. Of the total of the animals examined, 37.5% were diagnosed with flea allergy, 31.25% with food allergy, 12.5% with an allergic reaction from other insects, and those diagnosed with tickle allergy, atopic dermatitis and dermatitis Contact represents 6.25% of the total. In cases of intense pruritus-induced allergies, diphenhydramine was used as antihistamine therapy precisely for its anti-anxiety, sedative effect in order to prevent auto-implantation in susceptible animals.*

Key words: *antihistamines, anxiety, allergic inflammation*

Introduction

Since the antiquity, aspects of allergic diseases have been noted and mentioned. In the 4th century BC, Hippocrates mentions that certain foods, although healthy and nourishing for most people, may have unwanted effects on a small group of people.

In the Middle Ages, at the middle of the 14th century, attention was drawn to reactions to external agents, with some cases of rose fever known today as hay fever [1].

The term allergy was introduced in medicine by Viennese pediatrician Clemens von Pirquet in 1906. Initially, the terms of allergy and "hypersensitivity" were equivalent, but with the progress of knowledge in the field of immunology, hypersensitivity reactions were classified into two categories: hypersensitivity Immunological and non-immunological. Today, the term "allergy" is used to describe allergic type hypersensitivity reactions [2].

Anxiety is a state of mental discomfort, a disorder of affectivity, manifested by anxiety, unmotivated fear, anxiety. These psychological changes lead to behavioral alterations that increase over time and often result in the social exclusion of the affected person [4].

Human medicine was much earlier in determining the disease than in veterinary medicine, but in today's society where people share their homes, food, and often devote much of their lives to ill-favored companions, manifestations of animal anxiety are accused More and more often by the owners.

This study looked at the efficacy of antihistamine medication in the pathology of dog and cat allergic reactions, as well as in the increasingly frequent anxious manifestations of these species [5].

Growth of pets, especially dogs and cats, has become more recent in the past and problems with allergies to different factors, separation stress or anxious states caused by fireworks or household appliances have become problems many owners face [6].

Materials and methods

In order to identify and diagnose allergic reactions to dogs and cats, 5 cats and 11 dogs were examined which showed a specific symptom such as: pruritus of different intensities, depilation, skin erythema or edema. For the examination of the animals, working methods were used in the following sequence: anamnesis, clinical examination, dermatological examination. Wood examination for skin lesions was performed in a dark room after the lamp had been heated beforehand.

The otoscopic examination, in the case of impaired auditory conduction, was performed with an otoscope that was connected to a computer which allows a better visualization of the images [7].

Also, sampling was performed for the microscopic examination in situations where a pathology other than the allergic reaction was suspected. In this regard, bacteriological examinations were conducted to identify the etiology and sensitivity of microorganisms isolated from cutaneous lesions, whether they were transient or residual, as well as coproparasitological examinations [2,7].

Confirmation of the diagnosis of allergic reaction was accomplished by eliminating the other suspected diseases based on anamnesis and clinical examination.

In order to alleviate allergic symptoms, four types of antihistamines, H1 inhibitors, were used in both the first and the second generation.

Compared with human medicine, where H1 antihistamines are the second generation of choice, because of the lack of sedative effect, H1 antihistamines in the first generation are preferred because they penetrate the blood-brain barrier and thus have a sedative, anti-anxiety effect. Their use in human medicine is avoided as far as possible because the sedative, anti-anxiety effect affects people's daily activities and this is not desirable. With regard to pet animals, simple pathological impairment, regardless of its nature, triggers a state of anxiety, which can be controlled by the administration of first generation H1 antihistamines. To observe their effect on anxious animals, were selected for two cases of allergy that the owners claimed to be very fearful and possibly aggressive under stress conditions, and we monitored them behaviorally during treatment[1].

Results and discussions

The diagnosis and antihistamine medication used in the 16 animals examined are shown in Table 1.

Table 1. Diagnostic and antihistamine used in case studies

Case number	Species / Breed	Age / Sex	Diagnostic	Antihistamine used
1	Feline European	7 years / F	Allergy to flea bites	Promethazine
2	Persian cat	8 years / M	Allergy caused by the tick bite	Promethazine
3	Feline European	7 years / M	Allergy caused by the sting of an insect	Promethazine
4	Feline European	10 years / M	Food allergy	Promethazine
5	Feline European	4 years / F	Allergy to flea bites	Promethazine
6	Canide metis	4 years / F	Dermatitis of contact	Diphenhydramine

7	Canide metis	12 years / F	Allergy to flea bites	Diphenhydramine
8	Canide Dogo Argentino	3 years / F	Food allergy + allergic conjunctivitis	Diphenhydramine + Ketotifen
9	Miniature Schnauzer	7 years / M	Allergy to flea bites	Levocetirizine
10	German Shorthaired Pointer	15 years / F	Allergy to flea bites	Diphenhydramine
11	Canide metis	3 years / M	Allergy to flea bites	Diphenhydramine
12	Amstaff	8 years / F	Allergy caused by the sting of an insect	Loratadine
13	Westie	4 years / M	Atopic dermatitis	Promethazine
14	French Bulldog	4 years / M	Food allergy	Promethazine
15	Canide metis	4 years / F	Food allergy	Promethazine
16	Canide metis	1 year / F	Food allergy	Promethazine

Of the total of 16 animals examined, 37.5% were diagnosed with flea allergy, 31.25 with food allergy, 12.5% had an allergic reaction from other insects and those diagnosed with tick allergy, atopic dermatitis and dermatitis contact represents 6.25% of the total (Figure 1).

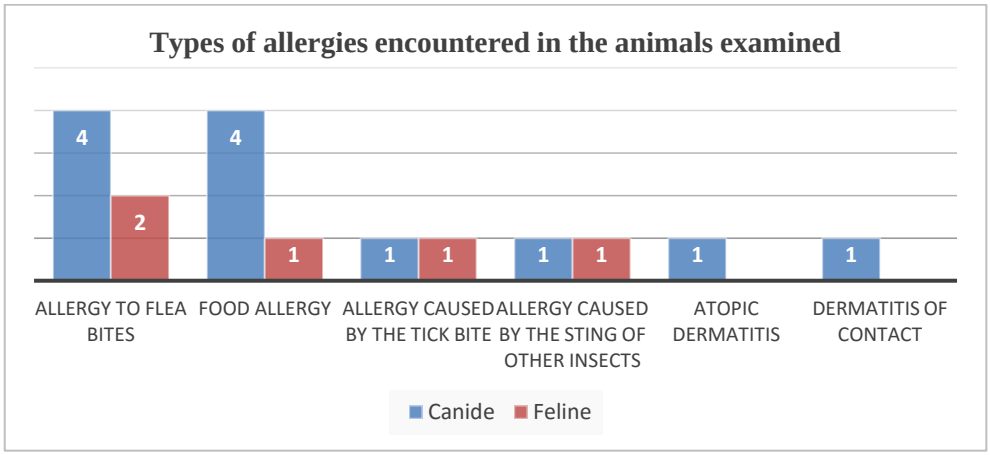

Fig. 1. Types of allergies encountered in the animals examined

In order to alleviate allergic symptoms in the 16 animals studied, four types of antihistamine H1 inhibitors were used in both the first and the second generation (Figure 2).

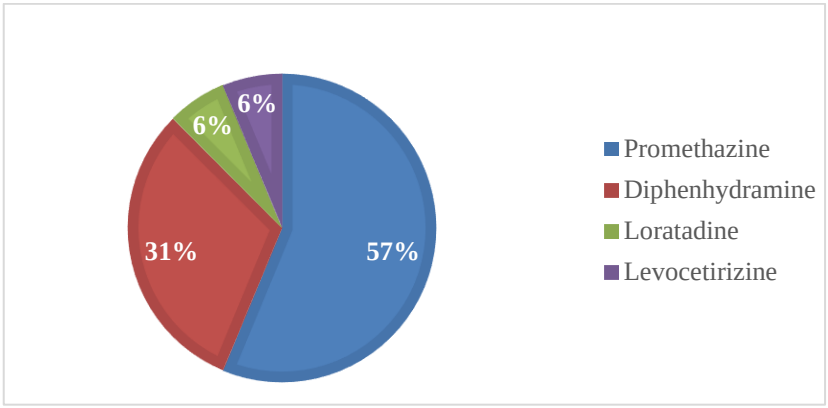

Fig. 2. Antihistamines used to treat the 16 cases studied

The treatments for the 5 cats diagnosed with allergy with different etiology consisted of: promethazine antihistamine, deep intramuscularly at 24 hours between 3 and 5 days, depending on the intensity of the symptoms; In 3 cases, prednisolone therapy for the immunosuppressive effect was established, 3 patients were treated with iodine for wound healing, and in one case also with amoxicillin and clavulanic acid antibiotics to combat overdose infections. At the same time, it was necessary to use a spot-on topical product with ceramide, fatty acids and cholesterol in 3 patients to restore skin integrity, hypoallergenic diets with dry food in one case and anti-parasitic collar in 2 cats (Table 2).

Table 2. The medication used in cases of allergies in cats

Medicines used	Case 1	Case 2	Case 3	Case 4	Case 5
Diphenhydramine	*	*	*	*	*
Prednisolone			*	*	*
Iodine povidone	*	*			*
Top spot on solution	*			*	*
External deparazitation	*				*
Antibiotic			*		
Diet				*	

In dogs, the following antihistamines were used: diphenhydramine in 5 cases, promethazine in 4 cases, loratadine 1 case, levocetirizine 1 case. In 9 cases, a glucocorticoid was also administered, chlorhexidine baths were recommended in 4 cases, external deparasite was required in 5 cases using spot-on fipronil or anti-parasite collision with imidacloprid and flumetrin. The monoproteic diet was established in 3 cases. Two patients were given a topical product with ceramide, fatty acids and cholesterol to restore skin integrity, and the same animals were given caffeine to combat overdose infections (Table 3).

As a result of the researches carried out, it was found that although in pet animals anxiety is a problem that owners are increasingly confronted with, they do not consider the manifestations of this kind to be of a pathological nature and for this reason they do not reach be examined by the veterinarian. However, in the case of the two cases monitored for anxiety, there was a marked improvement in the symptomatology following the treatments performed [3].

Table 3. Medication used in cases of allergy in dogs

Medication	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16
Antihistamine	D	D	D K	Le	D	D	Lo	P	P	P	P
Prednisolone	*	*	*		*	*		*	*	*	*
Chlorhexidine	*							*	*	*	
External deparazitation		*			*	*		*			*
Diet								*	*	*	
Top spot on solution										*	*
Antibiotics										*	*

Legend: D = Diphenhydramine; P = Prometazine; Le = Levocetirizine;
Lo = Loratadine; K = Ketotifen

From discussions with several animal owners it has been found that many of the pets have symptoms of anxiety caused by loud noises, electrostatic appliances or extreme meteorological phenomena, but never thought they should be treated.

Conclusions

1. Of the total of 16 animals examined, 37.5% were diagnosed with flea allergy, 31.25% with food allergy, 12.5% had allergic reaction from other insects, and those diagnosed with tick allergy, dermatitis Atopic and dermatitis contact represents 6.25% of the total.
2. Of the 4 cases of allergy caused by pharyngeal scabies in the dog, 3 were treated with Difenhydramine in combination with Prednisolone and topical medication and one case was treated with Levocetirizine as a single therapy.
3. All 3 cases of dog food allergy were treated with Prometazine in combination with Prednisolone and topical medication.
4. In only two cases, the use of antihistamine medication as a single therapy, both cases occurring in dogs.
5. In 57% of cases Promethazine was used as antihistamine, followed by Difenhydramine in 31% of cases. Loratadine and Levocetirizine were each used in 6% of cases.
6. In cats, 87.5% of cases used H1 antihistamines in 1st generation and only 12.5% of the animals tested were treated with H1 antihistamines in the second generation.
7. In cases of severe pruritus-dominated allergies, diphenhydramine was used as antihistamine therapy precisely for its anti-anxiety, sedative effect, in order to prevent auto-implantation in susceptible animals.
8. In two cases of dog allergy suspected of having anxiety, improvement was seen during treatment with first-line H1 antihistamines.

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Clinical and therapeutic interferences between diabetes and cataract

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Abstract

One of the most common non-communicable chronic diseases and the most common endocrine disease in both humans and animals, diabetes is characterized by disorders of the entire metabolism, especially carbohydrate metabolism and by complications affecting the eyes, kidneys, nerves and vessels blood. In many studies, dogs have been favored due to breeds of breed, genetic molding, and it is found to them that the living environment is important in acquiring diabetes over time, the disorders being heterogeneous by race and characterized phenotypically . The aim of the present paper was to identify the correct and early diagnosis of diabetic animals, to establish the mechanisms by which the secondary cataract of diabetes occurs, to establish a correct treatment, so that the diagnosis errors in the future will be as rare as possible. For this, dogs with diabetes were monitored even over a year, blood glucose levels were as close as possible to the normal range, using appropriate treatment for each patient, using appropriate diets by encouraging a proper exercise regimen , But not least by properly informing the owners of diabetic animals. The primary objective was to detect diabetes and secondary cataracts as early as possible by determining blood glucose, ketone bodies, ocular ultrasound, and electroretinography, followed by appropriate treatment for each patient with Mixtard-30 and / or Lantus insulin. Of the 25 significant cases studied, 14 were treated exclusively with insulin, ie insulin dependent diabetes mellitus, sometimes supplemented with oral hypoglycemia, 7 patients were treated exclusively with oral hypoglycemia and 4 cases were treated with insulin as well as with Oral hypoglycemia. Cataracts present in 56% of subjects were treated with good results using Quinax eye drops. Good results were achieved by observing a constant caloric intake at each meal, feeding half of the total caloric intake at each insulin injection, maintaining the same meal hours, avoiding dietary changes, and following a daily, daily exercise effort.

Key words*: cataract, diabetes, therapy*

Introduction

Diabetes mellitus is one of the most widespread chronic non-communicable diseases and the most common endocrine disease in both humans and animals. It is characterized by disorders of the entire metabolism, especially glucose metabolism and by complications affecting the eyes, kidneys, nerves and blood vessels. Essentially, diabetes is a disease in which the body does not produce enough insulin or does not use it effectively [3].

Over time, there have been many studies on the development of spontaneous diabetes in animals such as pigs, sheep, horses, cats and monkeys, with frequent cases, and unique cases have occurred in foxes, dolphins and hippopotamuses. In these studies, dogs were favored by race breeds, genetic molding, and found to be the fact that the living environment is important in acquiring diabetes over time, the disturbances being heterogeneous according to race and characterized by phenotypic [4].

In history, the possibility of developing spontaneous canine diabetes can be explained by the fact that they have lived and worked with people since prehistoric times, their social instinct and competence difference being good companions. With the wolf's domestication, the genetic profile has undergone numerous changes, thus defending a series of genetic irregularities, due to the environment in which he lived with man and food, and in time has developed this disease. It is essential to know the importance of this disease, precisely because of the need to control it: it has an epidemiological impact (The consequence of the increased frequency of diabetes among dogs),

biological impact (the risk associated with diabetes - cardiovascular risk) and an economic impact (due to the complications produced, it becomes costly for the owners and the animal owner) [4].

Progress has led to a prolongation of life by up to 7 years, which has allowed the study of the complications of this disease. Among these, diabetic cataract occurs with an increased frequency. The onset of diabetic cataract may be sudden, the decrease in visual acuity may occur within a few days or weeks, causing loss of vision [5].

The aim of our work was to identify the right and early diagnosis of diabetic animals, to establish the mechanisms by which secondary cataract occurs in diabetes, to establish a correct treatment, so that future diagnostic errors are as rare as possible.

To this end, dogs with diabetes were monitored even over a year, and blood glucose stabilization was attempted as close to normal as possible, using a patient-specific treatment with a proper diet, by encouraging a movement regimen Appropriate, but not least, by properly informing the owners of diabetic animals.

Materials and methods

The pursuit of the established objectives was aimed primarily at detecting the disease as early as possible and establishing the treatment adapted to each patient. Preciseness of detection is important because this also depends on the risk of possible complications, so that the more quickly the disease is diagnosed, the further complications will be easier to keep under control [3].

The diagnosis is mainly based on blood glucose determination. Normal blood glucose is 80-120 mg/dl in the dog and this is correctly determined when "fasting"; if the animal ate before blood collection, then values up to 140 mg/dl are acceptable.

Regarding the most common complication of diabetes, cataract, it will be controlled by ophthalmic solutions, aimed at preventing cataract evolution, to the mature cataract stage by regulating the lens's metabolism. If, however, the mature cataract stage is reached, surgical treatment by extracapsular extraction of the lens by focoemulsification will be used[5].

To determine blood glucose, classical methods can be used: colorimetric method with ortho-toluidine, enzymatic methods with hexokinase and glucose dehydrogenase. Harvesting targets venous blood, total capillary blood or venous plasma. Whole blood is taken over the fluoride and should be immediately centrifuged because it may undergo glucose depletion by up to 10%.

It is also possible to use reagent strips and "in situ" measuring devices called "glucose meters, hemoglucometry or hemoglucose" (Figure 1) [6].

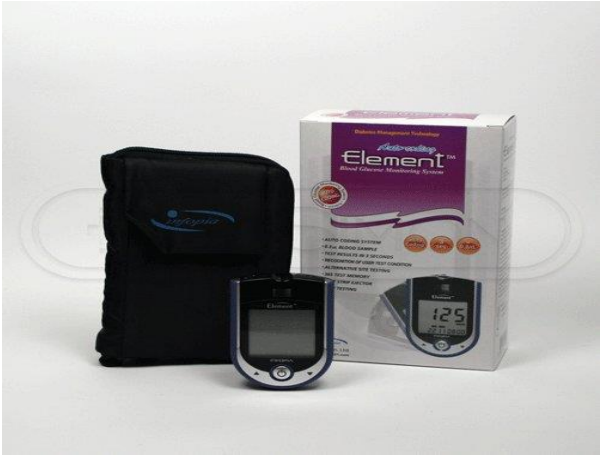

Figure 1 Glucose meter for quick determination of blood glucose

Another important aspect is the determination of ketone bodies which can be accomplished by using sodium nitroprusside impregnated strips (Labstix). Also, glucose, which occurs frequently in diabetes cases, can be determined for this purpose using qualitative (Fehling) or quantitative techniques (Ionescu Matiu method). There are also enzymatic techniques for determining glucose (Diastix, Ketostix) as can be seen in Figures 2 and 3 [2].

Figure 2 Ketostix

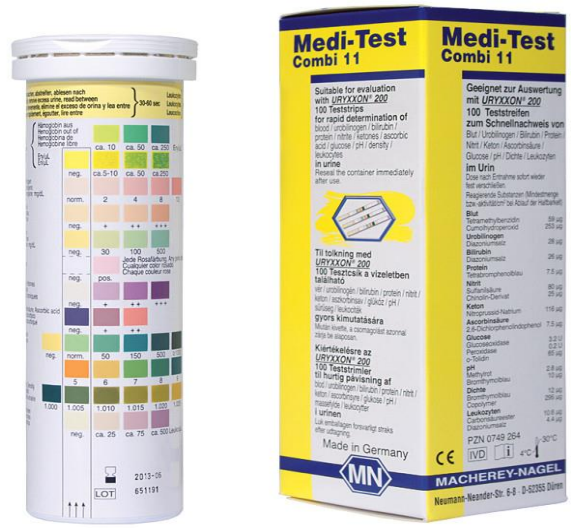

Figure 3 Combi Test

Regarding diabetic cataract, it will be diagnosed based on clinical signs, based on ocular ultrasound and electroretinography (Figure 4).

Mixtard-30 insulin, a 30% fast, and 70% retard type insulin, and insulin Lantus, a 24-hour glargine insulin human insulin, were used for therapy [3].

Oral therapy targeted the use of biguanides, the most commonly used Meguan which is well tolerated in the dog, and among the hypoglycemic sulphamides, Maninil. To prevent cataract complication, Quinax collagen containing sodium dihydroazapentacene polysulfonate sodium was used [4].

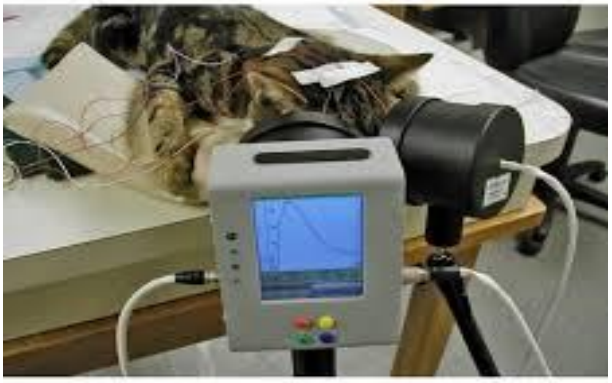

Figure 4 Electroretinograph

Results and discussions

All the animals under study followed the same protocol: anamnesis, direct clinical examination, blood biochemical analysis, urine summary, and other complementary methods to determine the degree of patient involvement and to detect diseases that may complicate diabetes.

Frequently, insulin-dependent diabetes mellitus occurs in dogs, thus expressing a complete destruction of betapancreatic cells, exogenous insulin requirements being essential for glycemic control, acidosis prevention and patient survival.

A higher prevalence of dibet in non-sterilized dogs was observed 61% versus 39% in sterilized dogs (Figure 5). Non-sterilized females have been investigated ultrasound for a possible pyrometer. Those with the negative result, as well as the other polyurized polydipsia animals, were subjected to biochemical blood tests and urine summary.

Apart from the fact that diabetes is a chronic disease and can cause serious complications, it is a costly disease because the treatment extends throughout life.

During 18 months, 86 patients diagnosed with diabetes were selected from which 25 cases were selected as the most significant. Of the 25 patients (Table 1), more than half (14 and 56%, respectively) presented as a complication of diabetes, unilateral or bilateral cataract.

Table 1 Cases of diabetes investigated in dogs

No. of cases	Females	Males
25	16	9
%	64%	36%

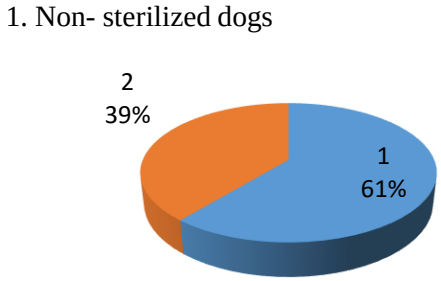

Figure 5 The ratio of diabetes cases between sterilized and non-sterilized dogs

It has been found that diabetes occurs more frequently in obese animals and that there is a predisposition by race, especially in dogs of Samoyed, Schnauzer dwarf, Caniche, or Boxer. There were also patients who had diabetes at 8 months, but the peak was found to be 8 years of age.

Polyuria - polydipsia was the predominant clinical sign that was present in most cases [1].

Table 2 Results obtained in the treatment of diabetes and cataract in the dog

No of case	Breed	Age/ Sex	Glucose before treatment	Complications	Glucose after treatment	Treatment	Obtained results
1	Cocker	11 y, M	197 mg/dl	Unilateral cataract	139 mg/dl	Diet food, Quinax drops	Improved
2	Bichon	8 y., F	310 mg/dl	Unilateral cataract	330-390 mg/dl	Maninil, then Mixtard insulin, diet food, Quinax	Stationary, Stabilized
3	Pekingese	3 y., M	430 mg/dl	Bilateral cataract	-	Diabetic coma	Death
4	Cherry	16 y., F	220 mg/dl	Bilateral cataract	< 200 mg/dl	Meguan, diet food, antidiabetic tea, Quinax	Improved
5	Caniche	15 y., M	240 mg/dl	Unilateral cataract, Cushing disease suspicion	-	Diet food Meguan, Quinax	Death
6	Half breed	5 y., F	410 mg/dl	Bilateral cataract	303 mg/dl	Mixtard insulin, Fitodiab, diet food, Quinax	Death
7	Samoyed	7 y., F	275 mg/dl	Unilateral cataract	140 mg/dl	Mixtard insulin, Quinax	Improved
8	Half breed	9 y., F	380 mg/dl	-	160-220 mg/dl	Mixtard insulin, blueberry capsules	Improved
9	Beagle	15 y., F	286 mg/dl	-	140 mg/dl	Dietă alimentară, Maninil, Silymarin	Improved
10	Border Collie	10 y., F	404 mg/dl	-	370 mg/dl	Mixtard insulin, Fitodiab, diet food	Stabilized
11	Dwarf Schnauzer	10 y., M	415 mg/dl	-	250 mg/dl	Lantus insulin, diet food	Improved
12	Caniche	10 y., F	284 mg/dl	Unilateral cataract	140 mg/dl	Mixtard insulin, diet food, antidiabetic tea, Quinax	Stabilized
13	Bichon	6 y., M	319 mg/dl	-	160 mg/dl	Fitodiab, blueberry capsules, , antidiabetic tea, diet food	Stabilized
14	Beagle	9 y., M	370 mg/dl	Bilateral cataract	140 mg/dl	Maninil, Silymarin, , antidiabetic tea, diet food, Quinax	Stabilized
15	Bichon	8 y., F	461 mg/dl	Bilateral cataract	160 mg/dl	Mixtard insulin, Fitodiab, diet food, Quinax	Stabilized
16	Caniche	8 y., F	350 mg/dl	-	240 mg/dl	Mixtard insulin, Fitodiab	Stabilized
17	Half breed	8 m., M	200 mg/dl	-	200-400 mg/dl	Mixtard insulin, diet food	Supposed genetic predisposition
18	Half breed	7 y., M	340 mg/dl	Bilateral cataract	160-200 mg/dl	Maninil, Mixtard insulin, Fitodiab, diet food, Quinax	Stabilized
19	Pekingese	5 y., F	402 mg/dl	-	140 mg/dl	Meguan, Fitodiab, diet food, antidiabetic tea	Stabilized
20	Caniche	10 y., F	410 mg/dl	-	150 mg/dl	Mixtard insulin, Fitodiab, diet food	Stabilized

21	German Shepherd	8 y., M	460 mg/dl	Bilateral cataract	180 mg/dl	Mixtard insulin, Fitodiab, diet food, Quinax	Stabilized
22	Bichon	10 y., F	210 mg/dl	-	130 mg/dl	Meguan, diet food	Stabilized
23	Caniche	8 y., F	380 mg/dl	-	170 mg/dl	Mixtard insulin, blueberry capsules	Stabilized
24	Dwarf Schnauzer	7 y., F	180 mg/dl	Unilateral cataract	140 mg/dl	Fitodiab, blueberry capsules, diet food, Quinax	Stabilized
25	Boxer	10 y., F	240 mg/dl	Bilateral cataract	122 mg/dl	Mixtard insulin, diet food, Quinax	Stabilized

Out of the 25 cases presented in Table 2, 14 were treated exclusively with insulin, involving insulin-dependent diabetes mellitus, sometimes supplemented with oral hypoglycemia, 7 patients were treated exclusively with oral hypoglycemia and 4 cases were treated with insulin and with oral hypoglycemia.

The treatment was completed in all cases with appropriate diets.

The objectives of the treatment are to balance the long-term blood sugar, to adopt a healthy lifestyle, to prevent complications. In most cases Mixtard-30 insulin was used for human use and the recommended dose was 0.5-1 I.U. /kg/day [4].

In diabetic dogs, persistence of clinical signs is the most common "complication" of insulin therapy. This persistence suggests that insulin is not effective, due to several reasons: dieting, not correctly choosing the type of insulin, incorrect insulin administration, and body insulin response. This response may be influenced by certain inflammatory, infectious or hormonal disorders.

The treatment was based on considerations such as: patients who had blood sugar levels up to 180 mg / dL at the time of presentation to the cabinet were treated with a dietary supplement if blood glucose reached 240 mg/dl, they added oral dietary and hypoglycemia (blueberry capsules, Maninil, Meguan, Fitodiab), and if the values exceeded 250 mg/dl or if the other glucose stabilization methods did not have the expected effect, insulin treatment was instituted.

Regarding cataract, in most cases it occurs suddenly, practically from one day to the next, the owner observes the opacity of the lens only when the animal strikes the surrounding objects. In most cases, when the cataract is detected in the incipient form, excellent results have been obtained by using Quinax ophthalmic drops to stop the development of the condition.

Commonly, insulin-dependent diabetes mellitus occurs in dogs, thus expressing complete destruction of betapancreatic cells, exogenous insulin requirements being essential for glycemic control, acidosis prevention, and patient survival.

There have been several cases where diabetes has been discovered in a routine exam, as well as some cases where the animals were already in a hypoglycemic coma.

Non-sterilized females have been investigated ultrasound for a possible pyometer. Those with the negative result, as well as the other animals with polyuria, have been subjected to biochemical blood tests, urine summary.

There have been cases in which patients developed insulin resistance when blood glucose levels remained at 250-300 mg/dl.

In diabetic cataract, it is preferable that the treatment be drug up to stabilize blood sugar when it can be surgically approached.

In addition to medication, lifestyle is important. Better results were found to be consistent with a constant caloric intake at each meal, feeding half of the total caloric intake at each insulin injection, maintaining the same meal hours, avoiding dietary changes, and following a constant

daily exercise effort. Existing studies show that the caloric requirement in dogs is 40-60 kcal/kg/day.

Conclusions

1. Diabetic cataract was most frequently found in diabetes complications, so 56% of patients with diabetes had cataract.

2. The onset of cataracts is sudden and the treatment of diabetes does not slow the progression and may be complicated by subluxation / dislocation of crystalline glaucoma, uveitis.

3. Diabetes treatment was performed with Mixtard insulin, Lantus insulin or oral hypoglycemic agents, and in the case of cataract, Quinax eye drops were used.

4. In both types of diabetes, a diet was established for each patient.

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Computed Tomography evaluation of peripheral nerve sheath tumor in an American Staffordshire Terrier – Case study

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Abstract

A 1 year old intact American Staffordshire Terrier male was brought to consultation being suspicioned by cervical compression. The neurological examination show proprioception deficiency of the front and hind legs and a diminution of the superficial proprioception was noted. Base on the neurological evaluation the lesion was located in the cervico-toracal area of the spine. A native CT was performed to identify the changes of the spine. The CT reveal a craniodorsal left paravertebral soft tissue mass associated with the segmental nerves of the brachial plexus and with extension into the vertebral canal through the left intervertebral foramen T2/3. A fine needle aspiration was performed from a formation localized intrathoracic in the close proximity of the nerve sheath tumor and the examination showed cell characteristically for a mesenchymal malignant process.

Keywords*: nerve sheath tumor, neurofribrosarcoma, dog, computed tomography*

Introduction

Peripheral nerve sheath tumor (PNST) in dogs can have origin in the Schwann cell, or other cell that surround the axons of peripheral nerves (3, 5, 7, 9, 10). Classification of those tumors was made in 1999 by the World Health Organization. PNST are divided into benign PNST (BPNST) and malignant PNST (MPNST) according to morphologic features and biological behaviour (1, 5, 6, 7, 11). Tumors of the peripheral nervosa system are not very common in animals, as location those usually can appear between C6 and T3, other locations are cited by the literature (2, 8, 10). The classification and terminology is confusing both in humans and animals medicine, the classification of peripheral nerve tumors in veterinary medicine is base of the type of cell that is involved and in cases of controversial ontogeny is simplified as malign or benign (10).

The purpose of this report is to describe the imagistic feature of a peripheral nerve sheath tumor in a young dog and to show the importance of CT examination in evaluation and diagnostic of peripheral nerve sheath tumor. The occurrence of nerve sheath tumor is often seen in middle to old age (3, 10).

Material and method

The biological material was represented by a 1 year old intact American Staffordshire Terrier male that was brought to consultation being suspicioned by cervical compression.

The CT evaluation was performed with the patient under sedation and restrained in a dorso-ventral decubitus. The CT examination was performed using a Siemens SOMATOM SCOPE CT (Siemens), with soft tissues and bone reconstruction windows. The images were obtained using 130 kV and a pitch factor of 1. After the CT, was performed an ultrasound guided fine needle aspiration, having as a target the metastatic formation located under nit the spine in the thoracic cavity.

Results and discussion

Computed Tomographic Findings

There is an elongated soft tissue attenuating craniodorsal mediastinal mass lesion of 5.5 cm length and 2.5 cm diameter to the left of the cranial thoracic vertebrae. Small multifocal intralesional mineralization is seen (fig. 1, fig. 2, fig 3).

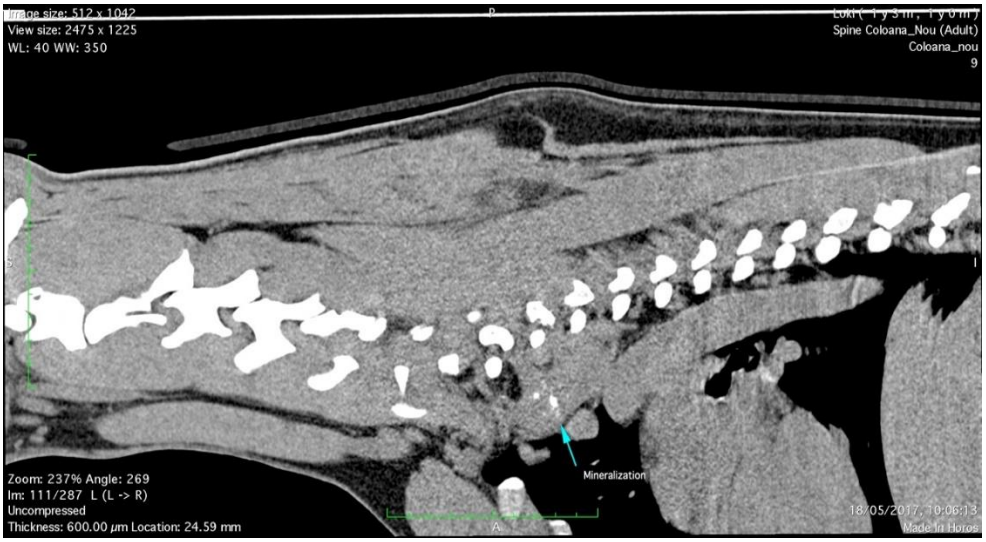

Fig. 1 Small multifocal mineralization

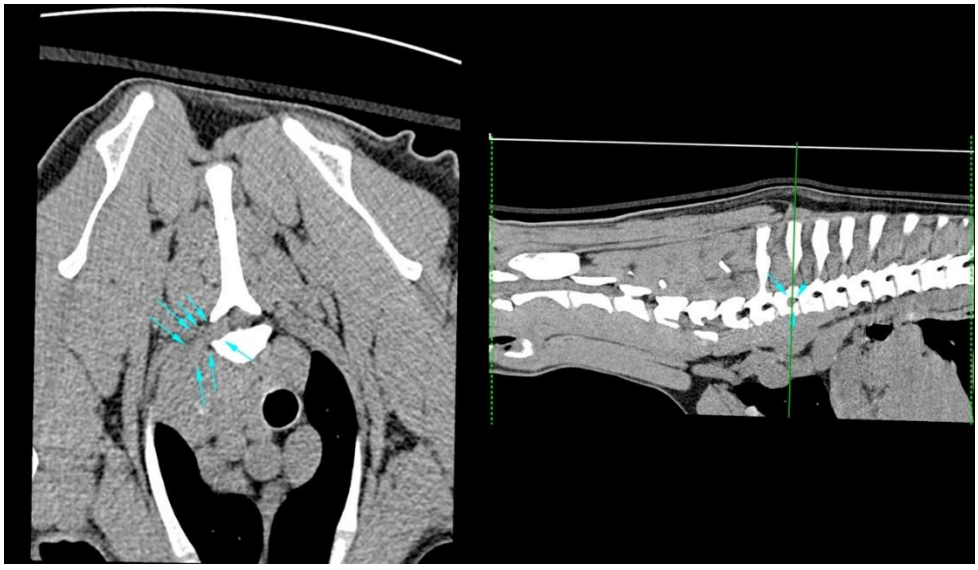

Fig. 2 Enlarged nerve root with presence of intramediastinal mass

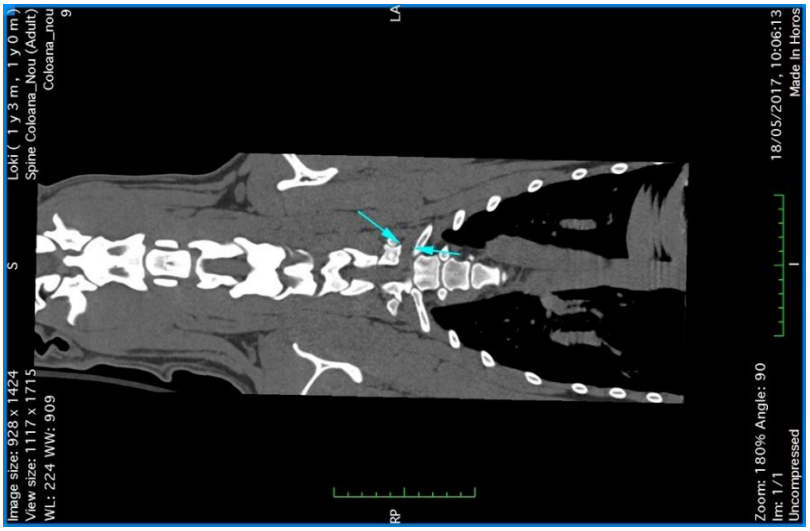

Fig. 3 Enlargement of the nerve rooth

The mass presents multiple thin finger-like extensions which appear to merge with the radicular spinal nerve branches of the brachial plexus nerves. The left spinal nerve root at T2/3 specifically presents marked centrifugal thickening and can be traced through the widened left neuroforamen. There is a moderate intradural mass effect onto the spinal cord from the mass at the same level.

The cranial mediastinal lymph nodes reveal moderate asymmetric enlargement at up to 2 cm diameter with faint mineralizations (fig. 4). The walls of the aortic root and brachycephalic trunk present faint mineralization as well.

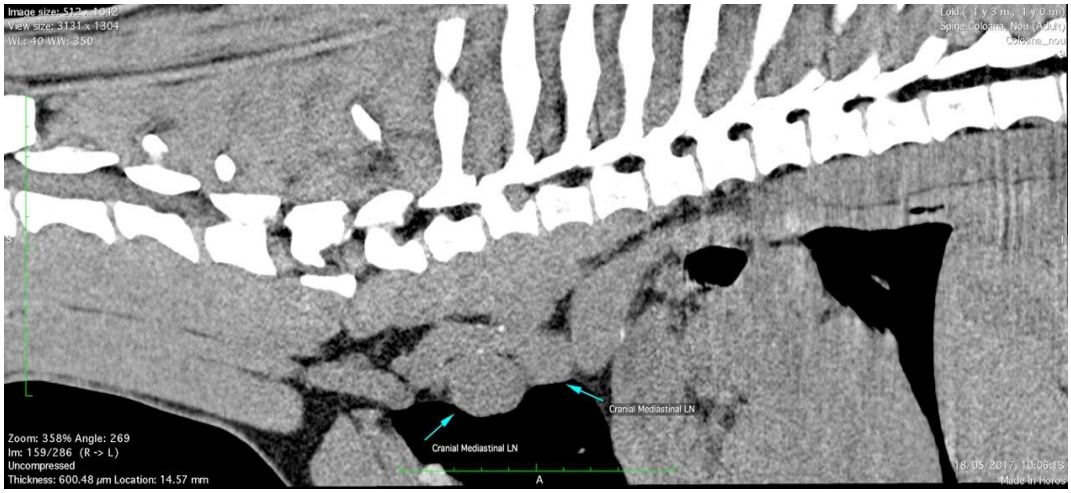

Fig. 4 Enlargement of the mediastinal limphnodes

Computed Tomographic Diagnosis

Craniodorsal left paravertebral soft tissue mass associated with the segmental nerves of the brachial plexus and with extension into the vertebral canal through the left intervertebral foramen

T2/3. Moderate secondary compressive myelopathy at the same level. Cranial mediastinal lymphadenomegaly meeting neoplastic criteria.

Computed Tomographic interpretation

We consider a primary neurogenic neoplasm such as peripheral nerve sheath tumor/neurofibrosarcoma with metastatic spread to the regional lymph nodes versus a secondary neoplasia such as lymphoma.

As to the patient demographics (age) and the involvement of the cranial mediastinal lymph nodes a round cell neoplasia seems more likely here.

The overall prognosis is guarded to poor as the lesion is probably not fully resectable due to the involvement of the component inside the vertebral canal and metastatic spread is very likely at this point.

The clinical signs are likely due to the spinal cord compression by the mass.

Fine needle aspiration

The content extracted from the formation by ultrasound guided biopsy was smeared on a glass blade and stained using Diff-Quik technique. A well-represented cell population is formed, consisting of round, elongated, fusiform, neoplastic cells arranged in groups. These shows marked cellular and nuclear polymorphism, anisocytosis, anisocaria. The nuclei are large, located centrally or paracentral, presenting large nucleoli, evidences of granular aspect of chromatin and presence of single or multiple nucleoli is marked. In some places, many multinuclear tumor cells can also be seen. There is also numerous mitosis. The cytological aspects described plead for a poorly differentiated malignant tumor of mesenchymal origin (sarcoma) (fig. 5, fig. 6, fig. 7).

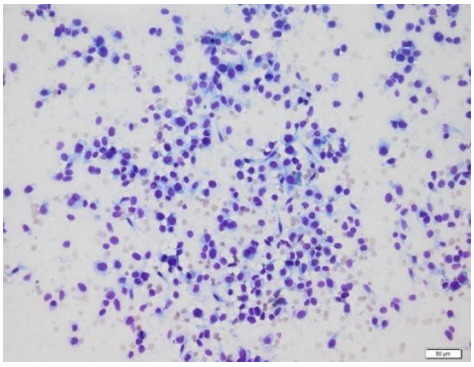

Fig. 5 Neoplastic Cell with various shape and size

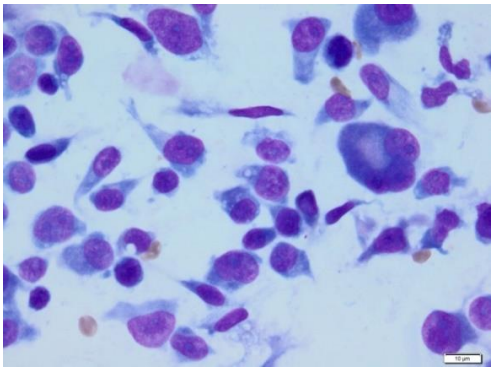

Fig. 6 Cellular and nuclear polymorphism

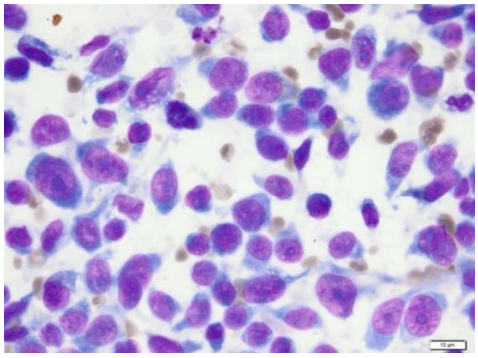

Fig. 7 Abnormal aspect of the cytoplasm and the nucleus

Conclusion

The neoplastic process located in the nerve root region is not limited to middle age or old individual but also can occur in younger individual. The aspect of cell (3) indicate a process with high aggressivity. The presence of calcification is not uncommon, being described along with the round and fusiform cell type in case of MPNST (3, 5). The presence of osteogenic tissue can mislead the diagnostic, but there is not presence of bone lysis, the new tissue being a result of PNST malignancy. The presence of cell with granular aspect is another indication that the origin of the tumor is of nervous origin (3, 4).

The fine needle aspiration technique is not sufficient to make possible an exact identification of the tumor classification. Taking in consideration the CT and cellular characteristic we can say that the most possible diagnostic in this case is schwannoma/neurofibrosarcoma.

Acknowledgement: the studies was conducted in the laboratory of Medical Imaging – Radiology and are part of the internal grand research conducted by the Radiology laboratory.

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The study of grazing behavior in goats

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Abstract

All living beings live their lives according to a scenario imposed by two programs: a hereditary one, due to the genetic code that includes the whole phylogenetic experience of the species from which the organism is part, and a second program stored in the brain that represents the amount of knowledge accumulated by the individual after confrontation with its environment. Behavior therefore has a dual causality: exogenous and endogenous, to the extent that it is dependent on external stimulation factors, but also on internal factors capable of triggering and justifying at the same time the emergence of a motivation, a pulse. In triggering and conducting behavior is required the concurrent participation of internal and external stimuli, whose synchronized actions will cause the critical threshold to be exceeded in intensity.

Keywords*: grazing, pasture, behaviour, goats*

Introduction

All living beings live their lives according to a scenario imposed by two programs: a hereditary one, due to the genetic code that includes the whole phylogenetic experience of the species from which the organism is part, and a second program stored in the brain that represents the amount of knowledge accumulated by the individual after confrontation with its environment. Behavior therefore has a dual causality: exogenous and endogenous, to the extent that it is dependent on external stimulation factors, but also on internal factors capable of triggering and justifying at the same time the emergence of a motivation, a pulse.

In triggering and conducting behavior is required the concurrent participation of internal and external stimuli, whose synchronized actions will cause the critical threshold to be exceeded in intensity.

Lorenz (1970) and other world-renowned ethologists believe that of all known instincts, only five are truly major: feeding, shelter, body care, sleep, and reproduction. Contrary to accredited opinions, social (grouping), aggressive, and environmental selection behaviors are not considered major instinctive activities, but only sub-instinctive (Decun, 2004). Their knowledge is important for animal breeders, veterinarians, engineers, but especially for ethologists.

The fact that references to behavior of goats are deficient in the literature is due to frequent classifications of small ruminant species (together with the sheeps), and research is often done on sheep, with indirect referrals to the goat species. This necessitates the initiation of behavioral studies in the goat species as a species is clearly defined by behavioral and maintenanceal features. Although there are plenty of similarities between certain sheep and goat behaviors, there are also behaviors specific to each species, which must be clearly differentiated.

Material and method

The biological material was represented by 87 goats out of which 54 adult females, 2 males and 31 kids. The goats surveyed were meticulous. The research was carried out in the commune of Bistra, Alba County, on a private breeder flock, the goats being grown in a semiintensive system.

Working methods used: observation, photography, sound and video recording, causal interpretation and analysis.

The study of pasture behavior is extremely difficult due to the diversity of individual responses, which is why we considered that the generally accepted minimum number would be 2 individuals in the lot, but the experiment may include all the flock that will be subject to individual observation by daily rotation.

The duration of the investigations was different, on some days the records and observations were intermittent, with varying durations, and on other days they were continuous. The research was designed to extend for a sufficient amount of time, including both daytime and nighttime. Suspension of night-time recording and observation can lead to erroneous interpretations and conclusions.

Observing grazing behavior includes grazing, ruminating, resting, going to water, and other activities whose duration depends on climatic factors, pasture condition, season.

The chosen methods of work have taken into account a major desideratum in ethological research, namely not to influence the behavior of animals by the presence of the ethologist or by the devices used in the research, which would lead to the obtaining of incorrect, incomplete information and implicitly to erroneous conclusions.

The basis of the behavior is the motivation, internal and external, defined as the disposition of action, the preparation or the tendency to execute a certain behavior. This availability is determined by a set of internal factors, some neurohormonal mechanisms, external stimuli and a history of active behavior (Cociu, 1999).

Results and discussion

The ingestion behavior of the goats studied was analyzed both in quantitative and qualitative terms. Under normal conditions, in the pasture, goats carry a cyclic diurnal activity, closely associated with sunrise and sunset.

At the flock under study, the grazing was done on the plots, bounded by an electric fence (fig. 1).

Fig. 1 Electric fence for the grazing area

Goats are very demanding in terms of nutrition, so when there is abundant food, selectivity to feed thereof is maximum, but there are extreme situations when goats can only survive with branches or bark of trees. Due to this biological particularity, when the goat receives a feed she does not like it, refuses it.

The goats are more active in light hours than during the night. Grazing in the spring and summer months is done in two distinct periods, one in the morning and the other in the afternoon. Between these two periods there is a period of time when the animals rest or ruminate. In days of precipitation or extreme temperatures this diurnal grazing scheme is severely disturbed.

At 25 ° C, grazing ceases, and in winter, if taken out on the pasture, the feed search period is reduced to 2-3 hours. During the night, the grazing period reaches 40-45% of the total time for this activity.

Grazing start time is not influenced by night time temperature when the temperature oscillated between -3 ° C and 9 ° C.

Rumination takes place cyclically, goats ruminate for a longer period during the night and at sunset, when the grazing activity is smaller. The ruminant time was 300-400 minutes / 24 hours, with an average of 63 mastication / bowl movements. The daily grazing period averaged 7-8 hours in the spring / summer months, and the grazing in the morning was about 3.5-4 hours. If the temperature is low or the weather is rainy the goats remain on the pasture for a shorter time, but the grazing can be extended in the afternoon to ensure the time required to ingest enough feed.

The amount of ingested green feed depends on the fullness of the digestive tract, the rate of advancement of the rumen content to the omasum and the water content of the plants. For plants that contain high percentage of water, goats allocate an increased time for grazing without any gastrointestinal disturbances, probably due to the high rate of feeding of these feeds from the rumen to the omasum.

Another factor influencing the amount of ingested feed is the time given to mastication, depending on the rate of digestion of the plants in the rumen. If the plants are lignified, the change in their chemical content also changes the ingestion behavior of the goats, which is to increase the time required for mastication and rumination. This phenomenon is closely related to the degree of digestibility of the plant, its content in nitrogenous substances, cellulose and water. During grazing, the goats generally select plants that are sweeter and avoid the fibrous plants necessary for normal rumen to function, so the fibrous index and rations are two indicators whose presence or absence can be markers of digestive problems in goats, especially for acidosis (Hirst, 2008).

The amount of feed ingested depends also on the vegetation stage of the plants and species to which they belong. Consumption of green plants increases directly in proportion to their nitrate content and inversely proportional to the amount of raw cellulose (Houp, 1998). For pastures where alfalfa, festuca, clover, were grown, the daily ingestion time averaged is 290-300 minutes, and the grazing period ranged from 3 to 12 minutes, averaging 4.5 minutes. Rumegation is directly conditioned by the raw cellulose content of plants.

Nutritional needs depend on the individual. Thus, pregnant or goat with kids as well as underweighted goats grant a longer grazing type, averaging 30 minutes to the other animals.

Due to the fact that the flock is made up of a single breed of goats, the behavior of feeding on pasture has been manifested by a pronounced individualism, both adults and kids, compared to sheep, which grazing pastures in a compact group.

On the pasture, we noticed that males prefer to move in the center of the group, the other animals being outfitted.

The goats of the local mixed breed, on the pasture, depending on the degree of dominance, the dominant ones occupying an end of the grazing plot, while the others occupying the opposite end. Extremely lively kids goat are placed near mothers; the degree of domination or subordination of the mother is automatically attributed to his own kids.

Taking into account that the flock has been costed from one race, we will make some clarification about the behavior of pasture feeding.

The goats of the Metis breed exhibit extremely selective behavior, searching for the young parts of the plants on the entire plot available. I have noticed that I'm not just choosing young parts of plants that are sweet, but they also often choose young parts of plants that contain high amounts of tannin, which greatly reduces the digestibility of the protein

During grazing, mothers' recognition of the kid goats is based on hearing communication; visual communication and olfactory communication. In a first phase, the mutual recognition of the goat and the kid goat is dependent on the visual organ. If the appearance of the two partners changes, and the recognition behavior will change.

Conclusions

Goats as well as sheep, although belonging to the same class of ruminants, their feeding behavior have certain species specificities. In our research, the fact that the goat was made up of individuals belonging to the same species, specific behaviors of this breed were identified.

The behavior of grazing is carried out according to a cyclic diurnal activity, closely associated with sunrise and sunset. They are more active in light hours than during the night. At 25 ° C grazing ceases, and in the winter if taken out on pasture, the search period is 2-3 hours. The rumination time is 300-400 minutes / 24 hours, and the mastication movements for a regurgitated bowl have an average of 63 movement. In comparison to sheep, goat in the pasture consume selective plants and most of the time prefer bushes, branches, leaves, sprouts or buds of trees. Mixed breed individuals are extremely dynamic on the pasture, being in a continuous movement in the need to consume a wide variety of plants.

Acknowledgement: the studies was conducted in the laboratory of Medical Imaging – Radiology and are part of the internal grand research conducted by the Radiology laboratory.

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Computed Tomography evaluation of occipital bone tumor in a Doberman – Case study

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Abstract
A 6.5 years old intact Doberman female was brought to consultation showing sign of medullary compression. The neurological examination concludes a lesion in cervical part of the spine, given the fact that the patient presents mobility deficiency on all four limbs, having trouble maintaining a standing position. The mental status, the behavior and the evaluation of the central nervous system haven't show any kind of changes. The CT examination of the spine show no changes in the vertebral alignment or sign of compression. The CT scanning of the head reveals an infiltrative osteolytic formation in the right side of the temporo-occipital bone of the skull,
Keywords*: cranial tumor, dog, occipital tumor, computed tomography*

Introduction

Osteosarcoma in dog is the most common type of bone cancer and have an incidence of 80% from all the bone tumor, followed by chondrosarcoma (10%) and less frequently are encountered fibrosarcomas and hemangiosarcomas (7%) (1, 3). The most affected are the large breed dogs (55%) and giant breed dogs (29%) (4). The prevalence for the lesion is 79-95% in the appendicular skeleton and 5-21% were located in the axial skeleton in the giant and large dogs breed. In the medium breed the prevalence of osteosarcoma in the axial skeleton in higher (33%) but the incidence in this category is lower (11%) (2, 4). Regarding the location of osteosarcoma in the head at the level of parietal bone only 2.3% were recorded from a total of 1215 examined dogs (2, 3, 5).

The chondrosarcoma is also more common found in the appendicular skeleton, but there were reported cases of chondrosarcoma located in the mammary gland, tongue, kidney, abdominal wall, urethra, mitral valve and aorta (1). Location of the chondrosarcoma at the level of the skull is rare representing only 0.1% in humans (3).

Material and methodos

The biological material was represented by a 6.5-year-old intact Doberman female that was brought to consultation for locomotor problem. The neurological examination concludes a lesion in cervical part of the spine, given the fact that the patient presents mobility deficiency on all four limbs, having trouble maintaining a standing position. The mental status, the behavior and the evaluation of the central nervous system haven't show any kind of changes.

The CT evaluation was performed with the patient under sedation and restrained in a dorso-ventral decubitus for spine evaluation and ventro-dorsal decubitus for head evaluation. The CT examination was performed using a Siemens SOMATOM SCOPE CT (Siemens), with soft tissues and bone reconstruction windows. The images were obtained using 130 kV and a pitch factor of 0.85.

Results and discussion

Computed Tomographic Findings

A mixed osteolytic and osteo-proliferative lesion with soft tissue component is seen at the right occipital and temporal bone. Multifocal permeative osteolysis is noted as well as chaotic osteo-proliferation and amorphous periosteal reaction. The lesion is perforating into the caudal and cranial fossa to the right of the skull base and is directly adjacent to the emergence of the right cranial nerves including the origin of the right trigeminal nerve (fig. 1).

Moderate right sided masticatory muscle atrophy is noted. The right tympanic bulla and the medial portion of the right ear canal are filled with hypoattenuating material (fig. 2).

The right medial retropharyngeal lymph node presents moderate enlargement with increased short-to-long axis ratio.

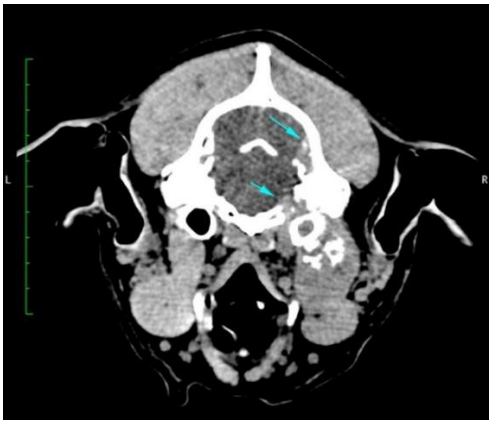

Fig. 1 Destruction of the bone and internal ear

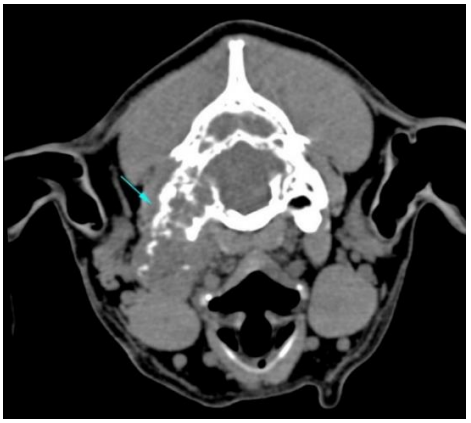

Fig. 2 Infiltrative character of the formation

Peripheral fat stranding is noted in proximity of the soft tissue component of the lesion as well as circumferential to the right medial retropharyngeal lymph node.

In the medullar canal at the atlas level a hyperattenuating material is present inside the medullary canal having a compressive effect on the spine (fig. 3, fig. 4).

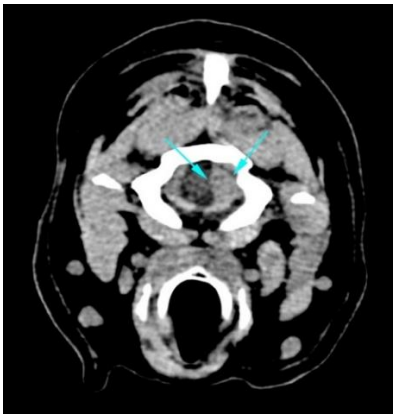

Fig. 1 Compression of the spine and retropharyngeal LN reaction

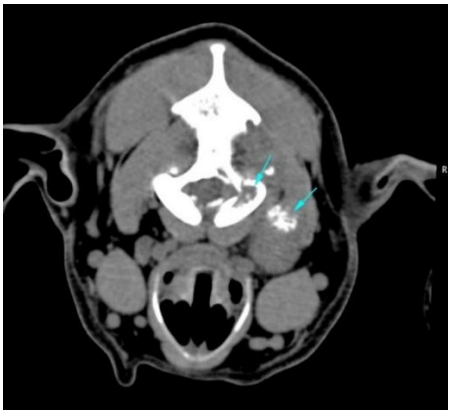

Fig. 4 Destruction of the occipital condyle

The osteolytic process is spread also to the occipital bone, there are new bone formation with infiltrative character on the right side of the occipito-atloidian junction (fig. 5).

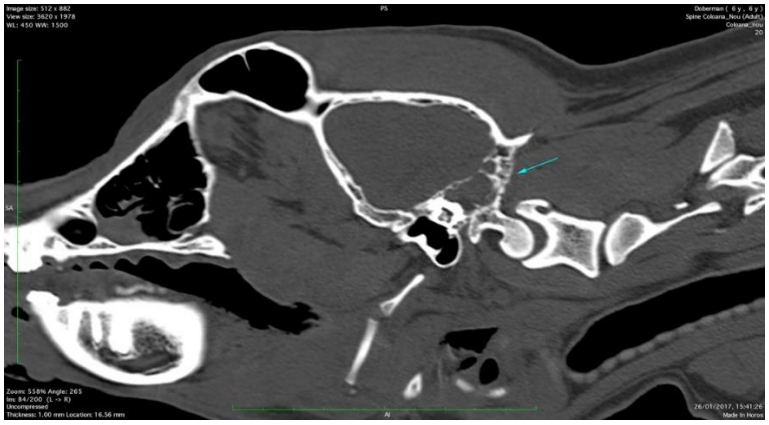

Fig. 5 Bone destruction in the occipital

Evaluation of the spine show no changes that could explain the patient symptoms.

CT Diagnosis

Neoplasia with aggressive biological behavior of the right occipital and temporal bone and intracranial extension. Possible metastatic spread to the right medial retropharyngeal lymph node. Secondary ipsilateral masticatory muscle atrophy. Secondary ipsilateral otitis media and externa.

Differential diagnoses

Squamous cell carcinoma, adenocarcinoma, osteocondrosarcoma, and lymphosarcoma are possible differential diagnoses. The lymph node changes are suggestive for metastatic spread.

Final diagnosis would require sampling. However, the long-term prognosis is poor. The lesion is not resectable.

Discussion

Unfortunately, the owner refuses a sampling from the formation in order to have a proper classification of the tumor and other form of treatment. According to the CT findings and the data from literature (2, 3), the radiographic presumptive diagnostics is of osteocondrosarcoma. The canine osteocondrosarcoma have relatively low metastatic rate and slow growing rate (1), that was proven also by the lack of other metastatic process in the body. Because of the slow rate of growing the spine and brain can adjust to the compression (1).

Because of the slow growing process the patients usually show clinical sign of at the late stage of the disease (4).

Acknowledgement: the studies was conducted in the laboratory of Medical Imaging – Radiology and are part of the internal grand research conducted by the Radiology laboratory.

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Contrast substance radiography of the digestive tract in goats

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Abstract
The gastro-intestinal anatomy of the goat is well known (3). Using contrast substance like barium sulfate the examiner is able to identify changes in the gastro-intestinal mucosa, also being able to follow the gastrointestinal passage of the content.
***Keywords**: digestive tract, radiography, contrast, goat*

Introduction

Due to the increasing demand on the consumer market for products and by-products resulting from goats, the growth of this species has been increasing in recent years (1, 2).

The lack of information on growing and exploitation technology among goat breeders is increasing, generating pathological disorders with serious repercussions on the animal and implicitly on the profit obtained by the breeder (1, 4).

Considering that the digestive system is most frequently affected, it is our goal to highlight by radiological examination, with the help of contrast agents, the time taken by the digestive content from the level of the rumen to the elimination.

Material and method

The biological material used in the research was represented by a group of 5 goats aged between 1 and 3 years.

The goats studied are of the native breed - Carpathina - and the hybrid between the Carpathian and Saanen breeds belonging to private breeder.

The method of working consists in preparing the contrast medium with water, reaching a viscous consistency and administered orally, to the animal in different amounts depending on the size of the patient. After individual administration of the contrast agent, individual serial exposures were performed at different time intervals from exposure immediately after administration to 1 hour, 3 hours, 5 hours, 24 hours and 48 hours.

The work parameters have been adjusted for each case, depending on the size of each.

Exposure locations were different by performing latero-lateral exposure with the pacient standing or in lateral decubitus as well as dorsal exposures of patients.

Results and discussion

In the first case, a 1 year old male from the Charpatina breed, the contrast substance was administered in a single dose of 200 ml barium solution. The animal was restrained in standing position and lateral decubitus for the radiographic examination and the dose were of 103 kV with 25 mAs (fig. 1, fig. 2, fig. 3, fig. 4, fig. 5, fig 6, fig 7).

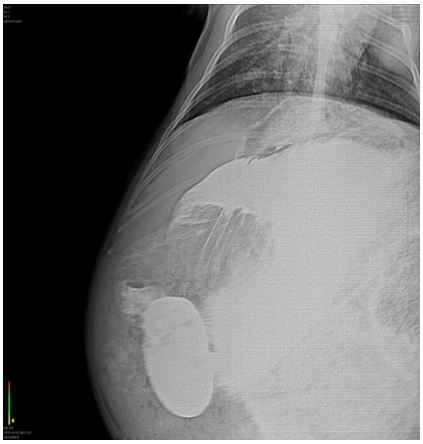

Fig.1 VD exposure after 200 ml barium ingestion (130 kV, 25 mAs)

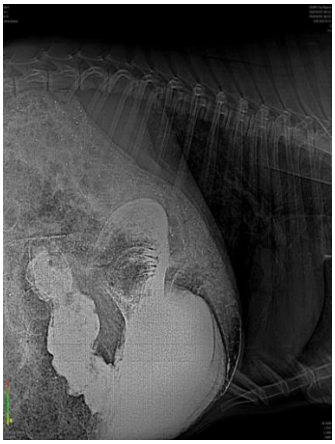

Fig. 2 Latero-lateral exposure after 1 hour post contrast

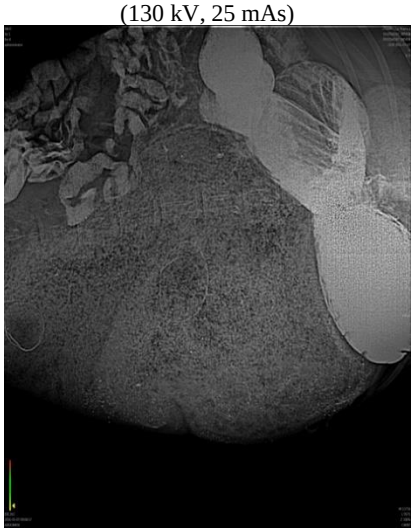

Fig. 3 VD exposure after 3 hours from barium administration

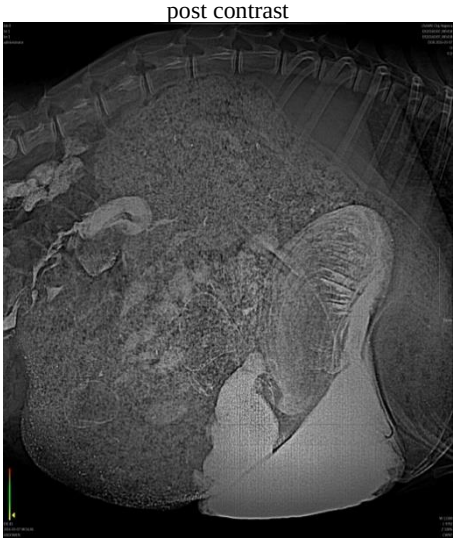

Fig. 4 Latero-lateral exposure at 5 hours post contrast

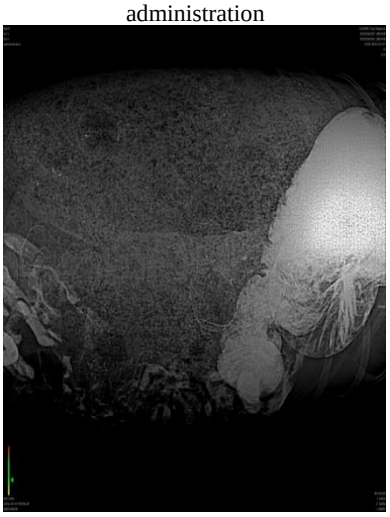

Fig. 5 Ventro-dorsal exposure at 24 hours post contrast administration

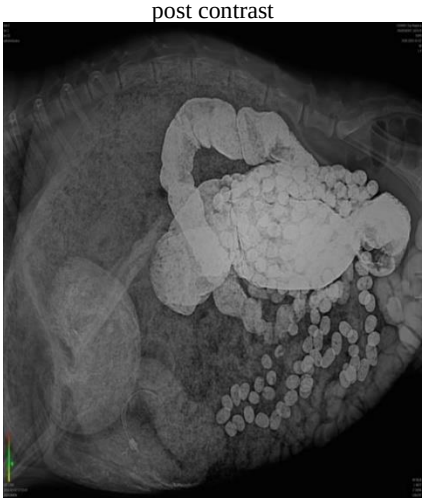

Fig. 6 Latero-lateral examination after 48 hours post contrast administration

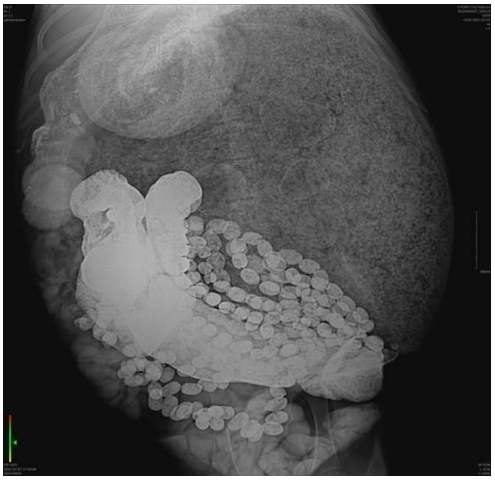

Fig. 7 VD exposure at 48 hours after contrast administration

For the second patient, the quantity of barium administered was 300 ml/animal and the parameters were of 106kV and 30 mAs. The third patient got 350 ml/ animal of barium and the exposures were made at 115 kV and 40 mAs. For the fourth patient, the barium quantity administered was of 400 ml and the exposures were made at 120 kV and 35 mAs.

For the last case taken in the study the barium quantity administered was 450 ml and the parameters used for exposure were 125 kV with 40 mAs.

One hour after administration, the contrast substance is almost entirely found in the reticulum, but we can very well observe both conducting the rest of the substance to the reticulum and passing the barium to the omasum (fig. 8, fig. 9).

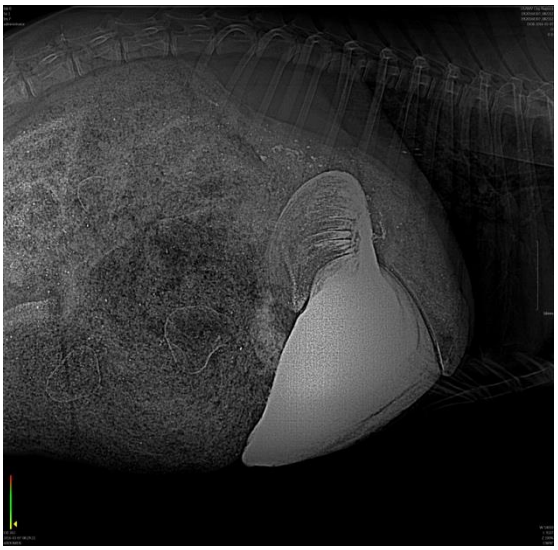

Fig. 8 Latero-lateral exposure at 1 hour after barium ingestion

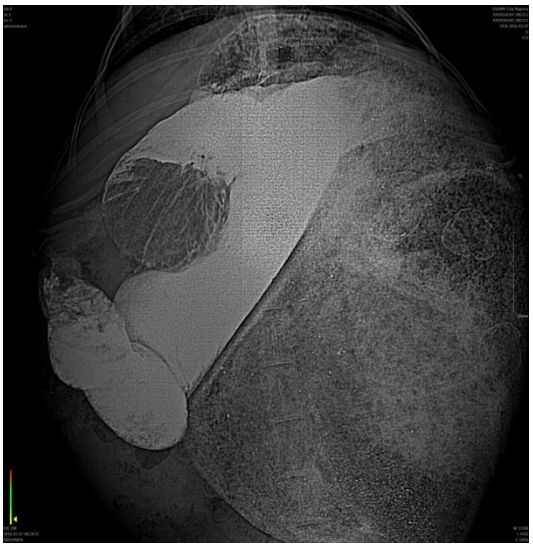

Fig. 9 VD exposure at 1 hour after barium ingestion

At 3 hours, all of the substance is at the reticulum level, the omasum blades are well highlighted, as well as the abomasum structure that passes the content to the small intestines (fig. 10, fig 11).

In lateral exposure 5 hours after administration of the contrast substance, it is visible at all gastric levels except rumen (fig. 12, fig 13).

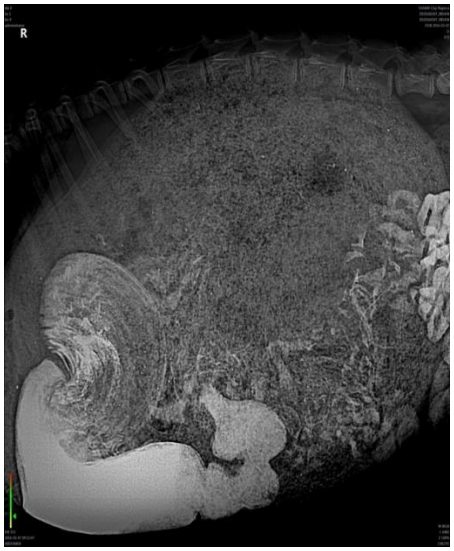

Fig. 10 Latero-lateral exposure at 3 hours after barium ingestion

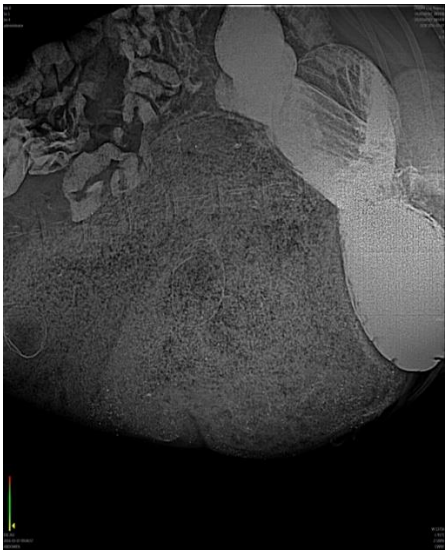

Fig. 11 VD exposure at 3 hours after barium ingestion

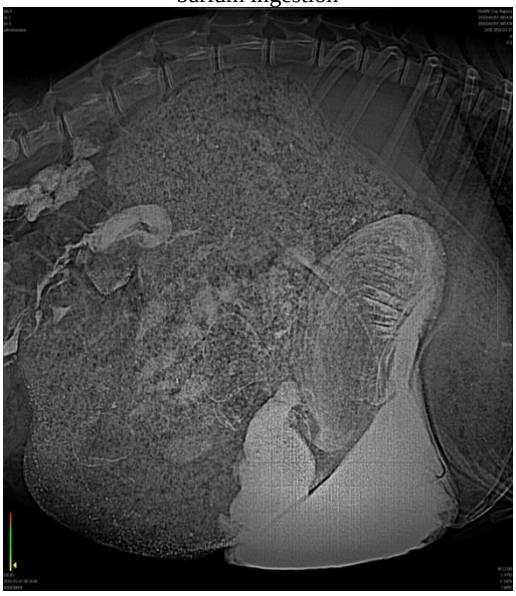

Fig. 12 Latero-lateral exposure at 5 hours after barium ingestion

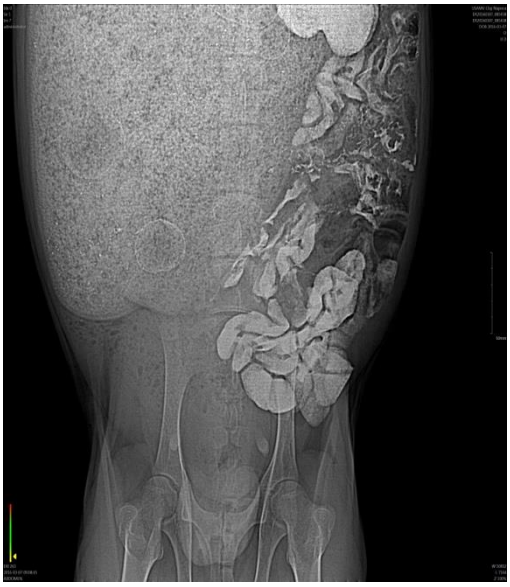

Fig. 13 VD exposure at 5 hours after barium ingestion

At 24 hours, the substance is still present, but with greater concentration on the small intestine (fig. 14, fig. 15).

At 48 hours, the substance is predominantly found in the large intestine and partially embedded in the faeces (fig. 16, fig. 17).

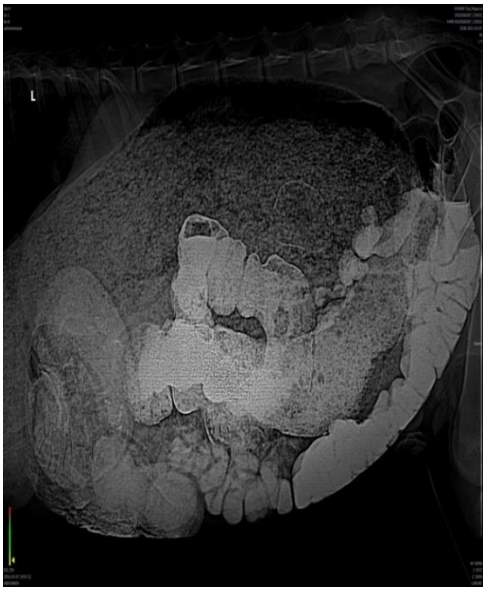

Fig. 14 Latero-lateral exposure at 24 hours after barium ingestion

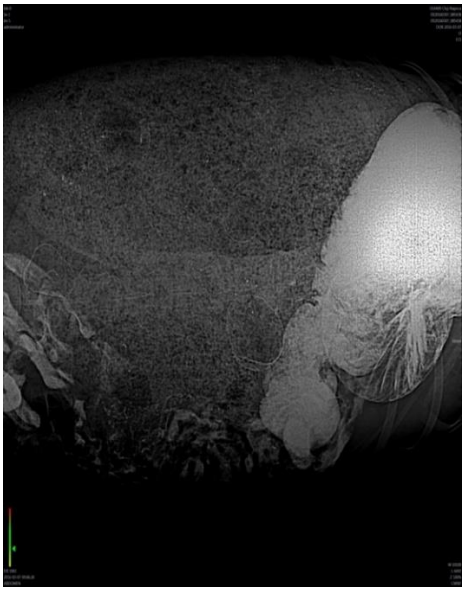

Fig. 15 VD exposure at 24 hours after barium ingestion

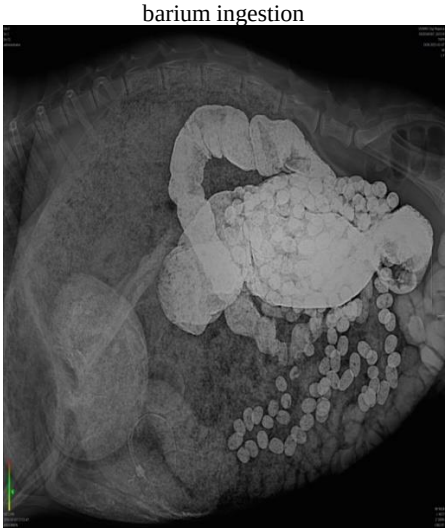

Fig. 16 Latero-lateral exposure at 48 hours after barium ingestion

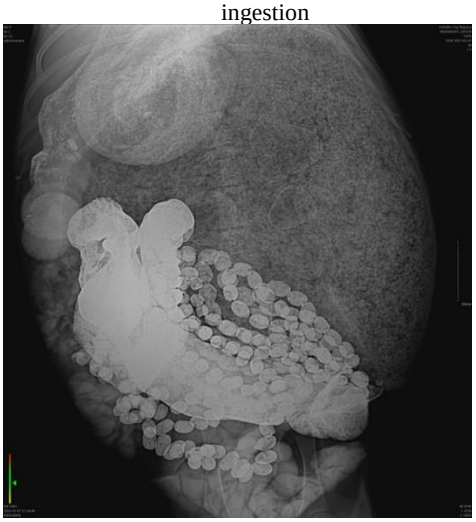

Fig. 17 VD exposure at 48 hours after barium ingestion

Conclusions

1. Barium sulfate doses ranging from 200-300 ml, provide good radioopacity to the digestive tract in goats.

2. Barium sulfate doses ranging from 350-400-450 ml / animal give excellent opacity for the radiographic view of the digestive tract in the goats, and can be used without restrictions in assessing digestive transit.

3. Dosing parameters (Kilovoltage and milliamperage) ranging from 106 - 110 kV and 30 - 32 mAs, provide good radiological imaging to interpret a possible diagnosis on the digestive tract.

4. Exposure parameters ranging from 115 - 120 - 125 kV and 35 - 40 mAs, give excellent radiological images capable of providing fine details about the entire digestive tract.

5. The exposure positions used (standing, latero-lateral and ventro-dorsal) are equally important in investigating digestive tract segments.

6. Exposure times at 1 and 3 hours after barium sulfate administration are excellent for visualization of transit across the reticulum, omasum and abomasum.

7. Exposures between 5 and 24 hours after administration are beneficial for the visualization of small and large intestines, and at 48 hours the radiological examination gives the possibility of investigating the rectum.

Acknowledgement: the studies was conducted in the laboratory of Medical Imaging – Radiology and are part of the internal grand research conducted by the Radiology laboratory.

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Comparative study of vascular arterial reactivity in several mammal species: 1. Reactivity of the arterial smooth muscle to vasoconstrictor agents

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Abstract

Vascular reactivity is one of the three pillars on which lies the regulation of arterial pressure in living organisms. Arterial pressure is one of the main determinants of the activity state of various organs and systems both in healthy and in pathologically-altered states. The present study aims at identifying similarities and differences between the resistance arteries belonging from various mammal species that are most involved in veterinary practice: rats, cats, dogs and horses. The arterial fragments harvested from animals dead due to various clinical and traumatic conditions unrelated to vascular pathology were normalized using a newly-introduced system of quantification, the force index system. This has been calculated using the wet-weight parameter and the force generated after administration of various pharmacological agents that cause vasoconstriction. The artery fragments were fitted in organ baths using the Krebs-Henseleit saline, thermostated at 37° C and bubbled with a mixture of 95% O_2 and 5%CO_2. Vascular endothelium was either kept or removed using gentle rubbing with moist filter paper. Control of endothelial removal was made both functionally, using carbachol (synthetic derivative of acetylcholine) and microscopically, after testing. The force generated was measured using isometric force transducers coupled to a computerized acquisition system. The pharmacological vasoconstricting agents used were phenylephrine (synthetic derivative of epinephrine), KCl (potassium chloride 40-80 mM, as depolarizing agent) angiotensin II, and vasopressin. The results were statistically investigated using the t-test and ANOVA testing. The preliminary results show a dependence of the force generated an the amount of muscle present in the various species from which the arteries were taken, a specifically increased response of feline-derived arteries to angiotensin and a specifically increased response of canine-derived arteries to vasopressin. These results will be used as controls for further testing in various pathological conditions and for various other pharmacological agents used in the therapy of vascularly-induced pathological states.

Key words*: vascular, reactivity, arterial, vasoconstrictor agent.*

Introduction

The present study aims to investigate the modifications commonly encountered in the veterinary practice in vascular reactivity of arteries that may be involved in the pathogenesis of different animal species. Our scope is to conduct a comparative investigation of the vascular reactivity in histologically and functionally similar arterial segments that have been collected from various mammal species that the veterinary pathology frequently deals with.

In the past few decades the investigation of the mechanisms underlying the adjustment of the arterial tonus and of the arterial smooth muscle fiber has relied on the well-known isometric transducers pattern and on that of the annular preparation of different arteries. The arterial duct typically used for these types of investigations is the rat aorta because it meets most of the conditions of stability, accessibility, disposability and controllability that a trustworthy investigation calls for. The price is also an important factor to be taken into consideration in this matter.

Although the aforementioned pattern is widely known, the rat still isn't a perfect model in what the cardiovascular modeling is concerned; it is not similar to humans and even less so to other mammals. This experimental model has been used as from half a century ago [3].

Hence an experimental comparative investigation was conducted using fragments of arteries collected from dogs, cats and horses as well as thoracic aorta rings taken from Wistar rats.

Material and method

The reactivity of the arterial rings was measured in terms of both absolute force, measured as force index (the force in mN of the preparation reported at its weight in mg) and relative reaction towards a standardized witness. Dose-response curves were also produced where possible (considering the availability of preparations) involving the majority of the known vasorelaxing and vasoconstrictor substances that are pharmacologically well characterized.

The comparative study was made on arteries that were similar in terms of size, and that were assigned to the resistance segment, namely branches from the gastric coronary artery or the superior mesentery which had similar dimensions: maximum length: 2 mm, Φ = 1 mm, weight 10-15 mg. The quantification of the contraction force was expressed as N/mg wet weight. The organ parts were taken from the Medical Clinique and the Surgery Clinique at the Veterinary Medicine Faculty and were collected from dead animals that had not been subjected to legal euthanasia nor had they affections with vascular implications.

After the dissection the vessels were exsanguinated, washed in physiological salt solution, sectioned in 5-10 cm length fragments and then put into Krebs-Henseleit serum (prepared according to the formula), and transported to the place of the experiment in 30 minutes maximum.

The aorta fragments were fixated using a metallic serfina on the bottom of the isolated organ baths where the ring was tensed through the verniers of the tensiometric stamps to an initial tension of 100 mN.

The vascular endothelium was removed by gently rubbing with a damp filter paper where the characteristics of the experiment called for it. The presence of the vascular endothelium was verified both pharmacologically (using carbachol) and by direct microscopy.

The aorta rings were mounted in organ baths containing 4 ml of Krebs-Henseleit physiological salt, (composition (mN): NaCl 118; KCl 4.7; 2.52; $MgSO_4$ 1.64; $NaHCO_3$ 24.88; KH_2PO_4 1.18; glucose 5.55), thermostated at 37°C and bubbled with carbogen (a mixture of 95% oxygen and 5% carbon dioxide).

Isometric force transducers connected to a computerized system for data acquisition were used to record the contractions of the vascular smooth muscles.

The preparations were allowed to equilibrate for 60-90 minutes under a resting tension of 100 mN.

The aorta rings were afterwards precontractated with phenylephrine ($10^{-7} – 10^{-6}$)M and K^+ (40-70 mM) and treated with carbachol (10^{-6}M) for releasing endothelial NO [**Eroare! Fără sursă de referință.**]. The absolute magnitude of the contractions was of 175 ± 25 mN for the phenylephrine (10^{-6} M) and K^+ (40-70 mM).

Results and discussions

Phenylephrine is a synthetic α-adrenergic used in the medical practice as mydriatic agent and nasal decongestant, and in the veterinary practice as cardiotonic agent. Its receptor is the adrenoceptor α 2 A.

It is a ubiquitous G protein-coupled receptor receptor localized in the sarcolemma of the smooth muscle. It produces effects by the medium of the inhibition of adenylate cyclase through the action of q-type G protein. Once activated, these proteins stimulate the activity of *Phospholipase C, stimulating the release of IP3 and DAG which act as secondary messengers that mediate the release of intracellular Ca^{2+} with immediate effect on the muscular contraction and, in subsidiary, activate PKC [4].*

It acts primarily on α2-adrenergic receptors in the arterial smooth muscle, regardless of their localization, producing vasoconstriction.

Administration of phenylephrine produces a strong and stable effect which can be replicated in all arterial preparations. This substance was elected also because it is completely hydrosoluble and stable in solution for several weeks, given that it is a synthetic substance (*fig. 1*).

Due to its availability, the substance was used as contraction witness in all subsequent experimentations.

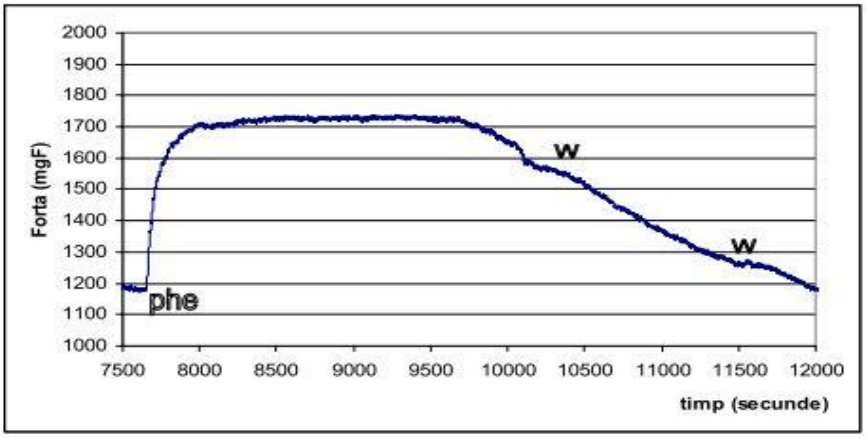

Figure 1 - Typical aspect of phenylephrine-induced contraction of an artery in the resistance segment

A dose-response curve to phenylephrine was done for preparations both in the presence as well as in the absence of the vascular endothelium (*fig. 2*).

The investigation of the effects of the vasoconstrictor substances was done as an evaluation of both the force index (F/gu) and the contraction relative to the 10^{-6} M phenylephrine-induced contraction, which was regarded as reference contraction (100%).

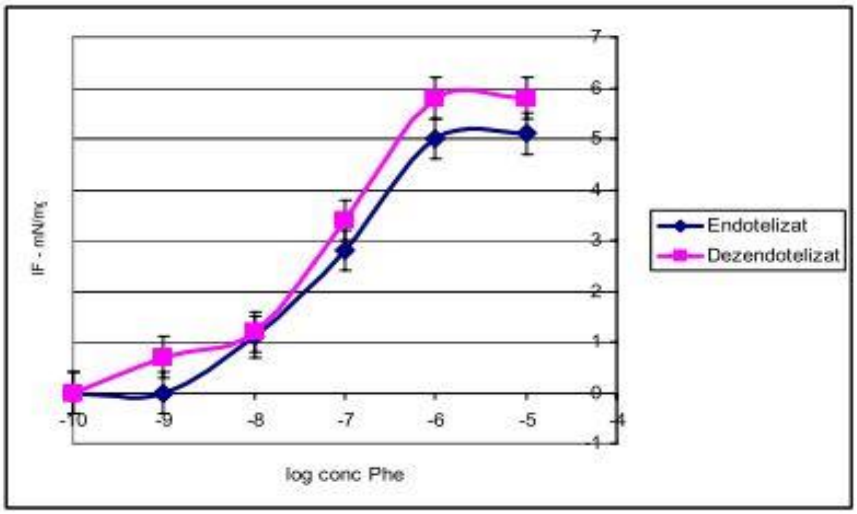

Figure 2 - Dose-response curve to phenylephrine - control group

Force indices of endothelialized vessels harvested from animals that had no vascular affections.

Rat – FI = F/gu = 2,5 gf/5mg – 25mN/5 = 5

Cat – FI = F/gu = 3,1 gf/5mg = 6,2

Dog – FI = F/gu = 3,4 gf/5mg = 6,8

Horse – IF = F/gu = 4,8gf/5mg = 8, 16

Where: *F* represents the force developed by the preparation, expressed as mN (N x 10^{-3}), and *gu* is the wet weight expressed as milligram tissue.

As it can be seen in *fig. 3*, the force of the arterial ring preparations harvested from big animals was also bigger. Therefore, the adrenergic responsiveness proved to be directly proportional to the quantity of the vascular smooth muscle in preparations.

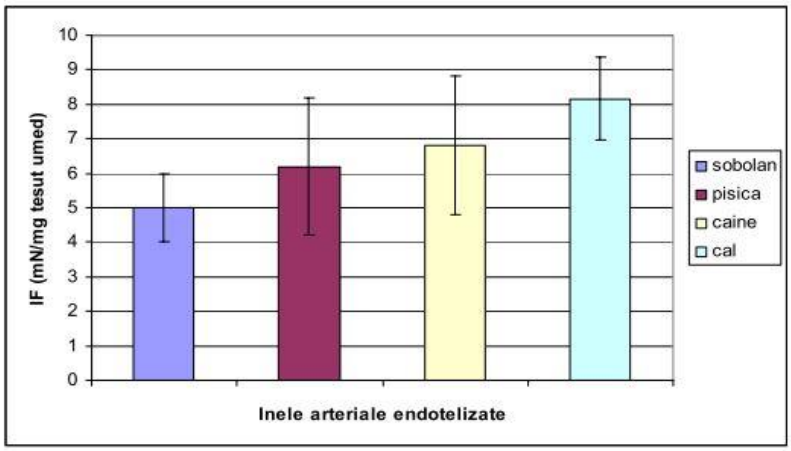

Figure 3 - Force index variation of arterial ring after administration of 10-6 M Phe

The response of *de-endothelialized* preparations to phenylephrine: as it can be seen in *fig. 4*, de-endothelialization led to an increased reactivity to phenylephrine by values ranging from 16% (rat), 9,25% (cat), 8,39 (dog) and 9,27% (horse). This phenomenon is in accordance with the results recorded by literature and with our expectations based on previous results.

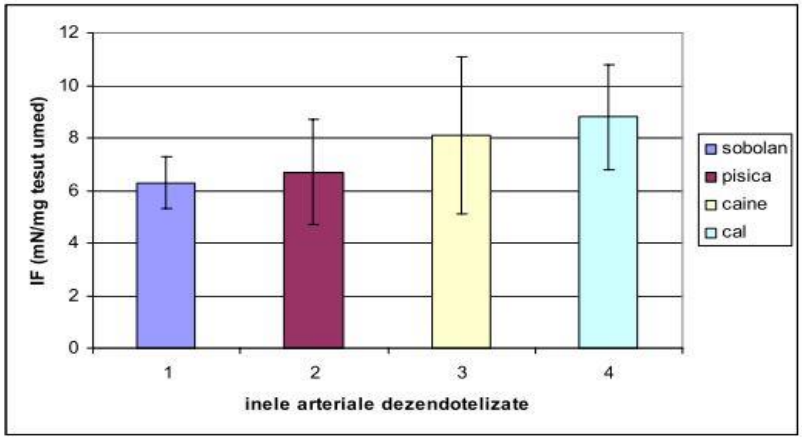

Figure 4 - Force index variation of de-endothelialized arterial rings after administration of 10^{-6} M Phe

Fig. 5 shows the cumulative graph of the two data sets. It can be seen that the stimulation of the contractile response is a more diminished effect in arteries harvested from big animals (horse, dog), which can imply that *catecholamines* involve the *basal vascular tone* regulation less *when resistance arteries show very large muscle masses.*

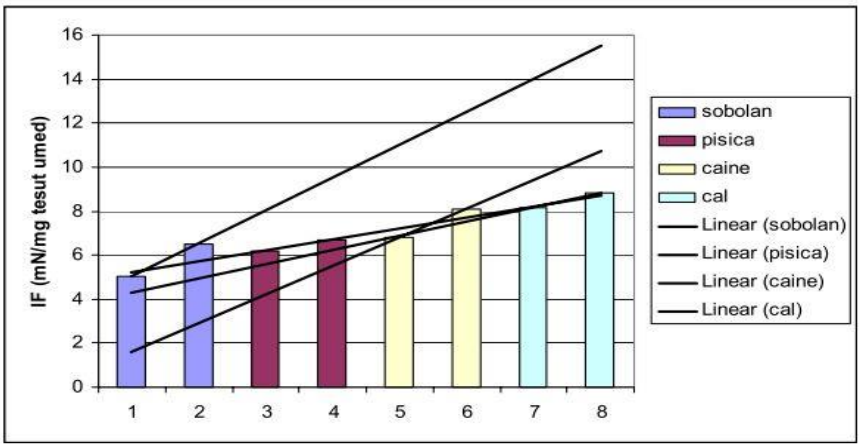

Figure 5 - Cumulative results (endothelialized and de-endothelialized) to which regression lines of the variations between the contraction of the endothelialized preparations and that of the de-endothelialized preparations were added

Conclusions

From the results shown above, the following can be inferred:

The α-adrenergic reactivity is similar in all species in the study, dose-dependent.

The only significant differences recorded are quantitative, possibly caused by the quantity of the vascular smooth muscle present in the different types of arteries used in the study.

Referring to histological data, it is safe to say that, given the percentage of smooth muscle, the results are within normal limits.

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Management of breeding sows in a farm in Tulcea County

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Abstract

The study was carried out between June 2016 – May 2017 in a private unit in Tulcea County, and involved 60 sows and gilts of different ages. The animals were divided into two experimental groups: group I involved 30 F1 gilts (20 gilts LWxL and 10 gilts LWxD) aged between 7-8 months and group II had 30 F1 sows (20 sows LxLW and 10 sows LWxL) aged between 28-36 months. Gilts were checked for estrus twice daily and were artificially inseminated. For the first group the mean value of piglets born alive was beetwen 7.9±3.84 for LWxL gilts and 9.85±2.73 for LWxD gilts. In group II the data recorded were 9±3.98 piglets for LxLW and 9.6±3.20 piglets for LWxL sows. Good results in terms of weaned piglets were recorded for LWxL gilts (9.7±2.73) and LWxL sows (9.1±2.96). In group II there were 13 cases of piglet mortality. The results of our study indicate that gilts presented higher values of the fertility parameters such that age is an important index to take into consideration.

Keywords*: gilts, piglet, sow.*

Introduction

It is already common knowledge among swine raisers throughout the world that crossbred are better than purebred sows. Sow longevity is important because litter size and piglet weights increase until the fourth or fifth parities, and the number of pigs weaned per sow per year increases until the sixth and seventh parities. Mature, structurally sound replacement gilts will most likely reach their fourth parity, at which time they are most productive for the swine operation (Stalder et al., 2003; Rodriguez-Zas et al., 2006). In a study presented by PIC, 2015, the average sow replacement rate was 45%. This high rate is due to failure of postpartum sows to return to estrus and conceive, poor reproductive performance, poor feet and leg soundness, and introduction of new genetic lines (Tomes et al., 1982; Lucia et al., 2000; Gill et al., 2007; Engblom et al., 2008).

Once gilts enter the sow farm, they must be managed in a way that does not restrict their productivity potential. Feed intake, stall acclimation, boar exposure, body weight at breeding, body weight gained in gestation, and first lactation management all determine the lifetime productivity potential of the female (Sow & gilt management manual, 2015).

The aim of this study was to identify and evaluate gilts and sows management in a productive sow farms. The following parameters were investigated: occurrence of estrus, number of AI needed for a gestation, pregnancy rate, piglets born per litter – alive and dead, weaned piglets.

Material and methods

The study was carried out between June 2016 – May 2017 in a private unit in Tulcea County, and involved 60 sows and gilts of different ages. The animals were divided into two experimental groups: group I involved 30 F1 gilts aged between 7-8 months and group II had 30 F1 sows aged between 28-36 months.

In group I 20 of the gilts were Large White crossed with Landrace boars (F1 - LWxL) and 10 gilts were Large White crossed with Duroc boars (F1 - LWxD). Regarding the group II 20 sows were Landrace crossed with Large White boars (F1 - LxLW) and 10 sows were Large White crossed with Landrace boars (F1 - LWxL).

The animals were monitored until first clinical signs of estrus were detected. Estrus was detected with boar exposure twice a day. Duroc boars and terminal sires with good fertilizing capacity were used. Semen was collected twice per week and volume, color and motility were evaluated. The semen was diluted in Merck III extender (Minitube), packaged in 100 ml bottles with 4.0 x 10^9 spermatozoa. Diluted semen was storaged at 18 ^{0}C.

Animals received first artificial insemination (AI) 12 hours after estrous was detected and second AI after 21 days. Pregnancy was determinated at 21 days observing estrus return. All pregnant gilts and sows were kept under observation and a week before parturition were moved in individual boxes.

The following parameters were investigated: occurrence of estrus, number of AI needed for a gestation, pregnancy rate, piglets born per litter – alive and dead, weaned piglets.

Results and discussion

In group I, 20 of the gilts were Large White crossed with Landrace boars (F1 - LWxL) and 10 gilts were Large White crossed with Duroc boars (F1 - LWxD). The LWxL gilts presented estrus at 7 months and 9 days, duration of estrus was 35.37±8.30 with individual values between 24-48 hours. Estrus return after first and second artificial insemination was 20% respectively 5%. In the present experiment the conception rate after three artificial inseminations was 100%. The LWxD gilts showed signs of estrus age between 7-7.6 months. The pregnancy rate after first artificial insemination was 70% and 30% of the gilts repeated estrus at 21 days. Performing the second artificial insemination pregnancy rate was 100%. The results of the fertility parameters are presented in table 1.

Table 1- Fertility parameters recorded for the two experimental groups

		No. of AI for a gestation	No. of piglets born alive	No. of piglets born dead	Weaned piglets	Large piglets	Medium piglets	Small piglets
Group I	LWxL	1.25±0.55	9.85±2.73	-	9.7±2.73	156	28	10
	LWxD	1.3±0.48	7.9±3.84	-	7.5±4.19	35	36	4
Group II	LxLW	1.75±0.85	9±3.98	6	8.6±3.87	148	7	6
	LWxL	1.2±0.42	9.6±3.20	7	9.1±2.96	83	4	4

In group II 20 sows were Landrace crossed with Large White boars (F1 - LxLW) and 10 sows were Large White crossed with Landrace boars (F1 - LWxL). The first signs of estrus were detected days 4 to 6 after weaning with a mean value of 5±0.45 days for LxLW sows and 5±0.47 days for LWxL sows. The mean duration of estrus was 36.74±6.00 with individual values between 24-48 hours. Regarding the results after performing artificial insemination in LxLW sows the data are the following: the conception rate after first AI was 50% and 75% after the second AI. In this category, it was necessary to carry out the third AI to achieve a 100% conception rate. Concerning the LWxL sows the results indicate that 8 out of 10 sows were diagnosed pregnant after first AI and one after second and third AI (table 1).

At weaning, piglets were classified in 3 classes according to their size: large piglets, medium piglets and small piglets. Our results indicate that the LWxL gilts showed higher mean of weaned piglets (9.7±2.73) compare with others categories. In group II there were 13 cases of piglet mortality.

Conclusions

The results of our study indicate that gilts presented higher values of the fertility parameters such that age is an important index to take into consideration. We recommend sows reformation to a number of 6-7 gestations, about 3 years - after this age, peak reproductive performance is no longer achieved.

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Incidence of clinical and subclinical mastitis in a dairy herd in Cluj County

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Abstract

The purpose of this work was to supervise the activity and health of the mammary gland in dairy cows in a dairy specialty unit in Cluj County. The objectives of this study can be summarized as follows: diagnosis of mastitis, carrying out a study on the incidence of mastitis according to some factors, performing the microbiological exam and treatment. Following the clinical examination, 6 cows (5.36%) were diagnosed with clinical mastitis. Depending on the type of lesion, it was found that acute mastitis had the highest rate (66.66%), and according to the symptomatology, there were found 2 cases (33.33%) with haemorrhagic mastitis, catarrhal mastitis and purulent mastitis. The highest incidence of clinical mastitis based on the number of lactations was in cows at 3th and 4th lactation (83.33%). Depending on age, all cases of clinical mastitis have been detected in cows aged 4-7 years. After the application of the Kerba Test method, a total of 10 cows (8.93%) were diagnosed with subclinical mastitis. The incidence of subclinical mastitis according to the age of the animals shows that 60% of the cases were recorded in cows aged 5-7 years. The incidence of subclinical mastitis regarding the number of lactations was 20% in cows at 5th lactation, 10% in those at 4th lactation, 30% in those at the 3th lactation, 30% in the 2nd lactation and 10% in the first lactation cows. Bacteriological exam revealed that the most commonly encountered pathogen was Staphylococcus spp. As regards the treatment of clinical mastitis, comparing the average of administrations, we observed a better efficacy of Masti Veyxym and Synulox LC therapy than in the treatment that used exclusively Synulox LC (2.5 vs. 4.5), although performed under the same conditions. In subclinical mastitis we achieved 100% healing after a single administration.

Keywords*: mamary gland, Kerba Test, mastitis, dairy cows.*

Introduction

Mastitis is defined as an inflammation of the parenchyma of mammary gland, which can reduce milk yield and alter milk composition (Souto et al., 2010). There are two main classes of mastitis. The first is clinical mastitis, which manifests signs observed from the animal or the milk. The other is subclinical mastitis, which produces no visible signs from the udder except when using diagnostic tools. The reduction in milk production attributed to sub-clinical mastitis may account for 70%–80% of the total losses (Philpot and Nickerson, 1991).

Mastitis control program can reduce economical loss and increase herd efficacy and milk hygiene. Epidemiological data including mastitis prevalence, mastitis causing organisms, predisposing factors and response to treatment, are necessary for the establishment of a mastitis control program (Hashemi et al., 2011).

The purpose of this work was to supervise the activity and health of the mammary gland in dairy cows in a dairy specialty unit in Cluj County. The objectives of this study can be summarized as follows: diagnosis of mastitis, carrying out a study on the incidence of mastitis according to some factors, performing the microbiological exam and treatment of the mastitis

Materials and method

The study was performed in a private dairy farm located in Cluj County, between September 2016 - May 2017.

Diagnosis of clinical mastitis. The diagnosis of clinical mastitis was based on the clinical examination, which consisted on inspection and palpation of the udder and milking test.

Diagnosis of subclinical mastitis. Subclinical mastitis detection was performed using the Kerba Test method.

Bacteriological examination of milk samples collected from cows suffering from bovine mastitis. For the isolation of microorganisms from mastitis milk samples, common culture media were used: broth, glucose agar 1‰, sheep blood agar 5%, selective media (Baird-Parker medium), and MacConkey gel for *Enterobacteriaceae*. The protocol has the following stages: collecting milk samples, preparation of samples, seeding samples and performing antibiogram method – diffusion technique. Standardized micro-tablets were used with the following antibacterial agents: amoxiclav, ceftiofur, mastidisc, enrofloxacin, florfenicol, penicillin, gentamycin, cloxacillin and colistin.

Treatment of mastitis. Treatment of clinical mastitis was performed with two products, depending on the type of disease: Nekro Veyxym, solution for injection and Synulox LC., intramammary suspension. For the treatment of subclinical mastitis the Tetra-Delta intramammary product was used.

Results and discussions

After the clinical examination, 6 cows (5.36%) were diagnosed with clinical mastitis (CM) and by the Kerba Test method, 10 cows (8.93%) were diagnosed with subclinical mastitis (SBM). In the present study, like some other studies, the majority of the cases of mastitis were subclinical (Almaw et al., 2008; Getahun et al., 2008; Hashemi et al., 2011).

After the organoleptic examination of the milk were identified 3 cases (50%) with catarrhal mastitis, 1 case (16.66%) with haemorrhagic mastitis and 2 cases (33.33%) with purulent mastitis. Analyzing the frequency of mastitis in cows, in relation to age, the highest value for subclinical mastitis was observed in cows ranging between 4-7 years - 60% and for clinical mastitis was 66.66% in cows aged beetwen 6-7 years. The occurrence of the mastitis is directly proportional to the number of lactations. For clinical mastitis, the maximum incidence was found during the fourth lactation (66.66%), while the minimum value was recorded during the second and third lactation (16.66%). The same report was maintained for subclinical mastitis with limits between 10% (first lactation) and 40% (fourth lactation). The highest incidence of subclinical mastitis depending on the affected quarter was recorded for the hindquarters (clinical mastitis – 83.33%, subclinical mastitis B - 80%).

Our investigations were conducted on a number of 10 milk samples selectively collected from cows diagnosed with clinical and subclinical mastitis. Samples were collected from a single quarter, usually the affected or all quarters when the entire mammary gland was affected. Bacteriological examination led to the isolation of microorganisms belonging to 5 different types: *Staphylococcus*, *Micrococcus*, *Bacillus*, *Escherichia*, and *Trueperella. Staphylococcus spp.* was isolated from 8 samples (80%), alone or in association with other species (*Escherichia coli*, *Bacillus*). *Staphylococcus* was isolated in pure culture from 4 samples (40%). *Staphylococcus* associated with *Escherichia coli* or *Bacillus* was isolated from 4 samples (40%) each. In 10% of the samples, were identified *Micrococus* alone or associated with *Trueperella pyogenes*. The results regarding the study of the sensitivity of isolated strains to various antibacterial substances are systematized in Table 1.

Staphylococci were the predominant bacteria as the cause of mastitis, which is in agreement with the findings of Meranzadeh et al. (2001) and Gharagozloo et al. (2003)

Treatment of haemorrhagic mastitis was performed with Synulox LC for 3-4 days, single administration/day. Clarification of the milk occurred between 7 and 9 days. In this type of mastitis,

repeated milking was not performed (only in the morning and in the evening). Regarding catarrhal and purulent mammals, the therapeutic protocol was carried out with two products: Nekro Veyxym and Synulox LC. The first product was administered once/day (in the evening), 0.4 ml/10 kg, intramuscularly, while the second product was administered 2 times/day, intramammary (morning and evening). A number of 5 cows (83.33%) were fully recovered after 2-4 days of treatment. A single cow (16.66%) with purulent mastitis was partially recovered, the affected quarters presenting clinical healing but with the reduction of glandular tissue in favor of connective tissue, the sclerosis of the quarter and reduced productivity. Comparing the average of administrations, we observed a better efficacy of Masti Veyxym and Synulox LC therapy than in the treatment that used exclusively Synulox LC (2.5 vs. 4.5), although performed under the same conditions.

In subclinical mastitis we achieved 100% healing after a single administration of Tetra Delta product.

Table 1 - The results regarding the study of the sensitivity of isolated strains

Strain/ number of the sample	AMC	EFT	NBT	ENF5	FL30	P10	G10	CLOXA (CX30)	CS
Staphylococcus spp. /530	R	15,01	26,10	26,21	22,43	10,80	18,66	28,14	R
Staphylococcus spp. /531	R	16,14	24,46	19,89	22,98	15,87	19,19	22,47	10,41
Staphylococcus spp. /533	R	14,02	25,59	25,74	19,67	R	17,40	19,18	R
Staphylococcus spp. /534	R	10,90	23,58	25,01	18,98	R	16,53	19,55	R
Bacil nehemolitic/534	13,52	19,51	22,70	23,44	25,82	24,79	20,34	20,64	13,16
Staphylococcus spp. /536	R	12,06	22,41	22,45	21,64	R	16,25	19,55	R
Staphylococcus spp. /537	R	14,64	22,18	18,75	22,59	15,28	19,79	19,22	R
Bacillus lickeniformis/537	R	11,25	30,03	26,51	27,25	14,38	20,95	17,29	R
Trueperella pyogenes/538	19,20 resistant colonies	25,84	34,80	32,04	30,30	36,26	27,60	23,92	R
Sensitivity	20%	90%	100%	100%	100%	60%	100%	100%	20%
Legend	AMC30: amoxicilin + clavulanic acid; EFT: ceftiofur; NBT 70: mastidisc (neomicin 30 μg, bacitracin 10 u.i, tetracyclin 30 μg); ENF5: enrofloxacin; FL30: florfenicol; P10: penicilin; G10: gentamicin; CX30=cloxa: cloxacilin; CS: colistin or colimicin; R= resistant.								

Conclusions

1. Sixteen cows (14.29%) were diagnosed as positive with subclinical and clinical mammitis;
2. Following the clinical examination a number of 6 cows (5.36%) were diagnosed with clinical mastitis, and by the Kerba Test method, a number of 10 cows (8.93%) with subclinical mastitis;
3. The most common pathogen was Staphylococcus spp .;
4. As regards the treatment of clinical mastitis, comparing the average of administrations, we observed a better efficacy of Masti Veyxym and Synulox LC therapy than in the treatment

that used exclusively Synulox LC (2.5 vs. 4.5), although performed under the same conditions.

5. In subclinical mastitis we achieved 100% healing after a single administration.

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Identification of *Microsporum canis* in cutaneous lesions of cats from Timis County

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Abstract

Dermatophytosis has a practical and medical importance both in the veterinary and human medicine due to its zoonotic potential causing economic problems worldwide. The aim of this study was to identify the etiological agents involved in the appearance of cutaneous lesions. 43 cats were examined, from eight locations in the Timis County, with or without cutaneous lesions at the age of 1 month to 11 years. 37 cats belonged to the European breed, one Birmanese breed, three were Persian and two were British Shorthairs. Samples of hair, squamae and crusts were collected from every animal and placed in Petri plates. Direct microscopic examination of hairs, squamae and crusts was done using the slide and coverslip method, with lactophenol and it was examined using the x10 objective. The sample were cultivated on Sabouraud agar gel and DTM (Dermatophyte test). Microsporum canis was the only one species indentified in the cutaneous lesions (group 1) in 35% out of examinated cats (7/20). In group 2 (asymptomatic cats), the only species identified was Microsporum canis in one individual (1/23 respectively 4.37%).

Key words: *Microsporum canis, cats, Timiș County*

Introduction

Dermatophytes are among the most common zoonotic agents. Dermatophytosis are considered the third most frequent cause for skin diseases in children younger than 12 years of age and the second most common cause in adults (9).

Bibliographic studies indicate a high prevalence of carnivore dermatophytosis, being the most widespread fungal disease with worldwide distribution. *Microsporum canis* is an agent commonly spread on a global level, most often association between man and his pet (6, 3, 2, 5).

The most common pets are cats and dogs and they can be the source for *Microsporum canis* and *T. mentagrophytes* infections (7).

Dermatophytosis affect 20-25% of the world population and in the past 15-20 years a significant rise in their prevalence has been observed which can be attributed to the change in migration models, in the development of tourism and the modification of socio-economic conditions (1).

Materials and methods

In the period October 2014-September 2016 a number of 43 felines from eight towns in the Timis County: Timișoara, Romanian Sânmihai, German Sânmihai, Pădureni, Variaș, Parța, Sânandrei, Cornești. The cats were aged from one month to 11 years. There were 13 males and 30 females, 37 of them belong to the European breed, one was *Birmanese*, three were *Persian* and two were *British Shorthairs*.

The cats were divided into two groups: one group was formed of 20 cats that presented cutaneous lesions and another group, 23 cats that were asymptomatic.

Samples of hair and squamae were collected from each animal from the two groups. The samples were collected and examined through direct microscopy. They were clarified using

lactophenol and they were cultivated on 43 Sabouraud agar gel and 43 DTM. The development of the colonies was followed on the aspect, colour and shape point of view (2, 4, 5).

Samples were collected from the colonies that grew and they were examined microscopically (with x40 objective). They were clarified with lactophenol and blue lactophenol.

Results and discussions

Clinical examination revealed distinct lesions located on: head (fig. 3), ears (fig. 4), body (fig. 2) and limbs (fig. 1). The lesions were dry and characterized by moderate erythema, squamae, depilation with no pruritus.

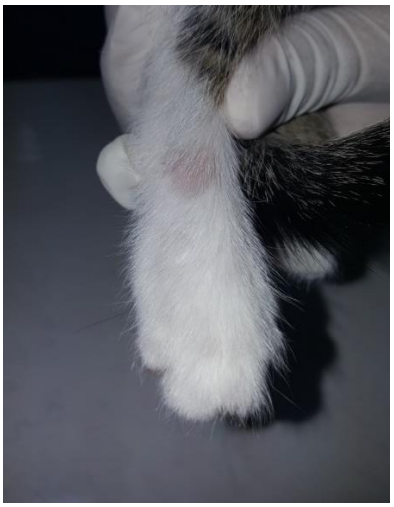

Fig. 1. Legs lesion

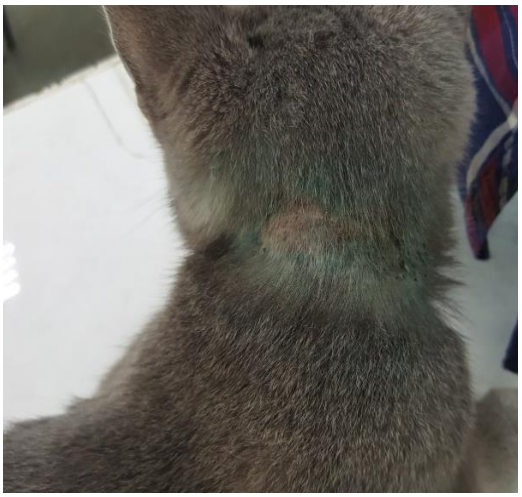

Fig. 2. Dry lesion on the body

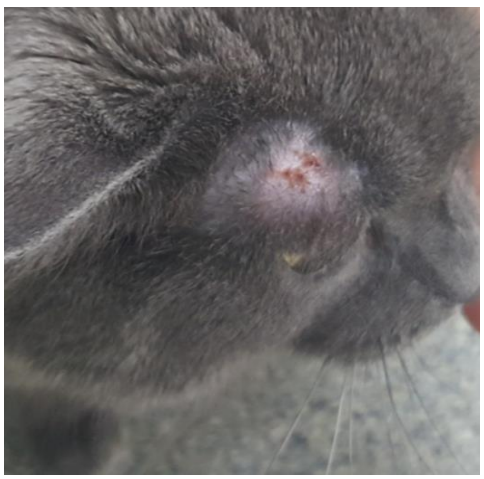

Fig. 3. Erythema and depilation

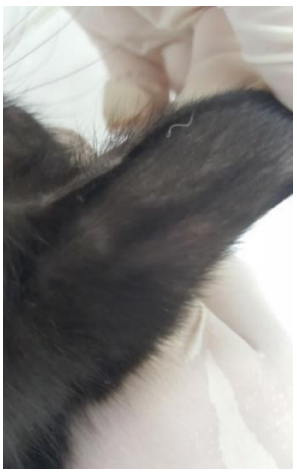

Fig. 4. Lesion on the ear

At the microscopic exam we did not identify characteristic parasitic elements: hife and spores. Ectoparasites were not present.

Macroscopic examination of Sabourad cultures allowed us to identify fluffy colonies and white-yellow pigmentation on 11,62% (5/43) out of the 43 samples examined (fig. 5).

Macroscopic examination of colonies grown on DTM revealed white fluffy colonies with turning color of the medium into red one (fig. 7) on 18,60% (8/43) out of the 43 samples examined.

Microscopic examination of collected samples from colonies grown on culture medium led to the identification of characteristic macroconidia (macroconidias in the form of a spindle with thickened walls, doubled, septal in several compartments) of the species *Microsporum canis* (fig. 6).

This dermatophyte was identified in seven colonies, of which four were both Sabouraud and DTM, and three were DTM only, in group 1 (35%) and in group 2 only in a single colony on DTM (4,34%).

Fig. 5. Sabouraud agar

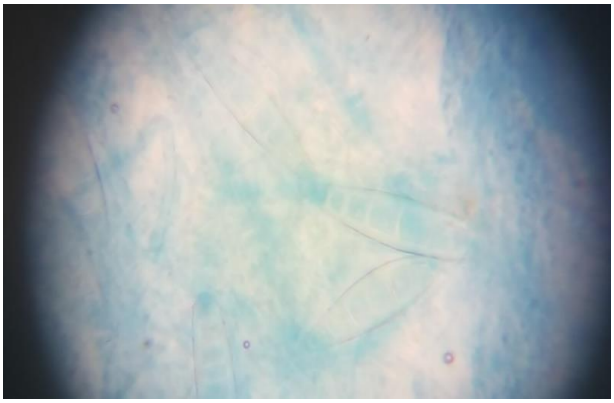

Fig. 6. *Microsporum canis* macroconidia

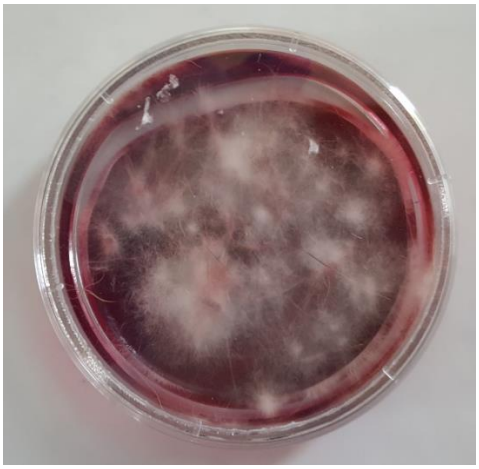

Fig. 7. Dermatophyte test

Dermatophytes are a major problem for public health in several countries and the drivers of distribution and transmission are: direct contact with animals, general hygiene and climatic conditions (7). Segal and Frenkel, 2016 (7) conducted a study stating that 100% of the cats examined in Italy living in the outside were positive for infection with dermatophyts, and in

Germany those living in the apartment, 21% were asymptomatic carriers of *Microsporum canis*, these being a source for animals and humans both.

It has been reported that the prevalence of dermatophytes infection also varies with temperature, humidity, season and geographical area (8). Some studies have indicated that the age of the animals is related to infection with dermatophyts and that animals less than one year old are more prone to these, so animals up to one year showed an isolation rate of 4.1%, and those aged between one and three years of 1.5% (7).

Conclusions

Microsporum canis was the only one species indentified in the skin lesions (group 1) in 35% out of examinated cats (7/20).

In group 2 (asymptomatic cats), the only species identified was *Microsporum canis* in one individual (1/23 respectively 4.37%).

The presence of *Microsporum spp*. in cats with skin lesions and asymptomatic demonstrates that they can be a source of infection for animals and humans both.

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Therapeutic efficacy testing of two topical products used in dry demodicosis lesions in dogs from Mehedinti County

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Abstract

Canine demodicosis is a serious dermatitis, difficult to treat, often recurrent, and the biology and pathogenesis of the aetiological agent prevent the action of drugs on skin. The aim of the study was to evaluate the therapeutic efficacy in dry canine demodicosis of two products: Ointment Canider (containing formamidine) and Demosymcan - gel whose components are natural and fixed in fast absorbing gels. The results obtained were statistically interpreted from the clinical and parasitological healing point of view (erythema, alopecia, squamae, hyperseboree, microscopic examination of the cutaneous scraping) and were calculated: scores averages, medians, standard deviations, minimum and maximum values. The clinical signs remission and negative microscopic examination of skin scraping from lesions were reported at 9 and 15 weeks in group I treated with the product based on honey, propolis, apple vinegar and extracts plant and 12 and 16 weeks, respectively, in lot II, where the product was based on amitraz and neomycin. Exacerbation of clinical signs and presence of mite in microscopic slides were revealed in the control group, to which a gel containing no ingredient was applied.

Key words*: dry demodicosis, dogs, therapy, Mehedinți County*

Introduction

Cutaneous parasitosis, demodicosis is diagnosed in a number of domestic and wild animals: carnivores, rodents, ruminants, insectivores. It is produced by *Demodex* species, the only genus of the *Demodecidae* family. A short history of the drug used in canine demodicosis over a hundred years confirms the claim that canine demodicosis is a serious dermatitis, difficult to treat, often recurrent, and the biology and pathogenesis of the aetiological agent prevent the action of drugs on skin (1, 8, 10, 11).

Current information on canine demodicosis is focused on therapy. While most authors use the acaricides treatment by spot-on (moxidectin-imidacloprid), oral or systemic administration of avermectin and milbemycin or topical applications of formamidines, other authors recommend non-irritating and non-invasive skin and body products (2, 3, 5, 6, 7, 10, 11, 12).

Taking into account the high prevalence of canine demodicosis in our country and in the world, the complex therapeutic approaches and the specialists emphased recommandation natural product, without irritating effects on demodectic skin, the aim of the study was to evaluate the therapeutic efficacy of two products: Ointment *Canider* (containing formamidine) and *Demosymcan - gel* whose components are natural and fixed in fast absorbing gels.

Materials and methods

A total of 62 dogs, different breeds, males and females, aged 3-48 months, were selected for this study. The dogs had dry localized cutaneous lesions manifested by: erythema, alopecia, fine squamae, whitish, hyperseboree. The lesion distribution was: periocular, cheek, ears, lips, ventral cervical region, olecranic region, anterior forearm, dorsal lumbar region, trunk, posterior legs. The dogs belonged to the Veterinary Districts of Greci, Şimian, Tâmna, Mehedinţi County. The dogs were divided into three groups.

Lot I - 22 dogs to which the product Demosymcan Gel was applied for the treatment of dry lesions in canine demodicosis

Gel for the treatment of dry lesions in canine demodicosis - is an original product produced by the Parasitology Department team - VMF Timisoara, in collaboration with *The National Research and Development Institute for Textile and Leather*, Bucharest. The gel is OSIM registered as patent application no. A 00075/ 1.02.2016. The invention refers to a gel for the treatment of dry canine demodycosis lesions produced in dogs by mite *Demodex spp.* and it based on of the main components from honey, propolis, apple vinegar and hydro-glycero-alcoholic extracts from the buds of various plants.

The product was awarded at the National and International Salons of Inventions (Iasi, Cluj, Timisoara, Bucharest, Brussels, Geneva, Barcelona) where it won 7 gold medals and 8 special prizes (Fig.1).

Fig. 1. Gel for dry lesions of canine demodicosis treatment

Lot II - 20 dogs to which the Canider Ointment product was applied (contains amitraz and neomycin sulfate). Lot III, the control group - 20 dogs to which a gel wich containing no ingredient.

The dogs were followed weekly for a period of 3 months. Monitoring began one week after the treatment onset and continued after clinical and parasitological healing.

The results obtained were statistically interpreted from the clinical and parasitological healing point of view (erythema, alopecia, squamae, hyperseboree, microscopic examination of the cutaneous scraping) and were calculated: scores averages, medians, standard deviations, minimum and maximum values.

Results and discussions

By corroborating the results of the anamnesis, the clinical and dermatological examinations, the diagnosis of demodicosis was established (Figures 2, 3, 4, 5).

Fig. 2. Descuamation

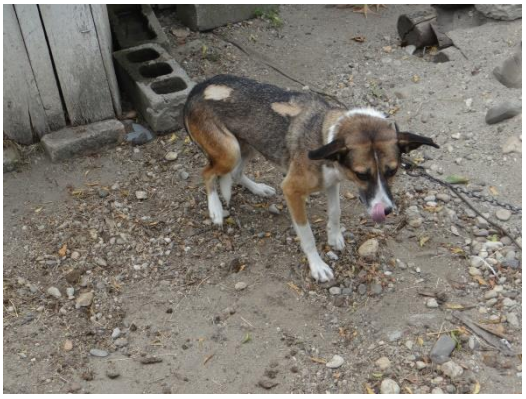

Fig. 3. Alopecia

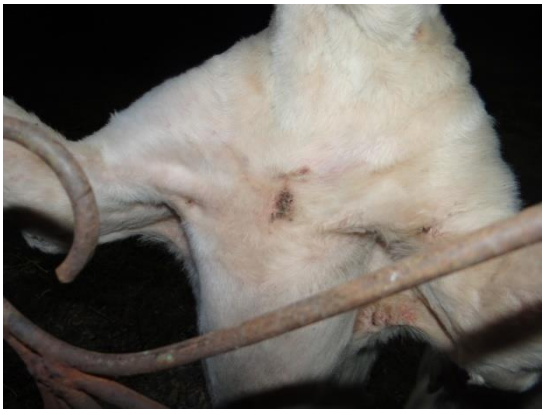

Fig. 4. Erythema

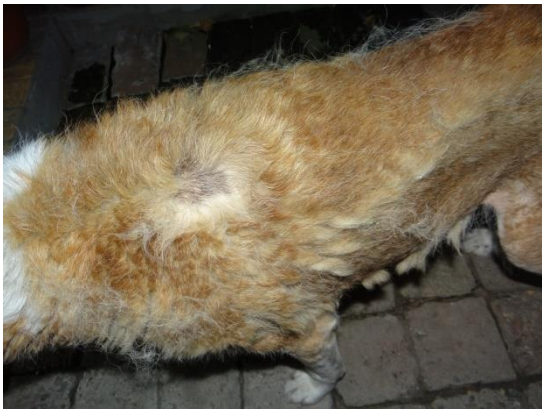

Fig. 5. Hyperseboree

The demodicosis diagnosis was confirmed by microscopy. The life stages of *Demodex canis* mite have been highlighted in slides clarified with paraffin oil or lactophenol (Fig.6).

Fig. 6. *Demodex canis*

Each patient's record sheet contained all patient information. Clinical signs (erythema, alopecia, squamae, hyperseboree) and microscopical examination of skin scraping have been evaluated weekly on a scale of 0-5.

The results of the clinical and parasitological evolution of each group during the 12 weeks of treatment are shown in Table 1.

Table 1: The clinical and parasitological evolution results

Săptămâna	Lot I (Demosymcan)					Lot II (Canider U)					Lot III (Gel)	
	E.	A.	S.	H.	P.H.	E.	A.	S.	H.	P.H.	C.H.	P.H.
1	5	5	5	5	5	5	5	5	5	5	5	5
2	4	5	5	4	5	5	5	5	5	5	5	5
3	3	4	5	3	5	5	4	4	5	5	5	5
4	2	4	3	3	5	4	4	4	4	5	5	5
5	1	3	1	1	5	4	3	3	4	4	5	5
6	0	3	0	0	4	3	2	2	4	4	5	5
7	0	2	0	0	3	3	2	1	3	3	5	5
8	0	1	0	0	3	2	1	1	2	3	5	5
9	0	0	0	0	2	1	1	0	1	2	5	5
10	0	0	0	0	2	0	1	0	1	2	6	5
11	0	0	0	0	1	0	0	0	1	1	6	5
12	0	0	0	0	1	0	0	0	0	1	6	5

Legend: E - erythema, A - alopecia, S - squamae, H - hyperseboree, P.H. - parasitological healing, C.H.- clinical healing

Descriptive statistic establishes significant differences between the three groups. In group I treated with Demosymcan Gel, clinical and parasitological recovery were significant ($p = 0.000 < 0.05$, respectively $p = 0.003$). The results of the statistic test indicate that Demosymcan Gel has the highest therapeutic efficacy.

It is widely accepted that the successful treatment of this parasitosis involves knowing the biology of the parasite, making it difficult to find an acaricide that is efficient against mites and, at the same time, harmless to the dog, the skin being a rather fragile structure when contacted with the therapeutic substances (1, 7, 10, 11).

The presence of *Demodex* mite leads to a change in skin microclimate: alkalinisation of pH, changes in the composition of the lipid layer, increase in the concentration of fatty acids, reactions leading to an irritant effect of most topical formulations. More over, the complex mechanism of skin absorption is influenced by the nature of the base, the condition of the skin and the biological factors.

15 years ago, the first therapeutic study in canine demodicosis was initiated using a non-acaricid product (8). The results obtained were re-evaluated and developed, and the final result is the Demosymcan Gel Patent Application for the treatment of dry lesions from canine demodicosis, product used for group I.

The results of the present study reveal a higher therapeutic efficacy of the above-mentioned product compared to the Canider U, the clinical healing and the negative microscopic examination of the skin scraping being noted in a shorter time in group I (at 8 weeks and 14 weeks after the onset of treatment), compared to group II.

At international level, there are therapeutic studies that support the treatment of canine demodicosis with herbal substances, plant extracts, non-irritating for skin but efficient in remitting clinical signs and in parasitic healing: the Maggacite product administered twice a day, the Homeopathic, Product Graphitis 200 administered daily, AV / EPP / 14, Gliricidia (*Gliricidia sepium* decoction), Dermanol, Anbioflam, Immuplus, Ectozee (6, 9).

This comparative therapeutic study between a synthesis product, Canider U, containing amitraz and neomycin (lot II) and a natural product, Demosymcan Gel, containing honey, propolis, apple vinegar and hydro-glycero-alcohol extracts from the buds of various plants (group I) revealed a remission of clinical sign at 8 weeks after the onset of treatment and a negation of the microscopic examination of cutaneous lesions at 14 weeks after the onset of treatment - group I over the longer time- it requires the treatment applied to group II, a result that joins the research that recomand natural, non-invasive therapy for skin.

A therapeutical study (4) conducted in Craiova on a group of 66 dogs diagnosed with demodicosis and treated with Demosymcan Gel (Lot I) and Canider U (Lot II) allows us to highlight the comparative results: remission of clinical sign (erythema, alopecia, squamae, hyperseboree) and the negative microscopic examination of cutaneous scraping from lesions were reported at 8 and 14 weeks in group I treated with the product containing honey, propolis, apple vinegar and vegetable extracts, and at 12 and 16 weeks respectively in group II, where the product was based on amitraz and neomycin. The results of this study reveal that clinical and parasitological healing have been achieved in a shorter time compared to the results of the therapeutic researches carried out in the dogs in Mehedinţi County (4).

Conclusions

The clinical signs remission (erythema, alopecia, squamae, hyperseboree) and negative microscopic examination of skin scraping from lesions were reported at 9 and 15 weeks in group I

treated with the product containing honey, propolis, apple vinegar and extracts plant and 12 and 16 weeks, respectively, in lot II, where the product was based on amitraz and neomycin.

Exacerbation of clinical signs and presence of mite in microscopic slides were revealed in the control group, to which a gel containing no ingredient was applied.

We recommend Demosymcan Gel for treatment of dry lesions in canine demodicosis, applied daily on lesions.

Acknowledgements

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Symptoms and management of indolent corneal ulcers in dogs. Overview of some medical and surgical options - case study

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Abstract

Indolent corneal ulcers are also known in the literature as chronic superficial erosions, recurrent epithelial erosions, superficial chronic corneal epithelial defects. The name itself underlines the recurrent, superficial and non-healing characteristics. The aim of this case report is to compare some of the medical and surgical management options available and their outcome upon corneal healing. The study was performed on dogs of different breeds and ages, presented in for ophthalmic examinations at the Surgical Clinic from the Faculty of Veterinary Medicine Iasi, Romania and private practices in Iasi. For this study, there were only taken into consideration dogs with chronic corneal ulcers that did not heal properly within 7-10 days after diagnosis and with clinical signs of loose epithelium around the margins of the lesions. Local medical treatment included the use of antibiotics, vitamins, hyaluronic acid and amino acids, artificial tears. Surgical options available were debridement, grid keratotomy and temporary tarsorrhaphy. Conclusions show that the evolution is longer if only local medication is applied. If owner complies and if overall health status of the patient allows a short, general anaesthesia, it is better to use the debridement and superficial keratotomy together, to allow new epithelium to attach to the anterior corneal stroma and the ulcer to heal faster.

Key words*: dog, indolent ulcers, debridement, grid keratotomy, temporary tarsorrhaphy*

Introduction

The cornea is the perfectly transparent, anterior component of the eye, playing the role of a convex -concave lens. From outside to inside, the cornea has 5 layers: epithelium, its basement membrane (Bowman), stroma, Descemet's membrane, endothelium (posterior epithelium). It is avascular and it has no pigments, but it has a sensitive innervation, provided by nasociliary nerves of the ophthalmic branch of the trigeminal nerve (cranial nerve V) (1, 3). The density of terminal nerves is higher in the center and lesser at the periphery of the cornea. The cornea plays many roles, such as: mechanical, optical, immunological and tissue healing. (2)

Corneal pathology in domestic carnivores summarizes a variety of disorders, which represent real challenges to the veterinary practitioner (4, 5, 6). Corneal pathology consists in several types of conditions: congenital, traumatic, inflammatory and neoplastic (4, 6).

Literature reviews offer data on corneal diseases that are related to specific disorders, such as indolent ulcers in dogs (4, 7), feline corneal ulcers (8), keratoconjunctivitis sicca or pannus (4), feline corneal sequestra (9).

One of the main reasons for ophthalmic consultation in dogs are corneal ulcers. This keratopathy has many predisposing factors, various symptoms and some treatment possibilities. The present paper resumes the symptoms of indolent ulcers and surgical management options.

Material and method

Research has been achieved on the number of cases presented for ophthalmic examination at the Faculty of Veterinary Medicine in Iasi, the Surgical Clinic, throughout the years 2007-2017 and at the private clinic Pets Land (Iasi). From the total number of carnivores, we collected those presented for a chronic superficial ulcer. A relevant and thorough history, completed by an orderly and extended ocular examination, gave a correct diagnosis and the possibility of successful clinical results.

Results and discussion

The clinical signs of an indolent ulcer that should draw the veterinarian's attention is the lack of resolution after 7-10 days of treatment, persistance of moderate ocular pain, translated by blepharospasm, and any ocular discharge (serous or mucoid). Around the ulcer itself, the corneal epithelium forms a lip, which is nonadherent and prevents the lesion to heal (*fig. 1, 3*). The fluorescein will run under the lip of the ulcer and form a halo (*fig. 2*). If the lesion has evolved for a period of time, neovascularisation may appear (in the characteristic form of superficial branch like vessels) (*fig. 4*). Corneal edema may (*fig. 4*) or may not be present. It is important to say that even if this type of pathology was first documented by literature in Boxer dogs, we have diagnosed it in different breeds, including French Bulldog, Chow Chow, Romanian Shepherd and older crossed breed-dogs.

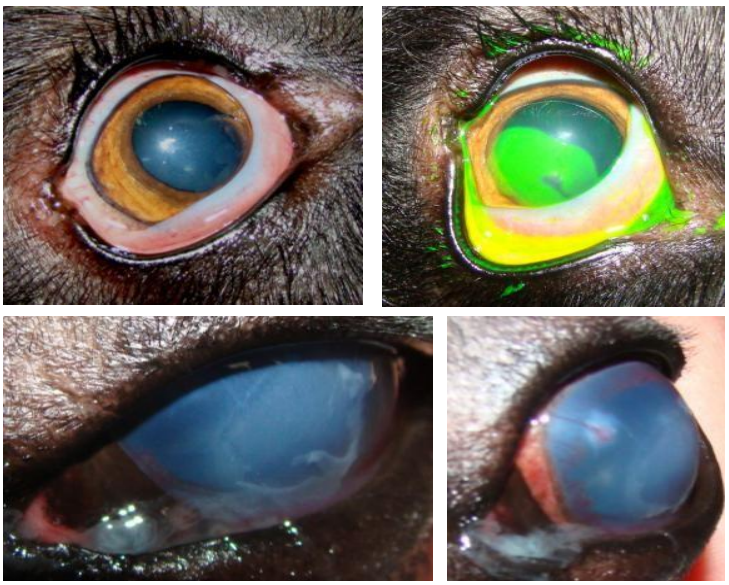


Fig. 1, 2 – Right eye of an 8-year old Romanian Shepherd presented for blepharospasm and ocular serous discharge. Fig. 1) Note the epithelial lip visible with the naked eye, lack of corneal edema and neovascularization. Fig. 2) After fluorescein staining, the surface of the ulcer becomes visible, and the dye is infiltrating under the lip.

Fig. 3, 4 – Left eye of an 5-year old French Bulldog presented for blepharospasm and ocular mucoid discharge. Fig. 3) Note the epithelial lip very visible at a glance and diffuse corneal edema. Fig. 4) Note the small superficial blood vessels arising from the limbus.

Medical therapy consists of local administration of antibiotics (large spectrum antibiotics in solution are preffered to ointments) and hyaluronic acid ophthalmic gel. These will be applied several times a day, depending on the severity of the ulcer. 5 to 10 minutes should be left between applying different products. Owner compliance is, at this stage, crucial for healing. Topical corticosteroids are avoided at all cost. General nonsteroidal antiinflammatory therapy is considered when pain is present and the animal shows signs of self trauma to the eye. An Elizabethan collar will be applied during the healing process.

Left alone, medical therapy usually gives no results, even when applied for weeks. It may be the cause of frustration for the owner. Time should be spared and surgical gestures are recommended, when the lack of healing is noticed.

After sedation, the first step is to apply a local anaesthetic and manually perform the debridement of the ulcer. For this, a cotton tiped applicator is used (*fig. 5, 7*). The loose epithelium is easily removed, and the area of the ulcer will become bigger (*fig. 6*). This step is very important and will ensure proper healing. If done superficial, the lesion will persist. Therefore, debridment is done all the way from the margins of the ulcer all the way to the corneo-scleral limbus.

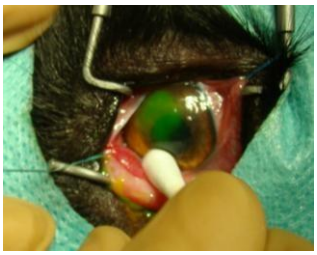
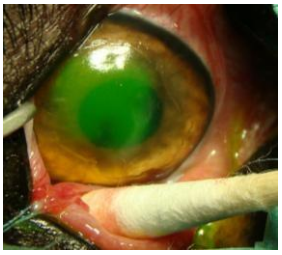
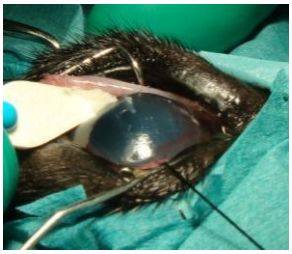




Fig. 5; Fig. 6; Fig. 7 – Corneal debridement

After this, using a 25-gauge needle (*fig. 8*), liniar striations are made to the surface of the cornea. Striations will go beyond the new margins of the ulcer, extending to normal epithelium. They must be visible (deep enough) and they should retain fluorescein at the end (*fig. 7*).

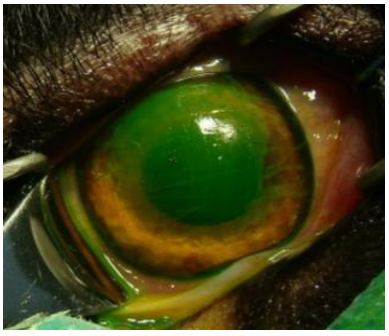
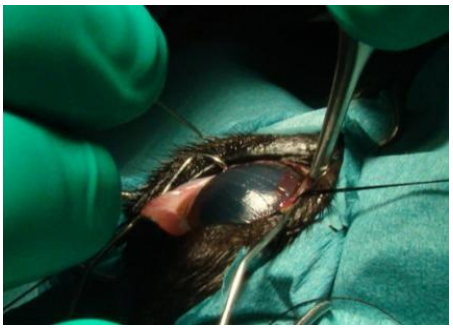



Fig. 7, Fig. 8 – Grid keratectomy performed with the tip of the 25-gauge needle

We placed a temporary tarsorrhaphy (3-4 suture points using USP 0 Polypropylene) for all cases of indolent ulcers, to ensure more protection to the cornea and to the healing process (*fig. 9, 10*).

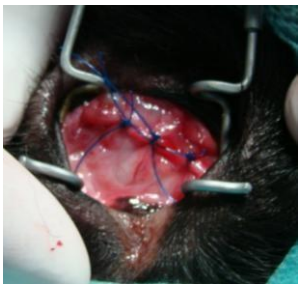
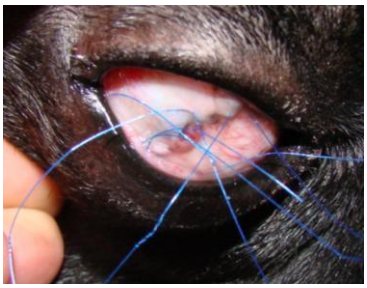
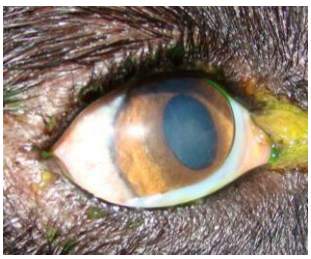



Fig. 9, Fig. 10 – Temporary tarsorrhaphy

Local therapy with antibiotics and healing agent (hyaluronic acid, vitamins E&A) has been continued afterwards until removal of the tarsorrhaphy. Sutures were removed after a minimum of 10 days, usually 14 days after the procedure. At this point, the maneuvre was done using local anaesthetic drops and good restraint.

Results have been satisfying in all cases. Healing was achieved by the time sutures were removed (*fig. 11, 12*). However, minimal scarring or corneal melanosis were present in some animals (*fig. 13*). Relapses are uncommon (we haven't noted any during our clinical experience).



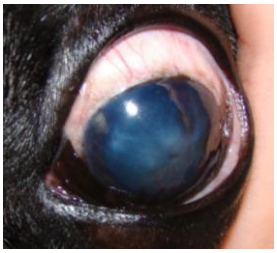


Fig. 11, Fig. 12, Fig. 13 – Healing of the indolent ulcer, without any scaring. In fig. 13 we can see some degree of corneal melanosis.

Conclusions

1. Indolent ulcers are chronic lesions that should draw the veterinarian's attention if lack of healing is seen after 7-10 days of treatment of a superficial ulcer.
2. Around the ulcer itself, the corneal epithelium forms a lip, which is nonadherent and prevents the lesion to heal.
3. Superficial neovascularization and corneal edema may be present, depending on the duration of the evolution.
4. Medical therapy consists of local administration of antibiotics (large spectrum antibiotics in solution are preffered to ointments) and hyaluronic acid ophthalmic gel.
5. Surgical management consists of corneal debridement, grid keratotomy and temporary tarsorrhaphy.

6. Tarsorrhaphy should be in place for 10-14 days. Healing was achieved by the time sutures were removed.
7. However, minimal scarring or corneal melanosis were present in some animals.
8. Relapses were uncommon (we haven't noted any during our clinical experience).

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Application of estrus synchronization and artificial insemination during the reproductive season in goats

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Abstract

The aim of this paper was to test the effectiveness of an estrus synchronization method during the reproductive season in goats kept under extensive conditions as well as to evaluate the effectiveness of natural breeding versus artificial insemination with freshly collected and diluted semen. Estrus synchronization was performed using 2 successive administrations of PGF2alpha in 20 cycling does. Next, 2 groups of 10 does and 2 fertile bucks each were randomly created. In group 1 the females were naturally covered by the two bucks, as soon as heats were expressed. In group 2 semen was collected from the two bucks by electroejaculation, diluted using an original extender based on skimmed milk and artificial insemination was performed. Pregnancy diagnosis was performed in all females using ultrasounds and all pregnancies were monitored until full term. Thus, after performing estrus synchronization followed by natural breeding or artificial insemination in the two experimental groups, the following reproductive indices were obtained: group 1: conception rate 80% and prolificity 170%; group 2: conception 90% and prolificity 220%. The two biotechnologies - estrus synchronization and artificial insemination - are thus complementary, with a major advantage resulting from grouped parturitions, which fully justifies their use in goats breeding.

Key words: *estrus synchronization, artificial insemination, goats.*

Introduction

Estrus synchronization and artificial insemination are commonly used in bovines and represent a key factor for the genetic progress of this species. For the same purpose, these biotechnologies represent a useful tool for goat breeders as well, being nowadays frequently performed in the intensive system (Gordon, 2004). Estrus synchronization allows a faster estrus detection prior to artificial insemination and moreover, enables timed artificial insemination, when all synchronized females are bred without heat checking, which is extremely labor and cost effective. Nevertheless, not all breeds have the same response to such hormonal treatments and technologies, while the rearing and feeding system, as well as nutritional value of the ratio are also key components in the success of such endeavors (Noakes et al., 2001).

Estrus synchronization in goats can be achieved using synthetic progesterone such as 6 - methyl -17 alpha acetoxy progesterone (MAP) or fluorogestone acetate (FGA) as well as natural progesterone by injection, oral administration or intravaginal sponges and pessaries. The success rate varies among studies, between 80-100% (Ishwar and Pandey, 1990). Since prostaglandins became available, another option of synchronizing cycling does is luteolysis. The success rate reported by several authors also varies between 80-100% (Perera et al., 1978; Ott et al., 1979; Bretzlaff et al., 1983; Alacam et al., 1985; Ishwar and Pandey, 1990).

Therefore, the aim of this paper was to test the effectiveness of an estrus synchronization method during the reproductive season in Romanian mixed breed goats kept under extensive conditions as well as to evaluate the effectiveness of natural breeding versus artificial insemination with freshly collected and diluted semen.

Materials and methods

The goat farm included in this study is part of a family type farm, which includes 40 does and 4 bucks, all mixed breed. The experiments were performed on 20 females and 4 males, which

were subjected to a general clinical examination, all of which were declared clinically sound. Two experimental groups were created, each consisting of 10 does and two bucks.

The estrus cycle was synchronized in all females with prostaglandin F2alfa, given that the experiments were performed during the breeding season. In order to achieve this, we applied a hormonal treatment, which consisted of administering 0.375 mg (1.5 ml) from a synthetic analogue of prostaglandin F2alpha (Cloprostenol 25 mg/100ml, Roflavol, Romvac), twice, at 13 days interval. They were completely separated from males throughout the whole duration of the treatment.

Natural breeding was performed on a group of 10 synchronized does (group 1). Within 24-48 hours of the last dose of prostaglandin, goats were carefully monitored in order to detect clinical signs of heats. All females were diagnosed with normal intensity behavioral estrus, and therefore the two bucks were introduced for free mating. During two days, all does were mounted several times by the two bucks.

Artificial insemination was performed on another group of 10 synchronized does (group 2). The bucks that were selected for semen collection were 3 and 3.5 years old, with proven fertility, as they had been used for natural breeding during the previous years. They were prepared for the reproductive activity by adequate nutrition and management. Semen was collected using a Premier 1 electroejaculator for small ruminants. Electroejaculation was preferred instead of the artificial vagina method, since males were not trained for such procedures.

The macroscopic examination of semen was performed right away, by assessing the volume, color, consistency and impurities of the ejaculate, immediately upon collection, by direct inspection of the collection bottle.

The microscopic examination required assessment of sperm waves, motility and density of spermatozoa, using an optic microscope. Thus, a drop of semen was deposited on a degreased, dry and preheated slide and subsequently covered with a coverslip. Examination was performed with the 10X objective. The first step was the visualization of the wave movement of the sperm. Density was assessed next, by observing the distance between neighboring spermatozoa and comparing it to the size of their heads. Ultimately, sperm motility was assessed by subjectively establishing the percentage of sperm with vigorous forward movements. Eosin-nigrosine colored smears were also performed to reveal the percentage of dead and abnormal sperm. Subsequently, the concentration of spermatozoa was determined using a Burker-Turk counting chamber.

The freshly harvested semen from the two bucks was mixed (to eliminate the individual factor but also to obtain the competitive effect) and then diluted with an original extender made from skimmed UHT cow milk (Parmalat) 0.1% fat, supplemented with antibiotic-antimycotic (Sigma) at a concentration of 1%. No egg yolk was added, due to its known negative impact on goat semen. The volume of extender was calculated based on concentration, so the final dose would contain 200 million sperm/ml. An initial dilution was made at 37°C (1:1), and after equilibration, the final volume of extender was added at room temperature.

After 48-56 hours from the last prostaglandin administration, estrogen-specific behavioral changes were observed (females were restless, loud, and refused to eat) as well as local changes in the vulva through swelling and congestion. Artificial insemination was performed exactly 56 hours after the last administration of prostaglandin, in order to obtain an adequate synchronization with the time of ovulation. The method chosen for artificial insemination was intracervical, with the help of a vaginal speculum.

Pregnancy diagnosis was performed in all females 45 days after insemination using ultrasounds and all pregnancies were monitored until full term. Conception rate as well as prolificacy were recorded for both groups.

Results and discussions

Following the estrus synchronization treatment performed in 20 mixed breed goats during the breeding season with prostaglandin F2alfa, heats were observed in all females within 32-40 hours of the last administration (Figure 1). Estrus was normally expressed, both behavioral (agitation, intense bleating, appetite suppression) and local signs (swelling and congestion of the vulva). The duration of the estrus was 20 ± 3 hours (Figure 2).

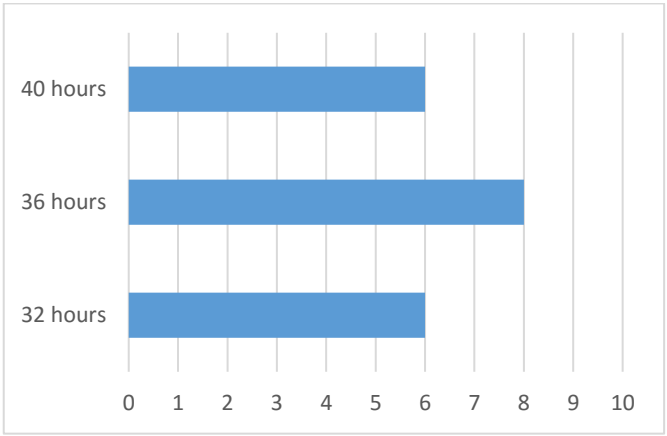

Fig. 1 Interval of estrus occurrence in synchronized does

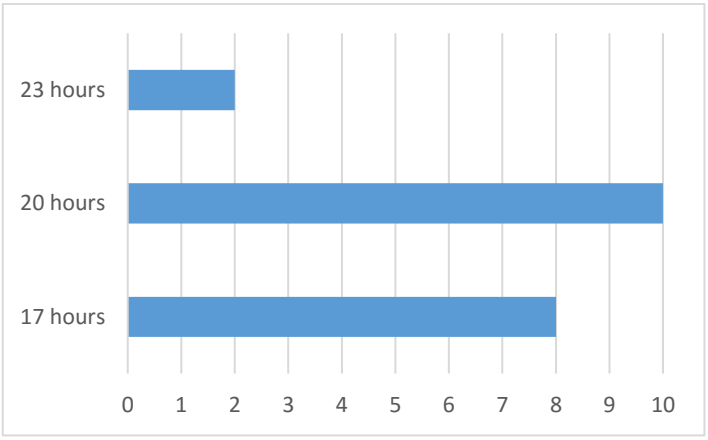

Fig.2. Estrus duration in synchronized does

Group 1, made up of 10 does prepared for natural breeding, was placed in the same stall with two bucks, allowing free breeding. The bucks showed adequate sexual reflexes, physiologically breeding the whole group of goats.

Does in group 2 were completely isolated from males, and 56 hours after the onset of estrus, artificial insemination was performed with freshly collected and diluted semen.

Semen collection was successfully performed in both selected bucks. After approximately 4-5 electric stimuli 1.3-1.5 ml of semen were obtained. Following the macroscopic examination of the freshly collected semen, the following results were obtained (Table 1):

Table 1 Macroscopic examination of semen obtained by electroejaculation

Macroscopic parameter	**Buck 1**	**Buck 2**
Volume	1.3 ml	1.5 ml
Color	Yellowish white	Yellowish white
Aspect	Creamy	Creamy

The microscopic examination of freshly collected semen, yielded the following results (Table 2):

Table 2 Microscopic examination of semen obtained by electroejaculation

Microscopic parameter	**Buck 1**	**Buck 2**
Spermatic waves	++++	++++
Subjective motility	95%	95%
Density	D	D
Sperm abnormalities or immature spermatozoa	<10%	<10%
Concentration	3.9 billions/ml	3,7 billions/ml

Since the ejaculates obtained from the two bucks met the macroscopic and microscopic examination criteria, they were pooled, diluted with the original extender and introduced intracervically, using an insemination catheter and speculum, to the 10 does belonging to group 2 (1 ml of diluted sperm containing 200 million spermatozoa).

Both experimental groups were carefully monitored to observe the percentage of females that returned to heats, which were considered non-pregnant.

Thus, in group 1 (natural breeding), the heats returned in 2 goats (20%) and in group 2 (artificial insemination) in one goat (10%). All of these females were naturally bred at the time of heat re-occurrence, after which anestrus was observed.

At 45 days from the date of the breeding or artificial insemination respectively, pregnancy diagnosis was performed using the ultrasound method. On this occasion, the following results were obtained:

Group 1 (natural breeding):

• 8 pregnant goats, of which 3 had twins and 3 had triplets;

• 2 goats with early gestation, diagnosed transrectally (resulting from the second breeding) (Figure 3).

Group 2 (artificial insemination):

• 9 pregnant goats, of which 5 had twins and 4 had triplets;

• 1 goat with early gestation, diagnosed transrectally (resulting from the second breeding) (Figure 4).

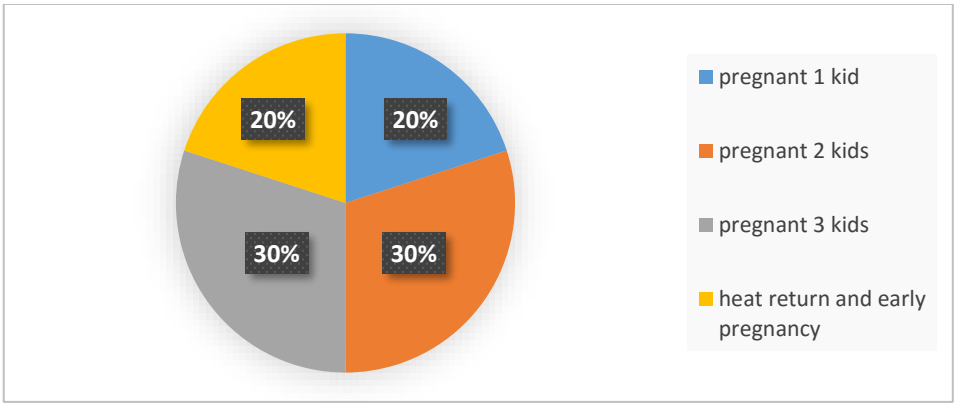

Fig.3 Pregnancy diagnosis in group 1

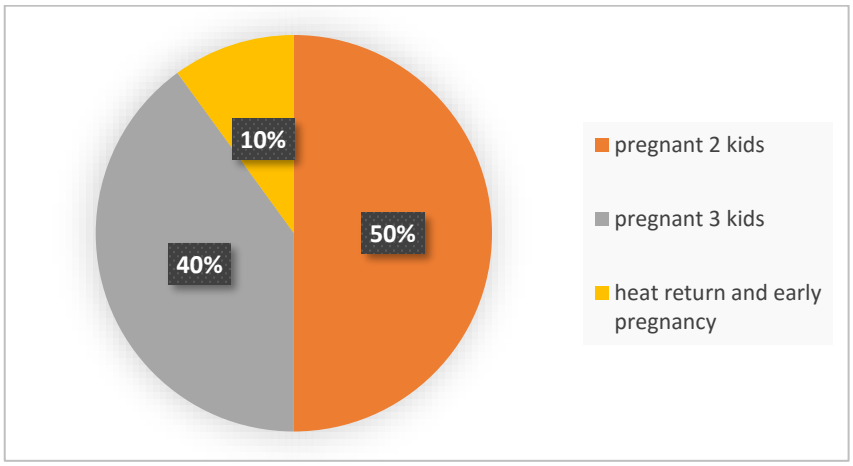

Fig. 4 Pregnancy diagnosis in group 2

Pregnancy advanced smoothly in both groups, without any complication, while the first parturition occurred at 148 days of pregnancy. Over the next 3 days, the 17 pregnant does from both groups that were initially diagnosed pregnant at 45 days had eutocic parturitions, without complications. The other 3 does, diagnosed with early gestation, gave birth about 20 days later.

Thus, following estrus synchronization and natural breeding or artificial insemination, respectively, in the two experimental groups, the following reproductive indices were obtained: group 1: conception rate 80% and prolificity 170%; group 2: conception 90% and prolificity 220%.

Estrus synchronization during the main breeding season (autumn) in goats is mainly used to facilitate artificial insemination. Conventional therapeutic protocols are based on a scheme that aims to administer progesterone for 14 days and to induce the Rebound effect (Hafez and Hafez, 2000). Our research has shown that estrus induction and synchronization during the breeding season with prostaglandin F2alfa is also highly effective, being cost-effective, easy to apply and with excellent results on heat manifestations in treated does. The timing of the estrus occurrence as well as heat duration were very well synchronized, as were parturitions in pregnant goats.

Accordingly, we consider it extremely appropriate to use prostaglandin F2alpha to synchronize estrus during the breeding season in goats, given its marked luteolytic effect.

Collection of semen from bucks by electroejaculation, yielded very good results; the ejaculates had superior quality and met the optimum quality parameters of this species. The extender based on skimmed milk (0.1% fat) was chosen for the dilution of semen, without the addition of egg yolk, which provided adequate sperm viability as shown by the high percentage of gestations obtained from artificial insemination.

The method of intracervical artificial insemination gave satisfactory results, given the high conception rate and superior prolificity obtained in the experimental group 2. The slightly inferior results regarding conception rate and prolificity obtained by natural breeding can be explained by the fact that all 10 females presented heats over a very short period of time, being repeatedly mounted by the two males, which probably led to their exhaustion and decreased ejaculate quality. Therefore, we consider it appropriate to use artificial insemination in case of heat synchronization of goats on a farm, because the number of females showing heats over a short period of time is high, which can lead to the exhaustion of males which are used for natural breeding.

Performing artificial insemination in goats is particularly recommended when improvement of genetics is desired, by using semen collected from males with superior traits, or when the number of males on a farm is insufficient to provide natural breeding of all females (Groza, 2006). At the same time, whenever estrus induction and synchronization is performed, artificial insemination appears to be a necessity due to the compression of estrus manifestation interval in females (a large number of females show heats over a very short time) and consecutive exhaustion of males used for natural breeding (Șonea, 2013).

The two biotechnologies - estrus induction and artificial insemination - are therefore complementary, the application of only one of them leading to unsatisfactory results. At the same time, the major advantage resulting from grouped parturitions fully justifies the use of these breeding biotechnologies in the caprine species.

Conclusions

The application of estrus synchronization treatments in goats during the breeding season with prostaglandin F2alfa followed by natural mating or artificial insemination with freshly diluted sperm, allowed the following conclusions and recommendations to be made:

1. The protocol of estrus induction and synchronization during the breeding season with the F2alfa prostaglandin is extremely effective, being cheap, easy to apply and with excellent results on heat manifestation;
2. The method of intracervical artificial insemination gave satisfactory results, given the high conception rate and superior prolificity obtained in the experimental group 2;
3. We recommend the use of prostaglandin F2alfa for the synchronization of estrus during the breeding season in goats, given its marked luteolytic effect;
4. We recommend dilution of goat semen with skimmed milk extenders without egg yolk, considering the positive results obtained in this study;
5. We recommend the application of artificial insemination to goats when improvement of genetics is desired, by using semen collected from males with superior traits, or when the number of males on a farm is insufficient to provide natural breeding of all females;

6. We also recommend the use of artificial insemination in case of heat synchronization of goats on a farm, due to the compression of estrus manifestation interval in females and consecutive exhaustion of males used for natural breeding.

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Use of ASYM® Plates to repair diaphyseal femoral fractures in two cats

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Abstract

Two intact domestic shorthair cats were presented at our clinic because of grade four signs of lameness at right forelimb. The cats have been hit by car and radiographs revealed a slightly displaced long oblique diaphyseal femoral fractures .The patients tolerated the implant and had a very good functional recovery. Radiographic follow-up after 60 days revealed sign of osseous union. The plates were not removed. This report describes the surgery and outcome of dyaphiseal femoral fractures in two cats repaired by ASYM® Plates.

Keywords: *ASYM® Plates, cat, dyaphisis, femur, fracture.*

Introduction

The incidence for fractures of the femur is about 20% to 25% of all fractures in most veterinary practices; this rate is higher than for any of the other long bones in the body. Diaphyseal fractures account for 56% of femoral fractures (Braden et al. 1995). In addition, femur fractures represent 45% of all long-bone fractures, a rate more than double that of other bones (Unger et al. 1990). Diaphyseal fractures are usually the result of direct trauma and are accompanied by various degrees of soft-tissue damage and hematoma (Brinker 1974; Olmstead 1984). Because of the eccentric loading of the femur during weight bearing, the surgeon must be most cognizant of the tension/compression cortices and their effect on implants in this bone. Bone plates are applicable to almost all diaphyseal fractures and are likely the most common implant used for their repair. Bone plates have the distinct advantage of providing uninterrupted, rigid internal fixation. Depending on the fracture type, the plate may be used as a tension-band compression plate in short oblique, transverse, and some segmental fractures; as a neutralization plate in long oblique and reducible wedge fractures; and as a buttress or bridging plate in nonreducible wedge fractures (DeCamp et al., 2016). When plating fractures in the proximal or distal third of a bone, unless the surgeon resorts to either using a shorter plate than desirable or modifying the plate to remove holes at one end, standard ‘off-the-shelf’ compression plates can be impossible to apply whilst maintaining the gap between the two sets of screw holes over the fracture line. This is because these plates are essentially symmetrical. Plate modification to remove holes is time consuming, wasteful and without the right equipment and attention to detail, can leave a sharp or rough end that can hold bacteria and irritate soft tissues or leave contaminants on the plate surface. ASYM® Plates (asymmetric plates) offer a simple 'off-the shelf' solution to this familiar problem and are available in the same standard thicknesses and widths as standard compression plates, but with an asymmetric hole arrangement that makes them significantly more suitable for these fractures

(veterinary-instrumentation.co.uk). The shorter side of ASYM® Plates carry either three or four round holes. Round holes have some benefits. They can be packed closer together than compression slots. This helps the surgeon to maximize screw count in the smaller fragment and makes ASYM® Plates more adaptable to fragment length (veterinary-instrumentation.co.uk). Round holes are also intrinsically more stable than compression slots and where space is at a premium, this may make all of the difference between success and failure. The longer side carries regular compression holes in standard spacing to permit compression as normal (veterinary-instrumentation.co.uk). ASYM® Plates are priced to match the equivalent length of compression plates. There are 70 plates in the ASYM® range, which covers 2.0 mm, 2.4 mm, 2.7 mm, 3.5 mm and 3.5 mm broad sizes. This report describes the surgery and outcome of dyaphiseal femoral fractures in two cats repaired by ASYM® Plates.

Material and methods

Two intact male domestic shorthair cats was presented for right hind limb lameness of approximately 1-week and 3 days duration. Owners reported no improvement in the lameness after these days of rest. On physical examination, pain and crepitus were evident on palpation of the right femur. No other abnormalities were recorded. The cats were admitted and a lateral and ventrodorsal radiographs indicated comminuted (cat 1) and simple (cat 2), severe displaced fractures at the femoral diaphysis (Fig. 1 and 2). A femoral plating was recommended. A craniolateral surgical approach to the femur shaft (Johnson, 2014) facilitated exposure and reduction. The two fragments were stabilized with one 1 mm Kirshner (K) wires in the cat with comminuted fracture (Fig. 3). An 8-hole 2.7-mm ASYM® Plates (Veterinary Instrumentation, Sheffield, UK) was applied to the lateral aspect of the femur in cat 1 (Fig. 3). A 9-holes 2.7-mm ASYM® Plates (Veterinary Instrumentation, Sheffield, UK) was applied to the lateral aspect of the femur in the second cat (Fig. 4).

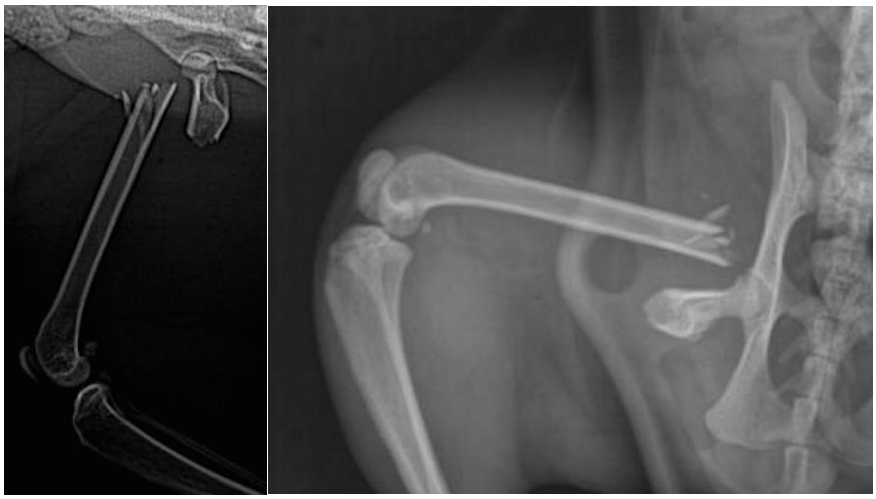

Fig. 1 Lateral and ventrodorsal radiographs of the cats demonstrating femoral dyaphiseal comminuted fracture of the right hindlimb with evidence of severe displacement (cat 1)

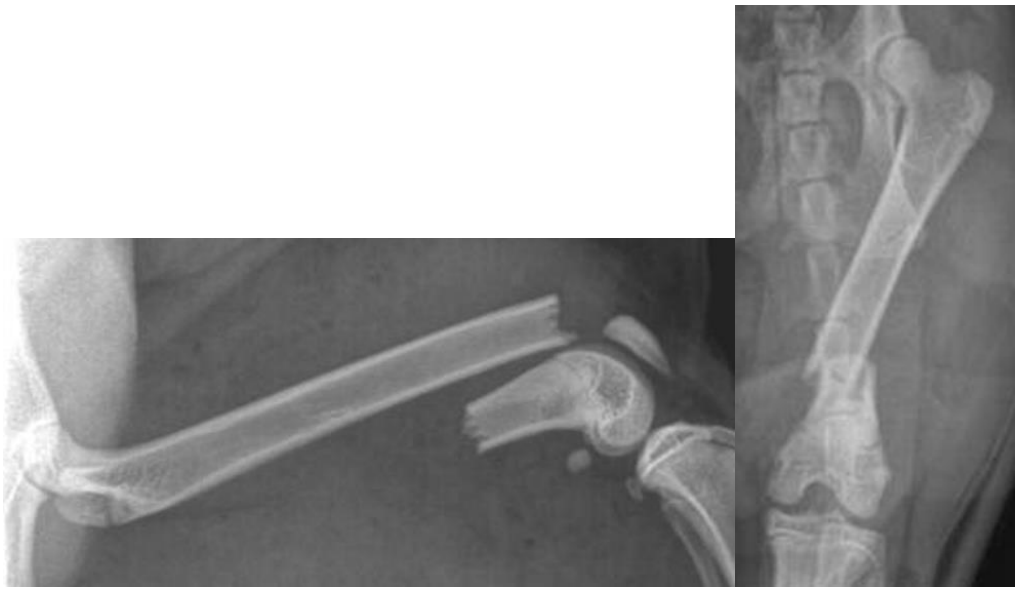

Fig. 2. Lateral and ventrodorsal radiographs of the cat demonstrating femoral dyaphiseal simple fracture of the right hindlimb with evidence of severe displacement (cat 2)

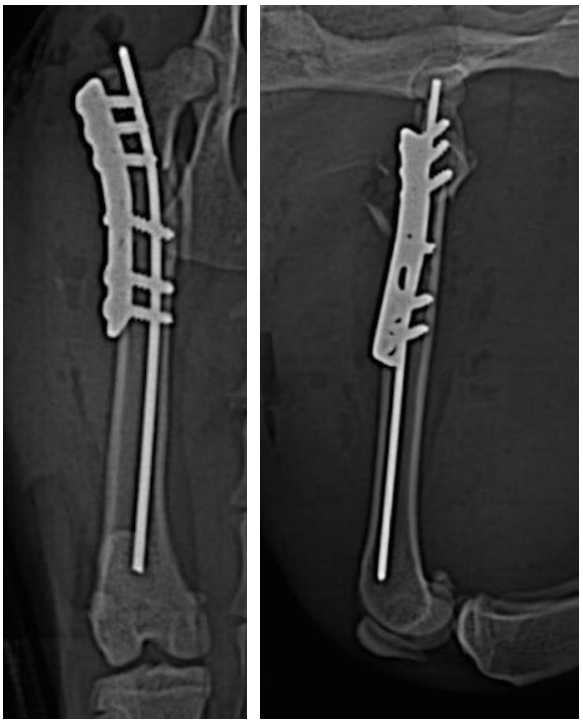

Fig. 3. Postoperative ventrodorsal and lateral radiographs showing fracture reduction and stabilisation with an 8 holes 2.7 mm ASYM® Plate (Veterinary Instrumentation, Sheffield, UK) and Kirshner (K) wire (cat 1).

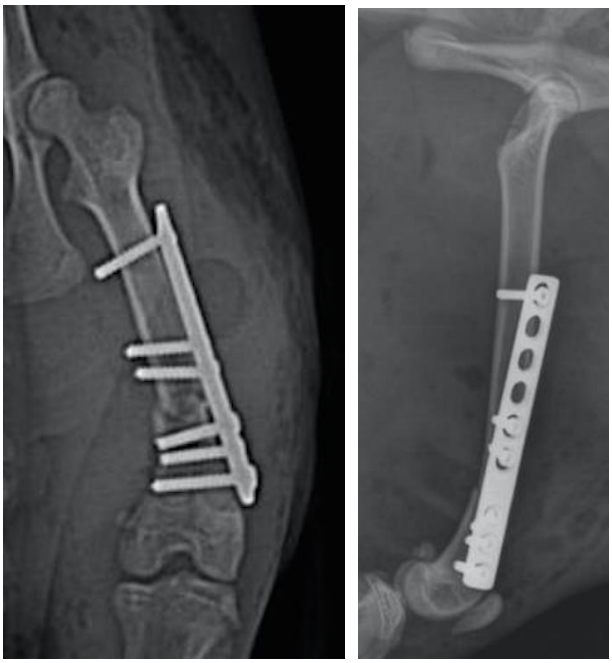

Fig. 4. Postoperative ventrodorsal and lateral radiographs showing fracture reduction and stabilisation with a 9 holes 2.7 mm ASYM® Plate (Veterinary Instrumentation, Sheffield, UK) (cat 2).

Results and discussions

Re-evaluation three weeks postoperatively revealed the cats to be very good weight-bearing on the affected hind limb with normal bone healing. Surgery time was estimated to be approximately 110 minutes in the first cat and 80 minutes in the second one. Oblique and spiral fractures are the most common fracture patterns recognized in patients of all ages, whereas comminuted and open fractures are more common in mature animals than in immature animals (Boone et al., 1986; Unger et al., 1990). Intramedullary pin fixation alone should be strictly limited to stable fractures in small dogs and cats with good healing potential (Piermattei, 2006). We used normograde pinning for case 1 to realign the fragments and to have the possibility to apply the plate accurately.

Today, the two implants that are most compatible with biological osteosynthesis are the bone plate, alone or in combination with an IM pin, and the angle-stable interlocking nail (DeCamp et al., 2016). With few exceptions, a lateral approach is used to expose the femoral shaft for reduction and internal fixation (Johnson 2014). In middle to proximal femoral fractures, the proximal fragment rotates caudally, inducing excessive anteversion of the femoral head. Oblique or multiple wedge fractures develop considerable overriding and can be very difficult to reduce, especially in large breeds or when several days have elapsed since the injury. We did not have this problem in the cat, because of the small size of the patient. The use of reduction by means of an IM pin was very helpful in our case. Using a lateral approach to the diaphysis, the pin was inserted in the proximal segment, the fracture was reduced and maintained by using self-retaining bone forceps, and the pin was then inserted into the distal segment. After clinical union, the IM pin may

be removed but we did not remove it in our case because no reactions appeared at the tip of the pin.

Contouring of the plate to closely fit the bone was critical especially in case 1 for satisfactory repair of fracture. Failure to contour can result in marked deformity of the limb. In the first case we applied the plate in a bridging mode. The plate for the second case was used with a neutralization role.

Because of the small amount of bone available for regular plate placement, the ASYM® Plates was a good option in our cases after reduction. Compression bone plate fixation is a very simple and highly effective method of treatment in animals of all sizes, especially in large and giant breeds dogs. The ASYM® Plates cannot be used in compression fixation because the holes are round. Anyway, the cats are small size species and we think compression in these fractures is not a key point.

Activity was restricted, and no additional fixation was indicated. Healing was rapid with clinically united in 3 weeks in our cases. Physical rehabilitation was recommended to maintain good range of motion, prevent the formation of adhesions, and optimize functional recovery during first three weeks.

In conclusion, the fact that we could maximize screw count in the smaller fragment in these fractures was the main advantage for using ASYM® Plates. We mention these advantages in tibial diaphyseal fractures in dogs also (Ober et al. 2017). Of course, the limitation of the study is the number of cats and the retrospective nature of it. An enough representative population of cats should be observed, to analyse the diferences between the outcomes after femoral fractures managed with ASYM® Plates compared with other types of plates. We agree that using ASYM® Plates is a good option to repair femoral fractures in cats, especially when the proximal or distal fragment is smaller.

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Determination of protein fractions in a sports horse with laryngotracheitis, nonspecifically stimulated with a phytotherapeutic extract (case study)

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Abstract

In a sports horse with signs of laryngotracheitis that hardly achieved the training program, a phytotherapeutic extract with immunomodulating properties was administered. Following the administration of the extract, on the second day, the horse showed a remarkable comeback in the sense that it did the normal training without the presence of fatigue and coughing. The horse was clinically examined, blood samples were collected and the extract was inoculated s.c., blood samples were collected at 24 hours, 7 days and 10 days for haematological, biochemical examinations and also for electrophoresis, in order to determin the protein fractions. Significant changes are noted for fractions β1, β2 and γ. In terms of albumin, they are found initially at physiological values, and 24 hours after the inoculation of the extract, very significant decreased throughout the experiment. The most spectacular variations of the protein fractions are those for gamma globulins: initially they are drastically decreased, and 24 hours after the inoculation of the extract, they increased spectacularly, about 3 times, and remain close to this level (in physiological parameters), throughout the experiment. The albumin / globulin ratio is clearly in favor of globulins.

Key words: *immunomodulation, phytotherapeutic extract, globulins, sport horses, laryngotracheitis.*

Introduction

Over time, the horse has been seen from several points of view: as a tool of battle, fieldwork, transport, then to become closer to the people and to become a symbol of the nobility among animals, winning the laudatory title of "gentleman" animal. However, with the domestication of these animals, the first diseases were found: digestive, locomotory, respiratory diseases. At the moment, respiratory disorders are becoming more frequent. They occur most often due to shelter conditions (microclimate, ammonia level), inadequate food (feed contaminated with mice) or even decreases in immunity in the case of horses subjected to a long effort, which will lead to a low economic yield of the animal. (4,5,8,11) Non-infectious respiratory disease is a common condition that limits performance and affects adult horses of different ages. Inflammatory airway disease is characterized by excessive tracheal mucus, high respiratory tract activity, and poor exercise performance in younger horses. Etiology is unclear, but viral respiratory infection (Equine Herpes Virus 1, 2, 5), allergy (exposure to organic dusts in older horses) and environmental factors may play a role in the pathology of the airway. The severity of clinical signs ranges from exercise intolerance to dyspnea at rest (2,6,7). Clinical manifestations are similar and include pyrexia, nasal discharge, submandibular lympha-denopathy, anorexia and cough. At the present, the choice of treatment for these conditions is chemotherapy, and it is estimated that for 2020 they will not be as effective as now. This creates the premise for the establishment of new therapeutic

protocols, based on plant extracts, to achieve a modulation of immunity with minimal side effects. In this case, immunomodulation in order to increase the immune potential of the body remains the only alternative to infection control. (3,9)

Non-specific immunomodulators are substances that induce a non-antigen-specific improvement in native or acquired defense mechanisms. Immunomodulatory preparations are most commonly used for the treatment of chronic, viral or bacterial infection, with secondary immunosuppression being evidenced. The mechanism of action of these products is macrophage activation and subsequent release of cytokines that improve cell mediated immunity. In equine medicine, immunomodulatory preparations are indicated for the prevention and treatment of chronic respiratory diseases rather than for the treatment of acute infections (1, 10)

Materials and methods

Research and experiences have been carried out within a private horse farm in Bucharest. The farm has 10 horses from the breeds: Andalusian, Lipitan and Arabian. They participate in various local, national and international competitions. All horses have two training sessions a day, morning and evening, consisting of one-hour training,. As food, the horses receive lucerne, hay, oats and barley, according to a well-timed schedule.

Blood samples were taken from four horses for paraclinical examinations, one of them showing clinical signs of laryngotracheitis (horse A, which received the phytotherapeutic extract), and the other three horses were clinically healthy, representing the control sample, as follows: Horse A: LEA - Arabian, female, age 11 years

Horse 1: ALFRED - Andalusian, male, age 4 years

Horse 2: NERO - Lipiţan, male castrated, age 12 years

Horse 3: MARCO - Andalusian, female, age 15

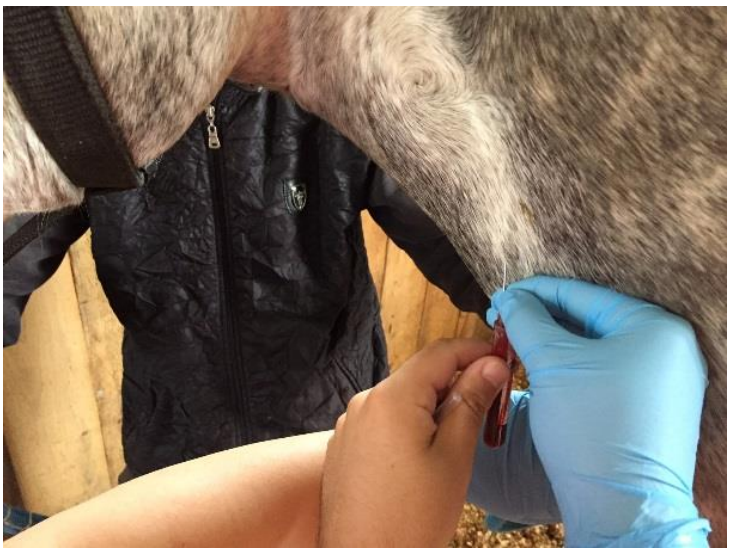

Fig. 1 Blood samples collection

The hematological examination was performed with the "Abacus Junior Vet 5" and the biochemical examination of the blood was performed with the "Arkray" analyzer. For electrophoresis it was used the EP SA200 electrophoresis system, all within the discipline of

"Medical Pathology and Clinical Lectures by Species" of the Faculty of Veterinary Medicine Bucharest. The phytotherapeutic product used has immunomodulatory action (Patent Application No. 467 / 20.04.2016) and has been used by subcutaneous administration in the horse A in the prescapular area.

Results and discussions

At the clinical examo, the horse A showed repeated and spontaneous coughing, triggered by exertion, at the exit of the pad, in contact with cold air, exhaustion in effort, resulting in a shortening of the training period, normothermia and absent nasal discharge. There were rallies in the thoracic auscultation, and the visceral tingling of the cheeks. The coughing challenge was easily accomplished by palpation of the laryngotracheal area, which demonstrates the sensitivity of the mucosa.

The experimental protocol focused on determinations (hematology, biochemistry, electrophoresis) at baseline (I) for the 4 horses, and for the horse showing the symptoms of a respiratory disease (laryngotracheitis), blood was harvested at 24 hours and 5 days and also at 10 days after administration of the phytotherapeutic product and the results are presented in Table 1.

Tabel 1. The Results of the Haematological Exam

Parameter	U/M	Physiolog. Values	Healthy Horses (I)			CLINIC CASE		
			1	2	3	(I)	24h	5days
WBC	$10^{-9}/mm^3$	5,4-14,3	7,96	8,39	7,87	8.75	9.5	8.4
LYM	$10^{-9}/mm^3$	1,5-7,7	2,94	2,05	2,26	**36.1**	**19.3**	**32**
MON	$10^{-9}/mm^3$	0-1,5	0,36	0,47	0,04	0.6	**4.7**	**4.7**
NEU	$10^{-9}/mm^3$	2,3-9.5	4,36	5,52	5,47	**58.2**	**75**	**65**
EOS	$10^{-9}/mm^3$	0,1-1	0,27	0,32	0,09	**4.7**	0.7	1
BAS	$10^{-9}/mm^3$	0-0,5	0,02	0,03	0,01	0.5	0.3	**0.7**
RBC	$10^{-12}/mm^3$	6,8-12,9	8,93	6,95	8,95	8.88	7.80	8.20
Hgb	g/dl	11-19	16,1	13,2	16,6	14.8	10.8	13.0
Ht	%	32-53	39,14	34,54	39,93	35.27	37.1	35.3
MCV	fl	37-59	44	50	45	40	47.6	42.3
MCH	pg	12,3-19,7	18,1	18,9	18,5	16.6	13.8	15.6
MCHC	g/dl	31-39	41,2	38,1	41,5	41.9	**29.1**	36.8
PLT	$10^{-9}/mm^3$	100-400	98	106	99	106	175	205

At 24 hours after inoculation of the extract, circumscribed spherical or ellipsoidal swelling was noticed, with increased of consistency, warm and sensitive, adherent to subcutaneous connective tissue at the site of inoculation, showed a slight decrease in appetite and exercise fatigue and body temperature increased by 1-2^{o}C. After 2-3 days, the overall condition of horse A has improved, appetite returned to normal, and the cough disappeared.

The results of the biochemical examination were not influenced, therefore Uric Acid (UA) was found below the physiological limits in all horses, with no clinical significance. Uric acid results from protein burning, being the final product of degradation of free purines: adenine, hypoxanthine, guanine. In our case, the horse's values have no pathological significance, these uricemic values being reported after uricosuric medication. Twenty-four hours after dosing there

was a significant increase in LDH levels, total protein and alkaline phosphatase, and the other parameters remained within physiological limits.

In the same time determinations of the protein fractions for horse A were also performed by electrophoresis. This is a method of analysis and separation based on the migration of solid particles dispersed in a liquid under the action of an electric field. Determinations were made at baseline (I) for all 4 horses taken into consideration, respectively at 24 hours, then at 5 and 10 days after inoculation of the extract. for the horse presenting the symptoms of laryngotracheitis, the results are shown in Table 2.

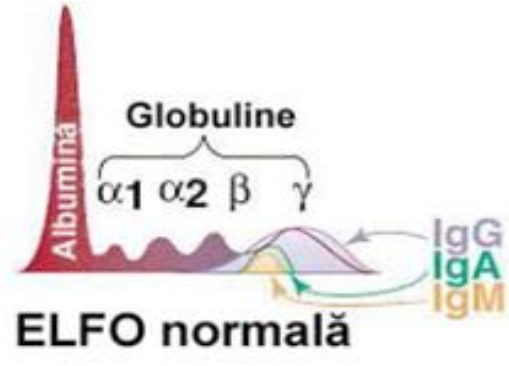

Fig. 2 Normal electrophoresis

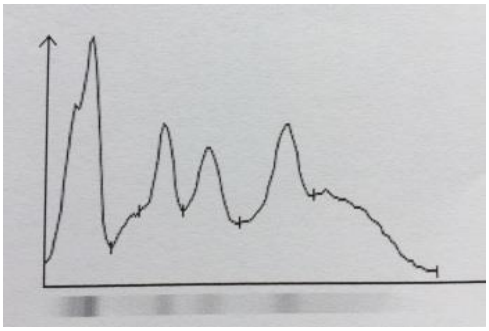

Fig. 3 Horse A Electrophoresis at 5 days postinoculation of the extract

Table 2. The Results of Determination of Protein Fractions

Fraction	U/M	Physiolog. Values	1	2	3	A
			(I)	(I)	(I)	(I)
Total Protein	g/dl	5,1-7,2	5,90	6,40	6,30	5.1
Albumin	g/dl	0,30-0,38	**0,22**	**0,21**	**0,20**	**3,20**
α_1	g/dl	0,19-9,31	**0,15**	0,25	0,25	0.42
α_2	g/dl	0,53-0,87	**0,49**	0,54	0,57	0.63
β_1	g/dl	0,28-0,73	**0,87**	**0,88**	**0,90**	0.61
β_2	gd/l	0,22-0,60	**0,18**	0,41	0,30	0.41
γ	g/dl	0,58-1,27	**1,97**	**2,19**	**2,20**	**0.29**
Alb/Glob Ratio	/	0,95-1,65	**0,50**	**0,49**	**0,61**	**0.66**

According to the data from table 2, Albumin are at a low level at all horses, except the horse A. The Albumin/Globulin ratio is also low. Hypoalbuminemia might be caused by deficiency of intake: nutrition, food deficiency, vomiting, diarrhea, digestive disorders, intestinal absorption

disorders, insufficient synthesis, hepatic failure, loss of albumin (glomerulonephritis, nephrosis), ascites, bleedings, shock. In our case, it can only be a matter of defective problems.

Globulins (α1, α2, β1, β2 and γ) are recorded and modified as follows:
Fraction β1 is slightly increased in all clinically healthy horses without clinical significance.
Fraction β2 is slightly low in horse 1, probably due to young age and within normal limits at the other horses; the ß-globulin fraction contains transferin, hemopexin, complement factors and beta and pre-beta lipoproteins (LDL and VLDL).

Fraction γ is significantly increased in horses from the control group. This shows an increased reactivity of the body and we can see it as a cosequence of the physical effort and not as a pathological problem (there are no clinical and laboratory data to prove it). The ɣ-globulin fraction contains lots of immunoglobulin (IgG, A, D, E and M).

Table 3. Determination of protein fractions in the horse with laryngotracheitis

Fraction	U/M	Physiolog. Values	Horse A			
			Initial	24 h	5 days	10 days
Total Protein	g/dl	5,1-7,2	**5.1**	**5.1**	**5.7**	**5.6**
Albumin	g/dl	3,0-3,8	**3,20**	**1.60**	**1.86**	**1.88**
α1	g/dl	0,19-0,31	**0.42**	0.25	0.24	0.29
α2	g/dl	0,53-0,87	0.63	0.49	0.54	0.58
β1	g/dl	0,28-0,73	0.61	**0.83**	**0.90**	**0.99**
β2	g/dl	0,22-0,60	0.41	**0.81**	**0.95**	**0.78**
γ	g/dl	0,58-1,27	**0.29**	**1.14**	**1.21**	**1.09**
Alb/Glob Ratio	/	0,95-1,65	**0.66**	**0.46**	**0.49**	**0.50**

The albumin/globulin ratio is almost constant below 1, indicating a constant disorder throughout the experiment, although the clinical condition has become very good after receiving the phytotherapeutic preparation. Albumins show a decrease, whereas globulins grow in the bloodstream and are mobilized for emergency situations.

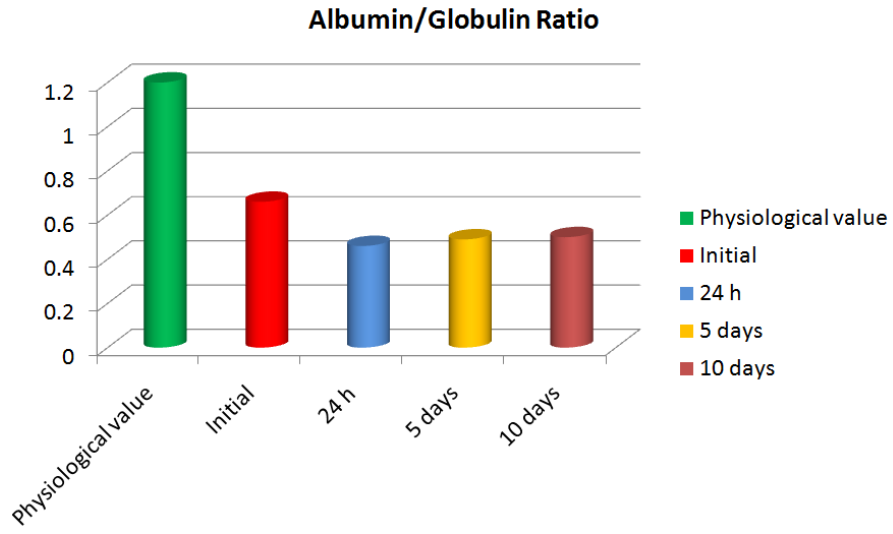

Fig. 4 A/G Ratio Variation Chart

The electrophoresis profile reflects the chronic inflammation demonstrated by the increased gamma globulin band. Fraction α1 shows growth over physiological values before the phytotherapeutic administration and starting to 24 hours, until ten days, the α1 was normal. The α1 fraction is recorded in all inflammatory reactions as the acute phase reaction. The α1 surface reflects the α1 anti-hypsal serum so that its reduction occurs in the α1 deficiency of the antitrypsin deficiency associated with a certain lung disease, probably at the onset of emphysema.

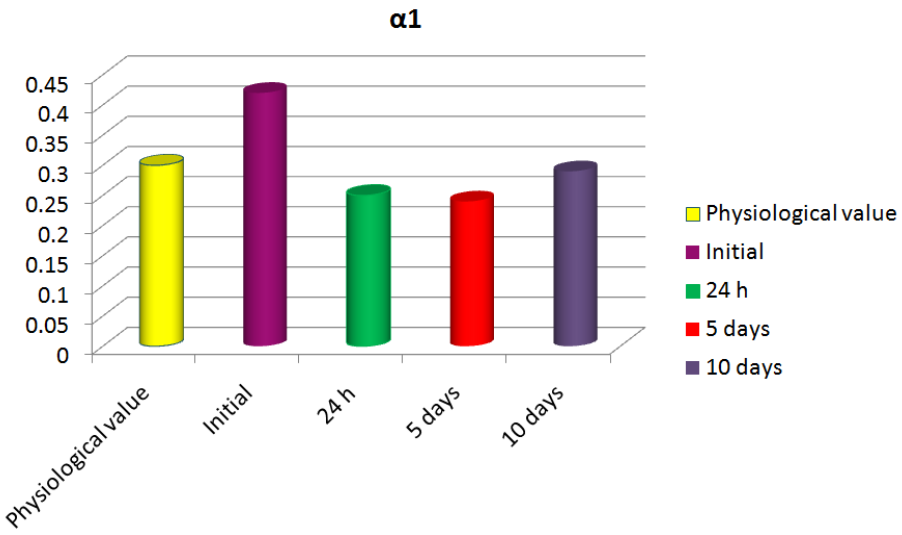

Fig. 5 α_1 Variation Chart

Fractions β1 and β2 remain elevated throughout the experiment. Fractions β include the C3 and C4 complement fractions, which are being elevated too throughout the experiment period.

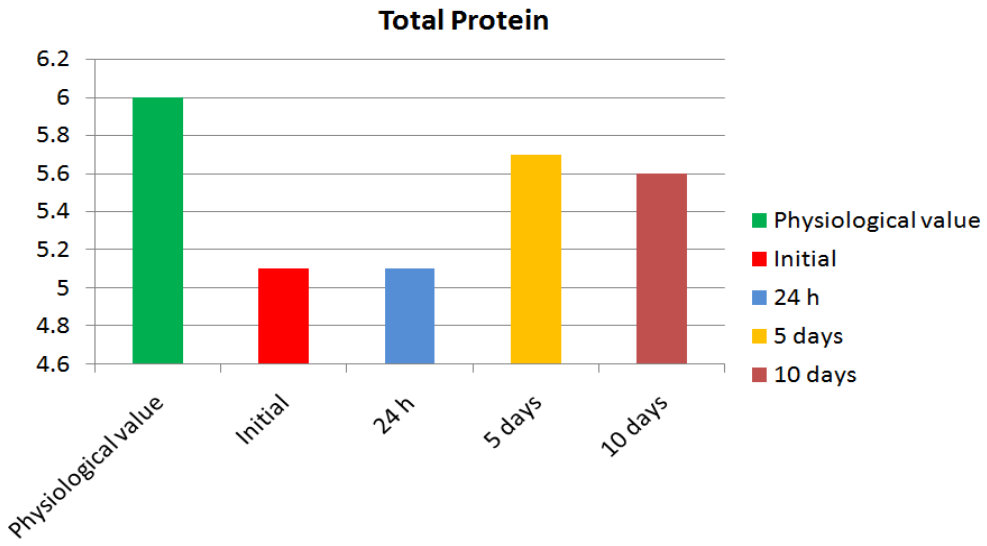

Fig. 6 Total Protein Variation Chart

The gamma area contains immunoglobulin (IgG, IgA, IgM, IgD and IgE). Increases in polyclonal gammaglobulins indicate a chronic immunological process.

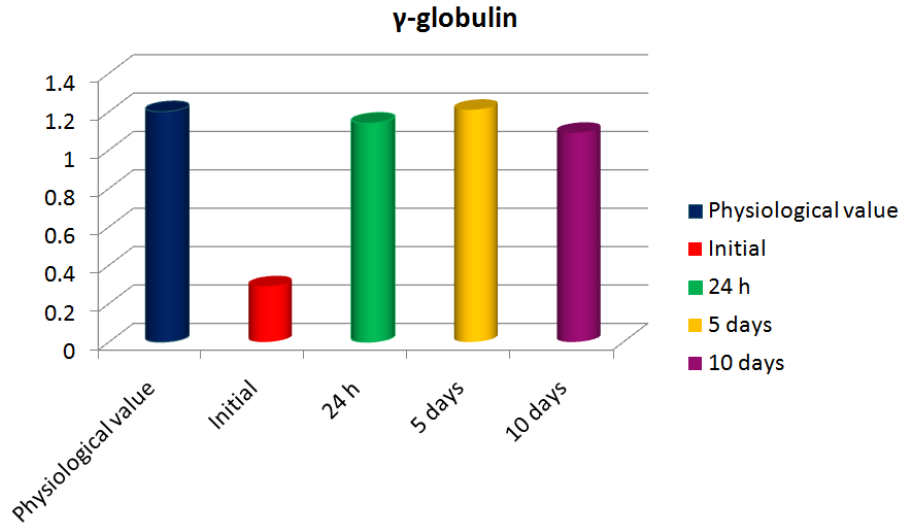

Fig. 7 γ-Globulin Variation Chart

Conclusions

1. At baseline, total proteins are at the lower limit compared to physiological values, and 24 hours after inoculation of the preparation, they reach normal values and remain throughout the study.
2. At the opposite end, the Albumin / Globulin ratio is decreased throughout the experiment, due to increased globulinemia and decreased albuminemia.
3. The fraction α1, if at the beginning of the experiment was very increased, after the inoculation of the phytotherapeutic extract, its values returned to normal until the end of the experiment.
4. Fraction γ had the most spectacular evolution, initially it was very low, than it returned to normal, from 24 hours after inoculation of the preparation up to 10 days postinoculation.

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Residual antimicrobial effect of week organic acids on spoilage psychrotrophs at pig carcasses

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Abstract

The aim of this researchwas to assess the residual antimicrobial effect of 3% lactic and acetic acid solutions regarding the load and configuration of psychrotrophs at porcine carcasses. During October 2016 and December 2016, 18 pork meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples. Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample. Experimental design were carried out over a 14-day period with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera Pseudomonas, Aeromonas, Yersinia and Enterobacteriaceae family. The most sensitive psychrophic bacteria regarding the decontamination effect of lactic and acetic acid were Aeromonas spp. and Yersinia spp., both species being completely inhibited after 24 hours since application. For all microbiological criteria analyzed, lactic and acetic acid shown an obvious residual antimicrobial effect during the shelf life of pork carcasses, when compared with control samples ($p<0.05$).

Keywords*: residual antimicrobial effect, organic acids, spoilage psychrotrophs, pork carcasses*

Introduction

Several studies have been conducted to reduce the level of microbial load from the surface and depth of the carcasses (Stradford *et al*., 1999; Alakomi et al., 2000; Castelo et al., 2001; Staruch et al., 2001; Strivarius *et al.*, 2002 a, b; Ockerman *et al*., 2001a, b; Pipek *et al*., 2004, 2006; Bosilevac *et al*., 2006). These studies were carried out in the context of extending the shelf life of meat, especially when packed in vacuum, when the level of microbial load should be extremely low. Different methods have been suggested for the decontamination of carcass surfaces (steam, chlorination, trisodium phosphate, pulsatile light exposure, pulsed electric fields or ionizing radiation, organic acid solutions) (Pipek *et al.*, 1997; Stradford *et al.*, 1999; Castelo *et al.*, 2000; Strivarius *et al.*, 2002 a, b). From the organic acid solutions, lactic acid, acetic and citric acid were mostly used in different concentrations (1-5%), principally based on reducing the intracellular pH of microorganisms, thus causing their death. Organic acids have the ability to inhibit the growth of some of the alteration germs, mainly Gram negative species of the Enterobacteriaceae family, Pseudomonas genus, etc. (Van Netten et al., 1998; Podolak et al., 1996; Smulders et al., 1998, Killinger *et al*., 2010). The use of these decontamination methods, well studied, authorized and widely used in comercial slaughterhouse in some countries (USA, Australia), often raises discussions about their effectiveness, the color changes induced at the surface of the carcasses and, last but not least, the economic aspects of scale utilization at industrial slaughterhouses for meat-producing animals. Since the reduction of the carcass microbial load of the meat-producing animal carcasses represented and still represents one of the most important foodsafety objectives of the food bussiness operator, our research has aimed to assess the residual antimicrobial effect of 3%

lactic and acetic acid solutions regarding the load and configuration of psychrotrophs at porcine carcasses.

Material and methods

During October 2016 and December 2016, 18 pork meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples (2.5-3 ml/100 cm^2). Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample (not decontaminated). Experimental design were carried out over a 14-day period (shelf life of the pig carcasses), with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera *Pseudomonas, Aeromonas, Yersinia* and *Enterobacteriaceae* family. Psychrotroph plate count was performed according with the protocol described by Nottingham *at. al* (1982). For isolation of psychrotrophs specific selectiv media were used, as follows: Aeromonas and Pseudomonas - GSP agar (Merck), Yesinia - CIN agar (Merck), Enterobacteriaceae – VRBD agar (Merck). Serial decimal dilutions (10^{-1} : 10^{-6}) were obtained from 10 grams of meat and 90 ml water buffered peptone. Spreading method was used to inoculate 0.1ml of inoculum on to the surface of 2 Petri plates. Incubation was realized at 20°C, for 72 hours. Biochemical confirmation test was realized using API 20 E and API 20 NE (Biomerieux). Statistical analysis was realized using Origin 8.5 software by comparison of means by analysis of variance through ANOVA test. The statistical interpretation of the results was realized according to the probability indicator: $p \leq 0.05$ (confidence level 95%). Result were depicted as log CFU/cm^2.

Results and discussions

The initial psychrotrophs load of the control sample was 3.83 ± 0.38 log CFU/cm^2, decrease after 24 hours to 3.53 ± 0.21 log CFU/cm^2, Afterwards showing an ascending trend throughout the experiment, reaching 5.67 ± 0.38 log CFU/cm^2 on day 14^{th}, maximum limit being exceeded on the 9^{th} day of the experiment. 3% acetic acid solution produced a markedly reduction of microbial load after 1 day, to 2.87 ± 0.31 log CFU/cm^2. Then the psychrotrophs presented an increasing trend in the following days, reaching 5.04 ± 0.26 log CFU/cm^2, at the end of the experiment. Similar with the effect of acetic acid, lactic acid reduced microbial load after 24 h to 2.29 ± 0.14 log CFU/cm^2, Afterwards an increasing trend was observed until the end of the experiment when the value of 4.87 ± 0.23 log CFU/cm^2 (Figure 1).

Enterobacteriaceae load showed a constant increase during the experiment, starting from an initial count of 2.31 ± 0.14 log CFU/cm^2 to 4.54 ± 0.2 log CFU/cm^2. Following spraying with 3% acetic acid solution, *Enterobacteriaceae* load decreased to 0.51 ± 0.21 log CFU/cm^2, then the evolution was ascendant by the end of the experiment, when reach 2.75 ± 0.26 log CFU/cm^2, the maximum admitted limit being exceeded on day 7. A similar evolution was noticed in the sample treated with the 3% lactic acid solution, but the inhibitory effect was less intense compared to acetic acid, but no significant differences between organic acid solution were identified ($p>0.05$) (Figure 2).

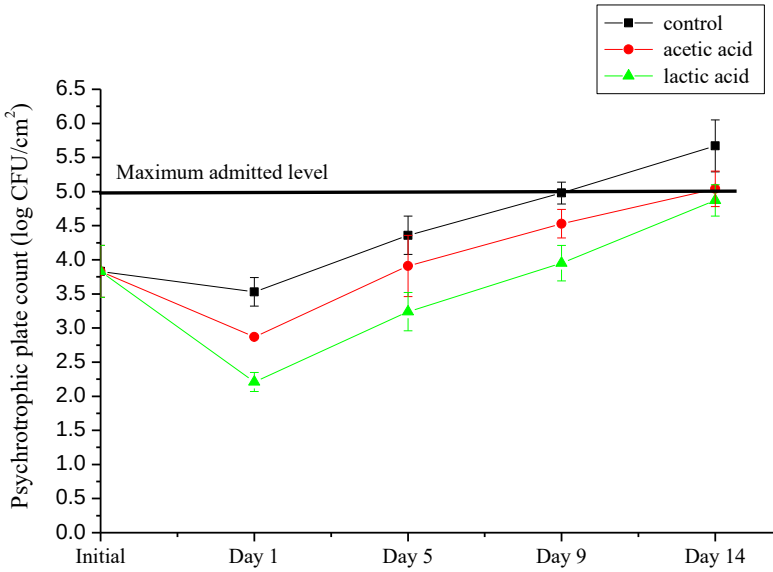

Figure 1. Decontamination effect of 3% organic acid regarding psychrotrophic plate count at the surface of pork meat (n=6)

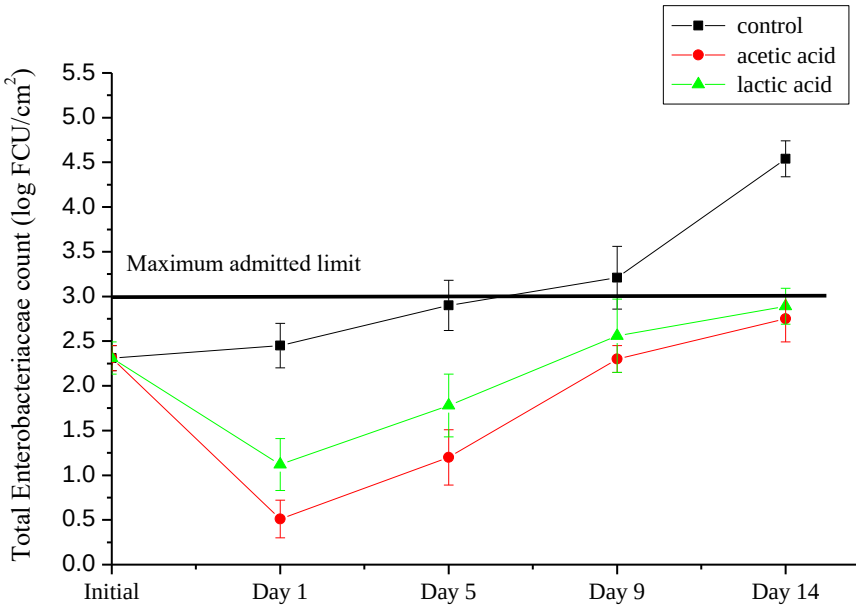

Figure 2. Decontamination effect of 3% organic acid regarding *Enterobacteriaceae load* at the surface of pork meat (n=6)

Following spraying of 3% lactic acid solution, initial microbial load of *Aeromonas* spp. decreased slightly from 2.40 ± 0.26 log CFU/cm^2, to 0.95 ± 0.14 log CFU/cm^2 after 24 h, to 0.76 log CFU/cm^2 in the 5th day of the experiment. Starting with the 9th day, *Aeromonas* spp. was totally inhibited. not on the last day of the experiment. Acetic acid solution produced total inhibition of *Aeromonas* spp. after 24 hours since spraying (Figure 3). Based on these results we can mention that aeromonads are very sensitive to the action of organic acids and especially to 3% acetic acid solution. Significant differences were recorded when compared acid with lactic acid ($p<0.05$).

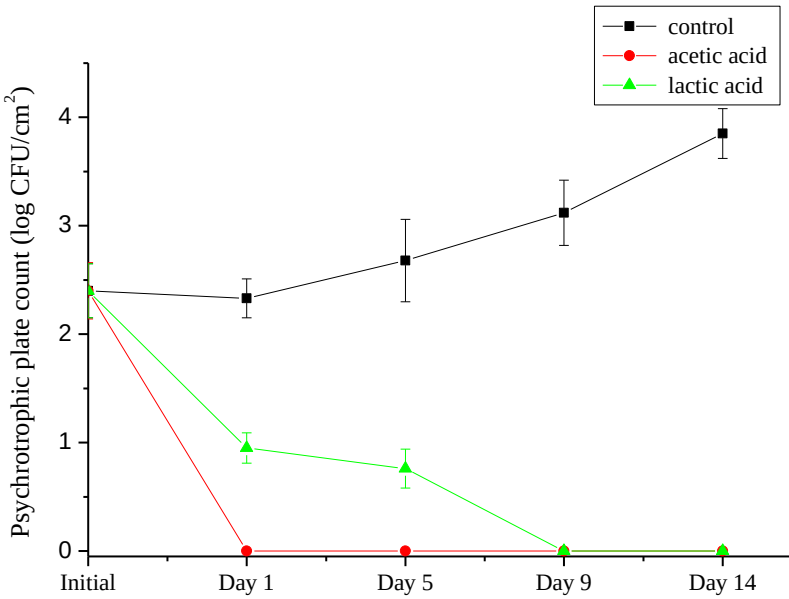

Figure 3. Decontamination effect of 3% organic acid regarding *Aeromonas* spp. at the surface of pork meat (n=6)

In the case of the control sample, *Pseudomonas* spp. load showed a moderate increase until day 5, when they reached the value of 4.06 ± 0.35 log CFU/cm^2, then the pseudomonads showed a more pronounced ascendant evolution, reaching at the end of the experiment 5.21 ± 0.39 log CFU/cm^2, the recommended maximum limit (5.0 log CFU/cm^2) being exceeded on day 13.

Both solutions of organic acids used caused a decrease of pseudomonads load after 24 hours (1.5 log CFU/cm^2 in case of 3% acetic acid). Afterwards, the results showed a more obvious effect regarding reduction of *Pseudomonas* spp. in case of acetic acid, but no differences were noticed between organic acid solution ($p>0.05$). Then, the evolution in dynamics was ascending until the last day of the experiment, when the pseudomonads reached 4.33 ± 0.32 log CFU/cm^2 in case of acetic acid, respectively 5.67 ± 0.40 CFU/cm^2 in case of lactic acid (Figure 4). Significant differences were recorded in case of control samples in comparison with those treated with lactic and acetic acid $p<0.05$).

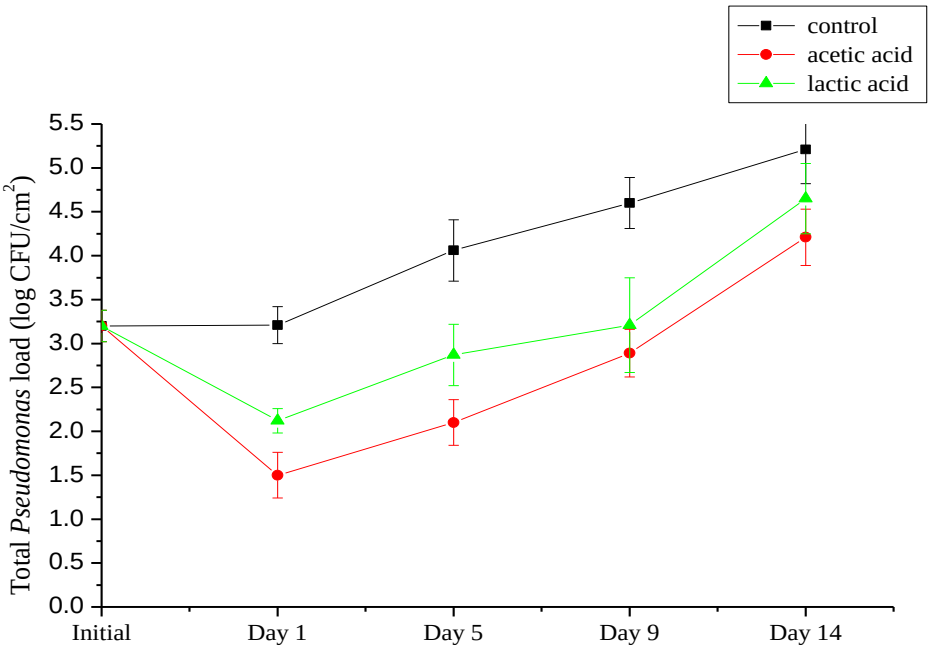

Figure 4. Decontamination effect of 3% organic acid regarding *Pseudomonas* spp. at the surface of pork meat (n=6)

In case of control sample, *Yersinia* spp. presented a ascendant evolution during the experiment, ranging from an initial load of 2.86 ± 0.28 log CFU/cm^2 to 4.78 ± 0.29 log CFU/cm^2 in the 14th day of the experiment. Acetic acid solution applied at the surface of pork meat samples produced after 24 hours a complete inhibition of *Yersinia* spp., while lactic acid solution produced a moderate reduction until day 9 when they did not were identified (Figure 5). Significant differences were recorded in case of control samples in comparison with those treated with lactic and acetic acid $p<0.05$). Similar results, reported by Mead *et al.* (1995), regarding preventive control methods for red meat, has shown that the use of 2.4% lactic acid solution for the decontamination of porcine carcasses has reduced the load with Enterobacteriaceae by 0.9 log CFU/cm^2 and the total number of germs with 1.0 log CFU/cm^2. Also, Pipek *et al*. (2006), highlighted the residual antimicrobial effect of the 3% lactic acid solution on the total germ load for a period of 5 days.

Castelo *et al.* (2001), in a dynamic study on various methods of inhibiting the psychotropic germs from the surface of pig carcasses, revealed that, following treatments with 2-3% lactic acid solutions, the total load remained constant up to 7 days at values of 3.52 log CFU/cm^2, the most effective inhibition method, which did not produce sensory changes in the carcasses, was the use of lactic acid after the final cleaning of the carcasses.

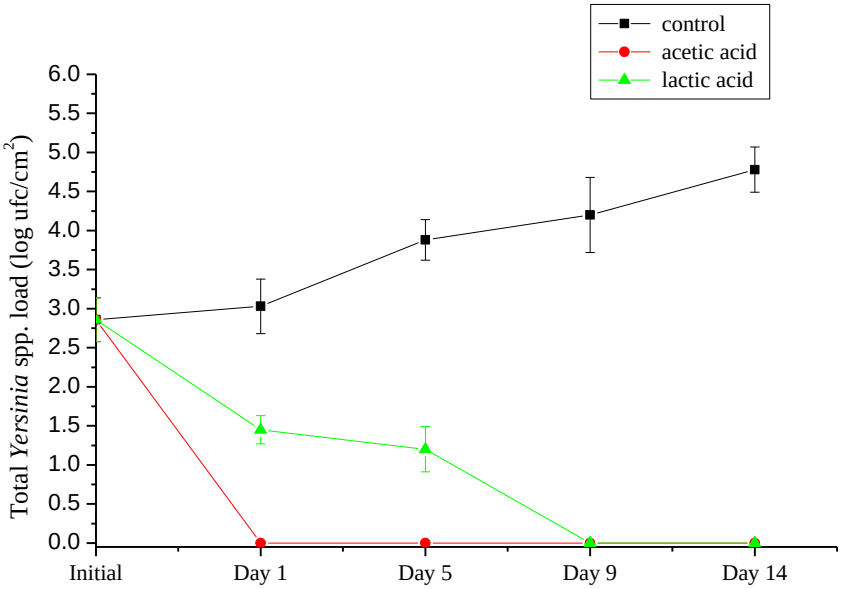

Figure 5. Decontamination effect of 3% organic acid regarding *Yersinia* spp. at the surface of pork meat (n=6)

Conclusions

The most sensitive psychrophic bacteria regarding the decontamination effect of lactic and acetic acid were *Aeromonas* spp. and *Yersinia* spp., both species being completely inhibited after 24 hours since application. For all microbiological criteria analyzed, lactic and acetic acid shown an obvious residual antimicrobial effect during the shelf life of pork carcasses, when compared with control ($p<0.05$). These methods of decontamination should not be used as basic methods of reducing initial microbial load of germs. It should also, not be considered as a substitute for the use of appropriate methods of preventing microbial contamination at the slaughter or processing units of meat, because most of these methods have only a limited effect of reducing germs or pathogens, which depends directly on the initial level of contamination.

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Influence on week organic acids on pathogens on swine carcasses

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Abstract

The aim of this research was to assess the efficiency of organic acids regarding the main pathogens as Salmonella, Listeria, Campylobacter, Escherichia and Yersinia on pork meat. The fresh meat samples were collected from a slaughterhouse located in Cluj County. The samples were sterilized using UV radiation for 30 minutes and afterword's contaminated with reference strains of above mentioned pathogens. The samples were subjected to decontamination operation by using solutions of organic acids (acetic, lactic and citric acid) in concentration of 1%, 2% and 3%. After decontamination were isolated and detected microorganisms. Statistic analyses of the results revealed that the investigated pathogens have a different sensitivity to the action of acid solutions, their sensitivity in ascending order being: Escherichia, Listeria, Salmonella, Yersinia, Campylobacter, so the strains of Campylobacter are the most sensitive to the action of the decontaminating agents and germs of Escherichia are the least sensitive. Among the organic acids, the most efficient appears to be lactic acid, followed by acetic acid and less efficient citric acid. The greatest reduction of germs was determined by the concentration of 3%.

Keywords*: pathogens, decontamination methods, organic acids, swine carcasses*

Introduction

Due to the it's chemical composition, meat is an ideal culture medium for growth of microorganisms, a rapid increase can be realized in the case of inproper monitoring of critical control points (CCPs) on slaughtering process (Gill, 1986; Gill and Jones, 1997; Smulders *et* Greer, 1998; Dan *et al.*, 2008; Sofos, 2008; Loretz *et al*., 2011). Microbial contamination may induce the early spoilage of carcasses, reduce its shelf-life, and, more seriously, may pose a public health risk by food borne ilnesses outbreaks. In the case of slaughtering, the contamination of pathogenic carcasses can be carried out mainly during skinning, evisceration, handling of the meat by the operators and refrigeration, in case of non-compliance with good hygiene practices (GHP) and good manufacturing practices (GMP) (Gill, 1986; Gill and Jones, 1997; Gill, 2002). In slaughter establishments, pathogenic bacteria can cause indirect contamination of carcases as a result of non-compliance with operator hygiene standards (Yen, 2003). Several studies have demonstrated the effectiveness of using decontamination methods in order to reduce the level of pathogens, among which the most commonly used are: organic acids (lactic acid, citric acid, acetic acid), alkaline solutions (trisodium phosphate) and steam (Castillo *et al*., 1999; Gonzales *et* Domongoz, 2006; Carpenter *et al*, 2011, Loretz *et al*., 2011). In this research we aimed to assess the efficiency of different organic acid solutions regarding pathogenic microorgaisms from *Salmonella, Camplylobacter, Escherichia, Yersinia* and *Listeria* genera from porcine meat.

Material and methods

Between January and May 2017, 16 samples of fresh refrigerated pork meat ware collected from a slaughterhouse located in Cluj County, in accordance with current legislation. The samples

were asseptically collected and transported in isothermal bags at 4° – 6°C to the laboratory of Food inspection and Control, within the Faculty of Veterinary Medicine Cluj Napocad. In order to achieve the above mentioned objectives, the following bacterial reference strains were used: *Salmonella enteritidis* ATCC 13076 (Microbiologics, USA), *Campylobacter jejuni* ATCC 29428 (Microbiologics, USA); *Escherichia coli* ATCC 25922 (Microbiologics, USA), *Yersinia enterocolitica* ATCC 23715 (Microbiologics, USA) and *Listeria monocytogenes* ATCC 19114 (Microbiologics, USA). For the contamination of samples with the above-mentioned pathogenic germs, the lyophilized cultures were suspended in a nutrient broth maintained at 37° C for 24 hours in *Salmonella* spp. and *E. coli* and TSYEB broth maintained for 48 hours in case of *Listeria monocytogenes*. After incubation, inoculation was performed on specific selective specific media (XLD - *Salmonella* spp., TBX - *E. coli*, ALOA - *Listeria monocytogenes*, mCCD and Karmali agar - *Campylobacter jejuni*, CIN agar – *Yersinia enterocolitica*), which were thermostated at specific temperetures, according to the specific pathogen for 24-48 hours. For each type of pathogen germ, 5 specific colonies were subjected to cultural and morphological confirmation tests. From the selective media, the colonies were cultured on nutrient agar, which was incubated at 37° C for 24 hours. Biochemical and serological tests were performed to confirm that the isolated strains were pure. In order to obtain microbial suspensions with a specific number of bacteria, colonies developed on the nutrient agar were homogenized in 5 ml sterile saline solutions in test tubes until a turbidity of 0.5 on the MacFarland scale was obtained, verified with the Densimat apparatus (Biomerieux). According to the manufacturer's stipulations, the value of 0.5 corresponds to a load of microorganisms of 150 x 10^6 CFU/ml. The collected samples were sterilized inside the microbiological cabinet using UV radiation for 30 minutes in order to inactivate any pathogenic germs. Three solutions of organic acids: lactic, acetic and citric in different concentrations (1%, 2% and 3% respectively) were used to assess the antimicrobial potential. In order to evaluate the decontamination effect of some organic acid solutions, the samples were processed as follows: each pork meat sample was divided into 11 sub-samples of 25 g, from which one was the negative (uncontaminated) control; 9 samples have undergone the decontamination process with the aforementioned organic acid solutions, and the latter has been a positive (non-contaminated) control; Samples 2-10 (25 g of meat each) were contaminated with 1 ml of microbial suspension (0.5 MacFarland), then homogenized for 30 seconds in the Stomacher device (230 rpm), leaving 30 minutes at 20°C to ensure the adhesion of bacteria to muscle tissue; Afterwords samples 2-10 were subjected to the decontamination procedure by adding 25 ml of 1%, 2% and 3% citric acid solution, acetic acid and citric acid respectively, followed by homogenization in Stomacher (230 rpm, for 30 seconds), being maintained for 1 minute at 20° C for the decontamination effect; For isolation of *Salmonella*, *Escherichia*, *Listeria*, *Campylobacter and Yersinia*, strains, 225 ml of buffered peptone water (BPW), semi-Fraser broth, Bolton broth, Irgasan–ticarcillin–potassium chlorate broth were added to samples 1-11, performing serial dilutions: 10^{-2}, 10^{-3}, 10^{-4}, -110^{-5}, 10^{-6}, 10^{-7}, 10^{-8}. For the isolation of pathogenic germs standardized methods have been used, according to the current legislation (SR EN ISO 6579 AC/2009, SR EN ISO 11290-1/A1/2005, SR EN ISO 16649-2/2007, SR EN ISO 10272/2006, SR EN ISO 10273/2003). Statistical analysis of the rsults was realized using Origin 8.5 software program by comparison of means by analysis of variance through ANOVA test. The statistical interpretation of the results was realized according to the probability indicator: $p \leq 0.05$ (confidence level 95%).

Results and discussions

The decontamination effect of organic acid solutions on Salmonella enteritidis

Based on the results, it was found that 1% acetic acid solution showed a reduction of *Salmonella enteritidis* from 6.78 to 5.54 ± 0.12 log CFU/g (1.24 log reduction), of 5.17 ± 0.13 log CFU/g in case of the lactic acid solution 1% (1.61 log reduction), and of 5.92 ± 0.09 log CFU/g (0.86 log reduction) in case of citric acid 1% (Figure 1). In case of using 2% organic acids solutions, the Salmonella load decreased at 4.47 ± 0.10 log CFU/g for acetic acid (2.31 log reduction), to 4.57 ± 0.09 log log CFU/g for lactic acid, respectively (of 2.21 log reduction), and 5.53 ± 0.13 log CFU/g for citric acid (1.25 log reduction) (Fig. 1).

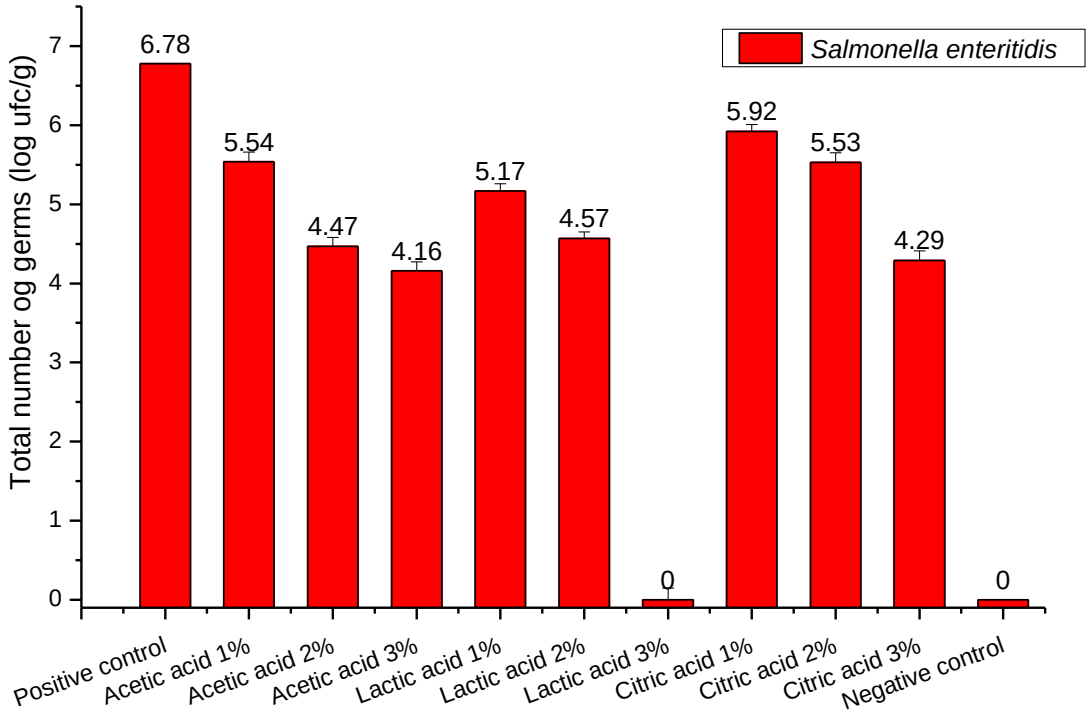

Fig. 1. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *Salmonella enteritidis* on pork samples (n=3)

Following the use of organic acids at 3% concentrations, a reduction in the initial load at 4.16 ± 0.12 log CFU/g was noticed after the use of acetic acid (2.62 log reduction), to a total inhibition in case of lactic acid. Citric acid solution reduced the initial load to 4.29 ± 0.29 log CFU/g (2.49 log reduction) (Figure 1). Statistical analyzes revealed significant differences regarding *Salmonella enteritidis* load between positive samples (contaminated) and decontaminated samples with organic acid solutions ($p < 0.05$). Significant differences were noticed regarding lactic and acetic acid, only in case of using 3% solutions ($p < 0.05$).

Similar results have been published by Seoknam *et al.* (2003), which found that the use of organic acids in concentrations of 0.5%, 1%, 1.5% and 2% resulted in a *Salmonella* prevalence

reduction of 0.4-1.4 log CFU / g in the case of lactic acid, with 0.6-1.5 log in the case citric acid, and 0.5-1.5 log UF /g in case of acetic acid. Unlike our results, which have shown that 3% lactic acid is most effective, Seoknam *et al.*, (2003) established that acetic acid was the most effective decontaminating agent.

The decontamination effect of organic acid solutions on E. coli

After the application of 1% acetic acid solution, the initial load of *Escherichia coli* decreased to 6.65 ± 0.09 log CFU/g (0.13 log CFU/g reduction), to 6.47 ± 0.09 log CFU/g after lactic acid use (0.31 log reduction) and 6.51 ± 0.16 log CFU/g after the use of citric acid, (0.27 log reduction), without significant differences (p> 0.05).

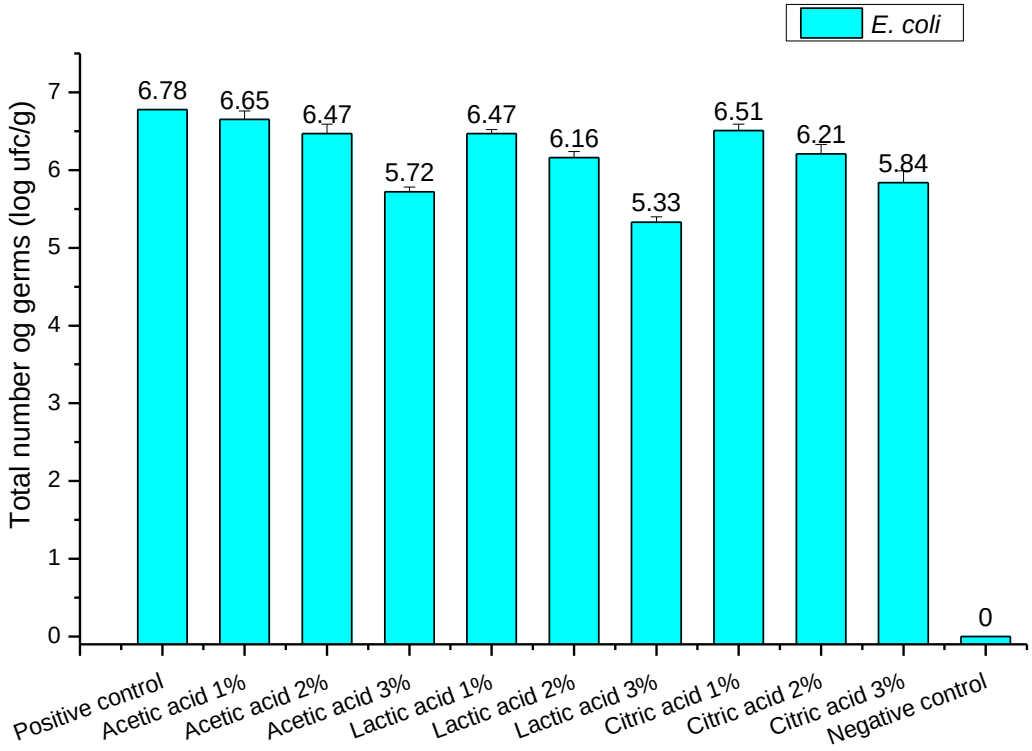

Fig. 2. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *E. coli* on pork samples (n=3)

After application of 2% organic acid solutions the initial *E. coli* load decreased to 6.47 ± 0.7 log CFU/g for acetic acid (0.31 log reduction) to 6.16 ± 0.18 log CFU/g in the case of the lactic acid (0.52 log reduction) and in the citric at 6.21 ± 0.21 log CFU / g (0.56 log reduction) without significant differences (p> 0.05) (Fig. 2). A more pronounced reduction regarding *E. coli* was noticed in case of using 3% organic acids solutions. Thus, acetic acid determined a reduction to 5.72 log ± 0.08 CFU/g compared to the control sample (1.06 log decrease), lactic acid to 5.33 ± 0.09 log CFU/g, (1.45 log reduction) and citric acid to 5.84 ± 0.1 log CFU/g (0.94 log reduction) (Figure 2). Significant differences between positive control samples and decontaminated samples

were revealed only in case of using 3% lactic acid and 3% acetic acid solutions ($p < 0.05$). Similar results were obtained by Ratkowsky *et al.* (1997), who observed a decrease of 0.5-2 log CFU/g in the prevalence of *Escherichia coli* following the use of organic acids in concentrations of 0.5%, 0.75% and 1%. Samelis *et al*. (2005), recorded a decrease of 1.2-1.3 log/g of Escherichia coli load following treatment with 2% acetic acid solution.

The decontamination effect of organic acid solutions on Listeria monocytogenes

The results showed a decrease in the initial load of *Listeria monocytogenes* from 6.78 log CFU/g (positive control) following the application of 1% organic acids solution to 5.38 ± 0.04 log CFU/g in case of acetic acid (1.4 log decrease), to 5.33 ± 0.05 log CFU/g for lactic acid (1.45 log reduction) and to 5.54 ± 0.21 log CFU/g for citric acid (1.24 log reduction) (fig. 3).

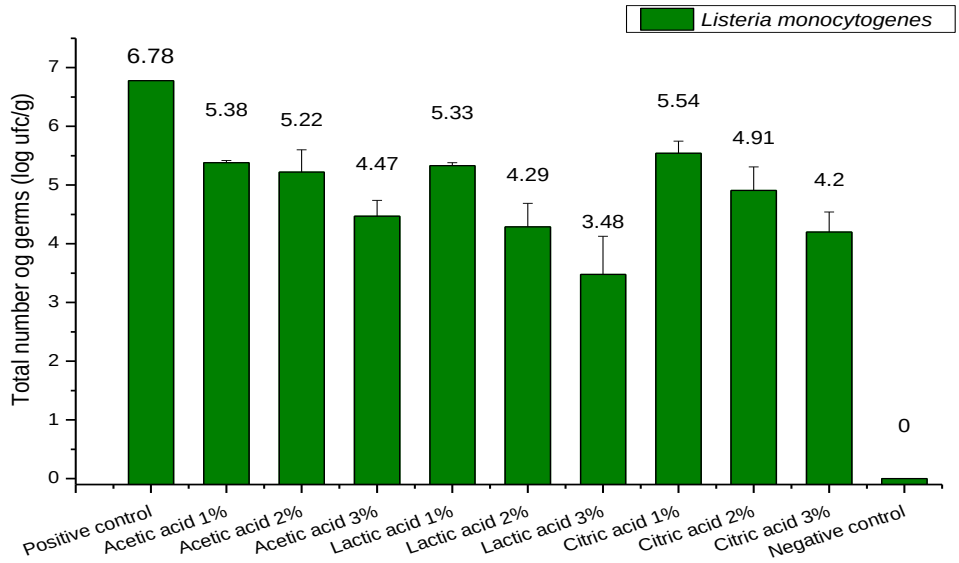

Fig. 3. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *Listeria monocytogenes* on pork samples (n=3)

In the case of application 2% organic acid solutions, a decrease in *L. monocytogenes* load from 6.78 log CFU/g to 5.22 ± 0.38 log CFU/g was observed for acetic acid (decrease of 1.56 log), 4.29 ± 0.40 log CFU/g for lactic acid (reduction of 2.49 log) and for citric acid to 4.91 ± 0.60 log CFU/g (1.87 log reduction) (Figure 3). Following the use of these organic acids in concentrations of 3%, *Listeria monocytogenes* decreased to 4.47 ± 0.24 log CFU/g compared to the control sample after acetic acid (decrease of 2.31 log), to 3.48 ± 0.65 log CFU/g in the case of the lactic acid (reduction of 3.3 log) and to 4.2 ± 0.34 log CFU/g in case of the citric acid (reduction of 2.58 log) (Figure 2). The differences regarding the load of *Listeria monocytogenes* in case of lactic acid treated meat samples in comparison with acetic acid treated meat samples were insignificant in case of 1% and 3% concentrations, but were significant in the case of 2% ($p < 0.05$). No significant differences ($p > 0.05$) were recorded when comparing the efficacy of citric acid with acetic.

Different results were highlighted by Greer *et al.* (1995), who found a 2.0 log reduction in pork meat contaminated with *Listeria monocytogenes* following the application of 3% lactic acid solution. These differences can be explained by the use of different strains of *Listeria monocytogenes* and different working protocol conditions.

The decontamination effect of organic acid solutions on Campylobacter jejuni

The results showed a reduction of the initial load of *Campylobacter jejuni* from 6.78 log CFU/g (positive control) to 4.19 ± 0.39 log CFU/g in case of 1% acetic acid solution (2.59 log reduction), to 4.15 ± 0.37 log in case of lactic acid (2.63 log reduction) and in case of citric acid to 5.40 ± 0.38 log CFU/g (1.38 log reduction), with no significant differences except 3% ($p > 0.05$) (Figure 4). Following the application of acid solutions in 2% concentrations, a decrease was observed compared to the control sample to 3.85 ± 0.39 log CFU/g for acetic acid (2.93 log reduction), to 3.65 ± 0.24 log CFU/g for lactic acid (3.13 log reduction), and in the case of citric acid to 4.07 ± 0.13 log CFU/g (2.71 log reduction) (fig. 4).

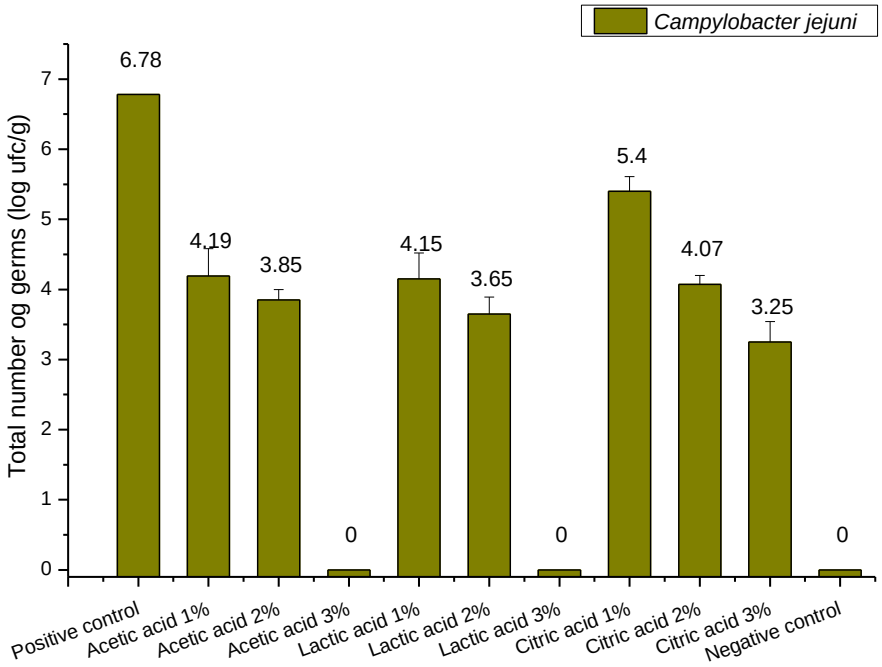

Fig. 4. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *Campylobacter jejuni* on pork samples (n=3)

The most obvious results regarding the decontamination effect of the organic acid solutions were recorded in case of using 3% organic acid solutions (Figure 4). Thus, the acetic and lactic acid solutions determined total inactivation of *Campylobacter jejuni*, and citric acid solution reduced the initial load to 3.25 ± 0.29 log CFU/g (3.53 log reduction). Significant differences regarding reduction of *Campylobacter jejuni* load were recorded in case of citric acid compared to acetic acid only for 1% and 3% concentration solutions ($p < 0.05$). Similar results were obtained by Chaveerach *et al.* (2002), which found after using acetic acid a reduction of 2.02 log (meat pH 4.5), 2.85 log (meat pH 5.0) and 3.24 log (meat pH 5.5). Also, using a 1:2:3 mixture of organic

formic acid, acetic and propionic acid, obtained a reduction of 3.03 log, and at a ratio of 1:2:5 a reduction of 3.22 log (meat pH 4.5).

The decontamination effect of organic acid solutions on Yersinia enterocolitica

The initial load of 6.78 log CFU/g *Yersinia enterocolitica* decreased as a result of using 1% organic acid solutions as follows: 5.39 ± 0.07 log CFU/g after using acetic acid (reduction of 1.39 log), to 5.05 ± 0.10 log CFU/g for lactic acid (reduction of 1.73 log) and 5.36 ± 0.12 log CFU/g after citric acid use, respectively a reduction of 1.42 log.

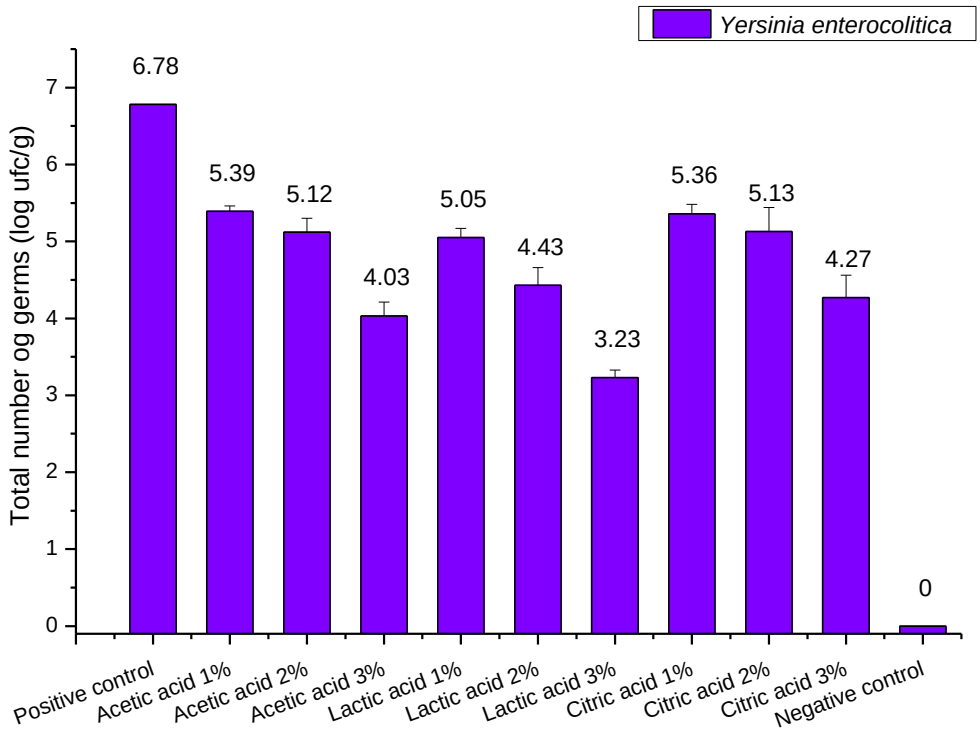

Fig. 5. Decontamination effect of 1%, 2% and 3% of acetic, lactic and citric acid regarding *Yersinia enterocolitica* on pork samples (n=3)

If 2% organic acid solutions were used, microbial load decreased to 5.12 ± 0.18 log CFU/g for acetic acid (1.66 log reduction), to 4.48 ± 0.23 log CFU/g in the case of the lactic, (2.3 log reduction) and in the citric to 5.13 ± 0.31 log CFU/g, meaning a reduction of 1.65 log (Figure 5). The use of organic acids 3% solution revealed a reduction in the initial load to 4.03 ± 0.18 log CFU/g following acetic acid (decrease of 2.75 log), to 3.23 ± 0.10 log CFU/g for lactic acid (reduction of 3.55 log), and for citric acid at 4.27 ± 0.29 log CFU/g (2.51 log reduction) (Figure 5). Significant differences ($p<0.05$) were noticed in case of decontaminated samples with lactic acid (1%, 2% and 3%) when compared with acetic acid (1%, 2% and 3%). Similar results have been mentioned by Greer *et al.* (1995), who reported a decrease in the prevalence of *Yersinia enterocolitica* with 3.5-4.0 log CFU/g, following the application of 3% lactic acid.

Conclusions

Based on our results we conclude that the pathogens have a different sensitivity to the action of organic acid solutions, as follows: *Campylobacter, Yersinia, Salmonella, Listeria, Escherichia*; the effectiveness of solutions of organic acids (in descending order) was: lactic acid, acetic acid and citric acid. 3% lactic acid and acetic acid determined total inhibition of *Campylobacter jejunii* and *Salmonella enteritidis*. Microbial load reduction ranged between 0.13 log-6.78 log, depending on to the organic acid, concentration and pathogen strain. Decontamination methods should be considered as complementary measures to increase the safety of meat, in addition to GMP and GHP.

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21. *** SR EN ISO 16649-2/2007, Metoda orizontală pentru numărarea *Escherichia coli* pozitivă la β-glucuronidază.
22. ******* SR EN ISO 10272/2006, Metoda orizontală pentru izolarea şi identificarea *Campylobacter* spp.

Microbial risk assessment regarding the prevalence of microbial pathogen on poultry carcasses destined for public consumption

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Abstract

The aim of this research was to perform a microbial risk assessment of potential pathogen bacteria in the poultry carcasses processed in slaughterhouses in Transylvania. Between July 2015 and June 2016, a total of 72 samples of poultry neck skin were collected from slaughterhouse located in Transylvania, 36 sample each year (six sample/month). Based on the analyses carried out on the samples collected in 2015, it was found that out of the total of the examined samples, 58.33% were compliant. From positive samples we isolate the following strains: Campylobacter jejuni, Campylobacter coli, Salmonella enteritidis, Listeria monocytogenes and E. coli. In case of samples collected during 2016, we recorded an insignificant increase regarding the prevalence of Salmonella enteritidis (p> 0.05) and a significant decrease of Campylobacter jejuni (p <0.05) between the samples examined in 2016 compared to 2015. Campylobacter jejuni represents the main microbiological risk isolated from chicken carcasses both in 2015 and 2016. The highest prevalence was recorded during the warm season of the year, respectively, from May to August, which indicates deficiencies related to the cooling system of slaughterhouse. The microbiological risk assessment of poultry carcasses demonstrates the very important role of pathogenic microorganisms in food borne illnesses outbreaks in case of non-compliance with hygiene standards and inadequate monitoring of the food safety management system.

Keywords*: microbial risk assessment, pathogens, poultry carcasses, slaughterhouse*

Introduction

Taking into account that meat is one of the most demanded food in many countries, it is important that processing units to respects the highest hygiene standards to be of the highest quality and not to pose a risk to the public health. Microbial load is of major importance in terms of quality, safety and freshness of the meat. Microorganisms, due to their characteristics, can reduce the quality of food, or even make it unwholesome, either by their pathogenic action or by the degradation and production of toxic metabolites (Bărzoi and Apostu, 2002). Microbial contamination may cause triggering of meat degradation processes, reduce their shelf-life, and more severely pose a risk to public health by episodes of food borne illnesses. The contamination of poultry meat with germs can be accomplished mainly during the stages of: scalding, plucking, evisceration and cooling as a result of not-respectiong of Good Hygiene Practice (GHP) and Good Practice (GMP) (Apostu and Stanescu, 2010; Bărzoi and Apostu, 2002; Rotaru and Mihaiu, 2002). As a result, improving HACCP programs, reducing contamination with pathogenic and spoilage microorganisms are very important food safety objectives for food business operators in poultry slaughterhouses (Morshedy and Sallam, 2009; Dan et al., 2008). Failure to comply with preventive procedures may in certain circumstances cause the spread of *Salmonella enteritidis/typhimurium, Escherichia coli, Campylobacter jejuni/coli, Staphylococcus aureus, Clostridium perfrigens, Clostridium botulinum, Yersinia enterocolitica, Listeria monocitogenes* at the surface and in the depth of the carcasses, causing the onset of food borne ilnesses (Bolder, 1998, Butterorth-Heinemann, 2000, Neil *et al*., 2002, Davies, 2004). The main sources of contamination of consumers with pathogenic bacteria are raw or undercooked meat (Mishu *et al.*, 1994, Mossel,

1988, Olsen *et al.*, 2000). Although in recent years there has been a decrease in the prevalence of pathogenic germs in poultry, globally, food borne illnesses outbreaks are reported annually, which leads to strict respecting of good hygiene practices at the farm of origin, GMP, GHP and compliance with HACCP in poultry slaughterhouses. Therefore, in our research we aimed to perform a microbial risk analysis of potential pathogens present in the poultry carcasses processed in slaughterhouses in Transylvania.

Material and methods

Between July 2015 and June 2016, a total of 72 samples of poultry neck skin were collected from slaughterhouse located in Transylvania, 36 sample each year (six sample/month). The sampling was done randomly, both from the beginning and end of working cutting programme on different days of the week, in accordance with current legislation. The samples were placed in sterile plastic bags and transported in insolathed bags at 2-4 ° C to the Laboratory of the Food Inspection and Control at the Faculty of Veterinary Medicine Cluj-Napoca, where they were procesed immediately. For the isolation of pathogenic germs standardized methods have been used, according to the current legislation (SR EN ISO 6579 AC/2009, SR EN ISO 11290-1/A1/2005, SR EN ISO 16649-2/2007, SR EN ISO 10272/2006, SR EN ISO 10273/2003). Statistical analysis was realized using Origin 8.5 software by comparison of means by analysis of variance through ANOVA test. The statistical interpretation of the results was realized according to the probability indicator: $p \leq 0.05$ (confidence level 95%).

Results and discussions

Based on the analyses carried out on the samples collected in 2015, it was found that out of the total of the examined samples, 21 were compliant, respectively 58.33% (Figure 1).

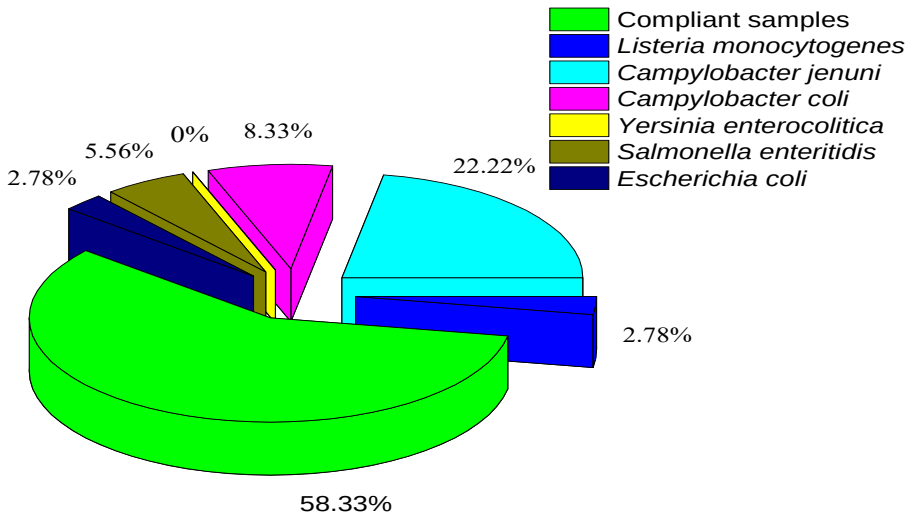

Figure 1. Pathogens prevalence at poultry carcasses during 2015 (n=36)

From the non-compliant samples (15 samples, respectively 41.67%) we isolate the following strains: *Campylobacter jejuni* (8 samples, respectively 22.22%), *Campylobacter coli* (3 sample,

respectively 8.33%), *Salmonella enteritidis* (2 samples, representing 5.56%), *Listeria monocytogenes* (1 sample, respectively 2.78%) and *E. coli* (1 sample, respectively 2.78%), as can be seen in the Fig. 1. Following the examination of samples carried out during 2016 we established a significant decrease regarding the prevalence of non-compliant samples to 33.33%, significant differences were recorded ($p < 0.05$), among the results obtained in 2016 compared to those in 2017. These results are different in comparison with another study performed during 2012-2013 in the same slaughterhouses, which showed a significant improvement in carcass sanitation in the course of 2015-2016 (Dan *et al.* 2015). From the total number of non-compliant samples (n=12), we isolate the following microbial strains: *Campylobacter jejuni* (n=6, 8.33%), *Campylobacter coli* (n=2, 5.56%), *Salmonella enteritidis* (n=3, 8.33%) and Escherichia coli (n=1, 2.78%), as we can see in Figure 2.

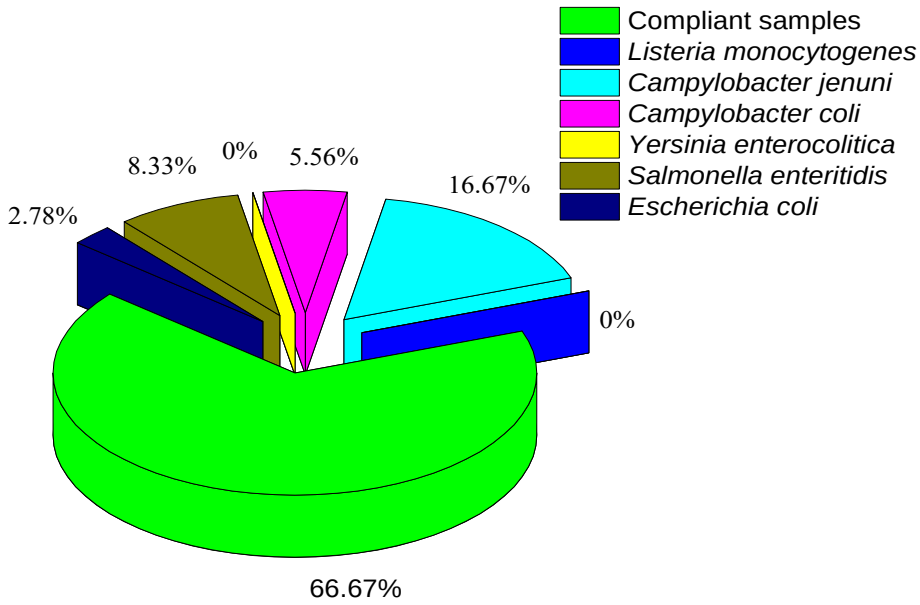

Figure 2. Pathogens prevalence at poultry carcasses during 2016 (n=32)

Analyzing the results presented in figure 1 and 2 we recorded an insignificant increase regarding the prevalence of *Salmonella enteritidis* ($p > 0.05$) and a significant decrease of *Campylobacter jejuni* ($p < 0.05$) between the samples examined in 2016 compared to 2015. No *Listeria monocytogenes* and *Yersinia enterocolitica* were identified in 2016 (fig. 3). Different results were reported by Dan *et al*. (2015) in another study regarding pathogen prevalence in the same poultry slaughterhouse. The overall prevalence of pathogens was much higher, 45.8% in 2012 and 43.05% in 2013. Similar results, with a higher prevalence of pathogens was mentioned by Fukushima *et al.* (1987), regarding contamination of poultry carcasses with *Salmonella enteritidis, Campylobacter jejuni* and *Yersinia enterocolitica*, in poultry meat in Japan. Out of 94 chicken samples, 78.3% was non-compliant. *Salmonella* spp. was identified in 35.0% of all chicken carcasses, *Campyobacte jejuni* in 50.0% of the samples, *Yersinia* spp. in 10.8% of the samples. Investigation has shown that these carcasses were frequently contaminated during the warmer

months of the year. It has been suggested that by inappropriate handling of chicken carcasses, they can become a source of infection with entero-pathogenic organisms, especially *Salmonella* spp. and *Campylobacter jejuni*, at the same time throughout the year.

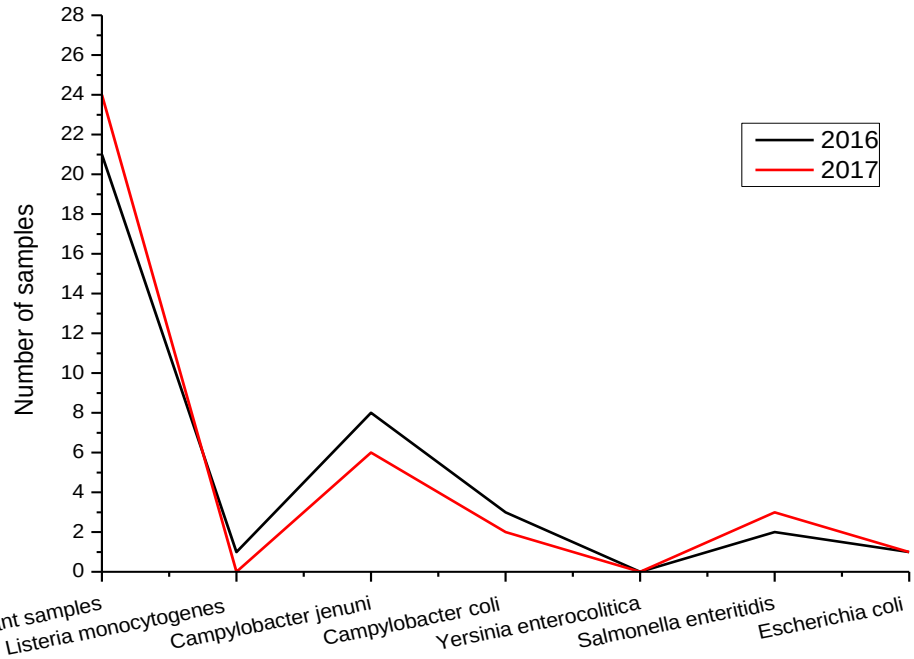

Figure 3. The evolution of the prevalence of pathogenic germs in poultry meat between 2016 and 2017

Other studies published by Rosa and Calleja (2002), regarding the prevalence of *Yersinia* spp., mentioned that out of the total carcasses studied, 26 were positive, of which 22 samples (55%) were contaminated with *Yersinia enterocolitica*. The incidence was higher than that obtained in poultry meat by the majority of the consulted authors. The results suggested that the presence of pathogenic *Yersinia* in poultry carcasses could pose a health risk to consumers in Spain and education of people involved in production, processing and finally preparation of poultry meat products are necessary to avoid cross-contamination. Moran *et al.* (2009), reported a higher prevalence of *Campylobacter jejuni* (64.6% of isolates), *Campylobacter coli* (27.4%), and *Campylobacter lari* (1%), which state prevention strategies to keep under control this pathogen had not a significant results in retail chicken meat. Also, Guyard-Nicodeme *et al.* (2015), reported that *Campylobacter jejuni* was detected in 76% of broiler meat products in a study performed in France during a monitoring plan throughout 2009. Similar results for reported by Korsak *et al.* (2015), in a study regarding prevalence of thermophilic Campylobacter in Poland during 2009-2013. The authors isolated thermophilic Campylobacter in 49.3% out of the total number of analyzed samples, from which *Campylobacter jejuni* was the most prevalent (46%). In case of turkey meat the most prevalent specie was *Campylobacter coli* (71.25). Skarp *et al*. (2016), in a review regarding the role of chicken meat in the incidence of campylobacteriosis, mention that the most important source of contamination of humans is represented by poultry meat, ranging from 46.3-70.9%, depending on the period and region. Also, the most important number of outbreaks in humans were reported

during the summer season compared with winter season, which was correlated with higher prevalence of Campylobacter isolates in poultry meat. According with EFSA report regarding trends and sources of zoonosis, zoonotic agents and food-borne outbreaks in 2013, the prevalence of *Campylobacter* spp. ranged from 11% in Finland and 71% in Austria.

These different results regarding the prevalence of pathogenic microorganisms at poultry carcasses are due to the involvement of certain factors, including: the use of different sampling methods, the geographical area in which the study was carried out, different isolation techniques, the degree of technologization of the slaughtering units, the level of training of the operators, the implementation and monitoring of the GHP, GMP and HACCP.

We can conclude that meat with a high hygienic quality can be produce only if strict hygiene and working practices are followed and rigorous monitoring of the food safety management system is achieved.

Conclusions

Campylobacter jejuni represents the main microbiological risk isolated from chicken carcasses both in 2015 and 2016. The highest prevalence was recorded during the warm season of the year, respectively, from May to August, which indicates deficiencies related to the cooling system of slaughterhouse. The microbiological risk assessment of poultry carcasses demonstrates the very important role of pathogenic microorganisms in food borne illnesses outbreaks in case of non-compliance with hygiene standards and inadequate monitoring of the food safety management system.

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Organic acids effect on spoilage psyhrotrophic microflora during the shelf life of bovine carcasses

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Abstract

The aim of this research was to assess the residual antimicrobial effect of 3% lactic and acetic acid solutions on the load and configuration of psychrotrophs at bovine carcasses. During October 2016 and December 2016, 18 bovine meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples. Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample. Experimental design were carried out over a 14-day period with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera Pseudomonas, Aeromonas, Yersinia and Enterobacteriaceae family. The initial psychrotrophs load of the control sample presented during the experiment an ascendant evolution, from 3.23±0.2 log CFU/cm^2 to 6.21 ± 0.25 log CFU/cm^2, maximum admitted level being exceeded on day 10. After application of 3% acetic acid solution, the total load of psychrotrophic germs decreases to 2.05 ± 0.15 log CFU/cm^2, afterwards showing a constant increase until the last day of the experiment to 4.89 ± 0.21 log CFU/cm^2. The most sensitive psychrotrophic bacteria regarding the decontamination effect of lactic and acetic acid were Aeromonas spp. Lactic and acetic acid solution shown an obvious residual antimicrobial effect during the shelf life of bovine carcasses, when compared with control ($p<0.05$). Although acetic acid has more pronounced residual antimicrobial effect, we recommend using 3% lactic acid because it is a natural metabolite of muscle tissue and does not induce organoleptic changes in meat compared to acetic acid. The use of these methods of decontamination of carcasses should be considered as complementary measures to ensure hygienic quality and meat, and must be integrated within HACCP systems.

Keywords*: residual antimicrobial effect, organic acids, spoilage psychrotrophs, bovine carcasses*

Introduction

The effectiveness of using a wide range of antimicrobial treatments to reduce the prevalence of spoilage and pathogenic bacteria from food producing aimals carcasses has been extensively studied and documented (Alakomi *et al.*, 2000; Castelo *et al.*, 2001; Staruch *et al*., 2001; Strivarius *et al.*, 2002; Ockerman *et al*., 2001a, b; Pipek *et al*., 2004, 2006; Bosilevac *et al*., 2006). Different procedures have been tested over the past 25 years to reduce microbial contamination from carcasses immediately after the slaughter. The most effective and practical methods for reducing microbial contamnation have been technically proven to apply solutions of diluted organic acids or hot water to the carcass surface, exposure to water vapor under pressure (steam pasteurization) and the use of steam or hot water in combination with the vacuum packaging of the meat. Other types of treatments have been tested, but with less extensibility, as follows: rinsing of carcasses with different chemical solutions (chlorination, trisodium phosphate, pulsating light exposure, pulsating electric fields or ionizing radiation (Pipek *et al*., 1997; Stradford *et al*., 1999; Castelo *et al.*, 2000; Staruch *et al.*, 2001; Strivarius *et al.*, 2002 a, b). Of the organic acids, the most used for reducing the microbial load on the carcass surface are: lactic and acetic acids in variable

concentrations, between 2-5%. By using the mentioned organic acids solutions on carcasses a reduction of microbial contamination was observed to 1.5 log. Some studies have shown that some meat pathogens are particularly susceptible to organic acids (*Yersinia enterocolitica, Aeromonas hydrophila*) while others are more resistant (*E. coli* O157: H7). One possible advantage of organic acid treatments compared to other types of treatments is that there is residual activity after applying them. Instead, some research has shown that reducing the bacterial load from the carcass surface was not correlated with improved meat hygiene, due to recontamination and microbial growth during post-processing and storage (Gill and Landers, 2003). Therefore, our research aims to assess the residual antimicrobial effect of 3% lactic and acetic acid solutions on the load and configuration of psychrotrophs at bovine carcasses.

Material and methods

During October 2016 and December 2016, 18 bovine meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples (2.5-3 ml/100 cm^2). Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample (not decontaminated). Experimental design were carried out over a 14-day period (shelf life of the bovine carcasses), with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera *Pseudomonas, Aeromonas, Yersinia* and *Enterobacteriaceae* family. Psychrotroph plate count was performed according with the protocol described by Nottingham *at. al* (1982). For isolation of psychrotrophs specific selectiv media were used, as follows: Aeromonas and Pseudomonas - GSP agar (Merck), Yesinia - CIN agar (Merck), Enterobacteriaceae – VRBD agar (Merck). Serial decimal dilutions (10^{-1} : 10^{-6}) were obtained from 10 grams of meat and 90 ml water buffered peptone. Spreading method was used to inoculate 0.1ml of inoculum on to the surface of 2 Petri plates. Incubation was realized at 20°C, for 72 hours. Biochemical confirmation test was realized using API 20 E and API 20 NE (Biomerieux). The statistical calculations were using program Origin 8.5. Data interpretation was made by calculating the monthly averages, based on standard deviation (6 samples/month). The statistical test used was ANOVA cathegorial monofactorial analisys. Result were depicted as log CFU/cm^2.

Results and discussions

The initial psychrotrophs load of the control sample presented during the experiment an ascendant evolution, from 3.23±0.2 log CFU/cm^2 to 6.21 ± 0.25 log CFU/cm^2, maximum admitted level being exceeded on day 10. After application of 3% acetic acid solution, the total load of psychrotrophic germs decreases to 2.05 ± 0.15 log CFU/cm^2, afterwards showing a constant increase until the last day of the experiment to 4.89 ± 0.21 log CFU/cm^2. A similar trend was noticed in the case of lactic acid. Total psychrotrophs counts decreased after 24 hours to 2.12 ± 0.19 log CFU/cm^2, followed by a steady increase up to 4.95 ± 0.16 log CFU/ cm^2 on day 14. Based on these considerations, we can mention that the residual antimicrobial effect of both organic acid solutions was maintained until the 14^{th} day, maximum admitted level vas not exceeded and no statistical differences ($p>0.05$) were recorded between acetic and lactic acid, , the maximum recommended limit being exceeded on day 12 (Figure 1). Similar results were obtained by Strivarius *et al.* (2002 a, b), in the dynamic studies performed to assess the effect of lactic acid and

and acetic acid, regarding the decrease of the total plate count at bovine carcasses. The study revealed that acetic acid lowers the microbial load after application, with 1.0 log CFU/cm^2 when compared with initial load, and the antimicrobial effect being maintained until day 7 of the experiment. Also, Pipek *et al*. (2006), in a research regarding the antimicrobial effect of 3% lactic acid on the psychotropic germs at the surface of the bovine carcass observed a reduction of 2.0 log CFU/cm^2, and the maintenance of this effect during the experiment (5 days).

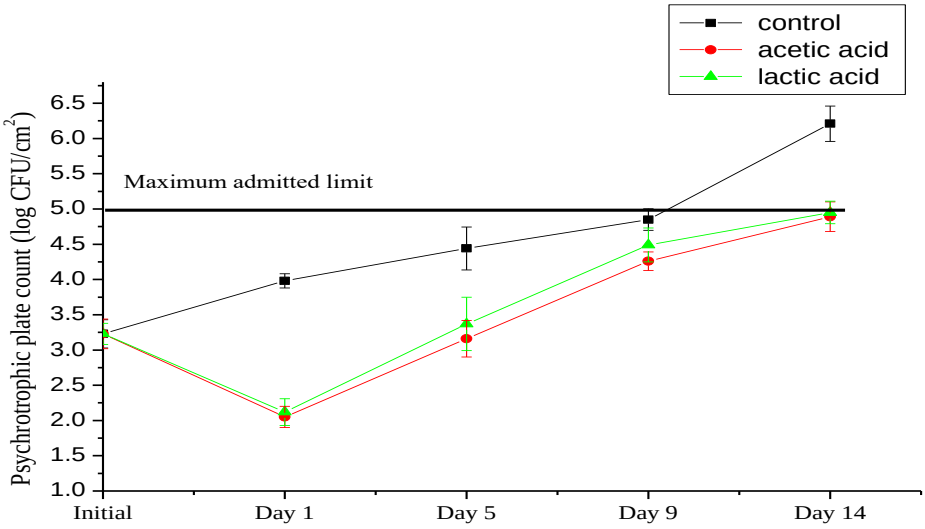

Figure 1. Decontamination effect of 3% organic acid solution regarding psychrotrophic plate count at the surface of bovine meat (n=6)

Regarding the *Enterobacteriaceae* psychrotrophic microflora, an ascending trend from an initial load of 1.89 ± 0.15 log CFU/cm^2 to 5.12 log CFU/cm^2 on the 14th day of the experiment was observed for the control sample, the maximum admissible limit being exceeded in Day 6 (Figure 2). Both organic acid solutions, acetic lactic acid caused after 24 hours a reduction of the number of *Enterobacteriaceae* at 0.87 ± 0.05 log CFU/cm^2 and 1.1 ± 0.41 log CFU/cm^2 respectively, followed by a constant increase up to 3.2 ± 0.25 log CFU/cm^2, respectively 2.89 ± 0.25 log CFU/cm^2 in the last day of the experiment (figure 2). Analyzing these results, we can assume that the antimicrobial effect of 3% acetic and lactic acid solutions was maintained for about 9 days.

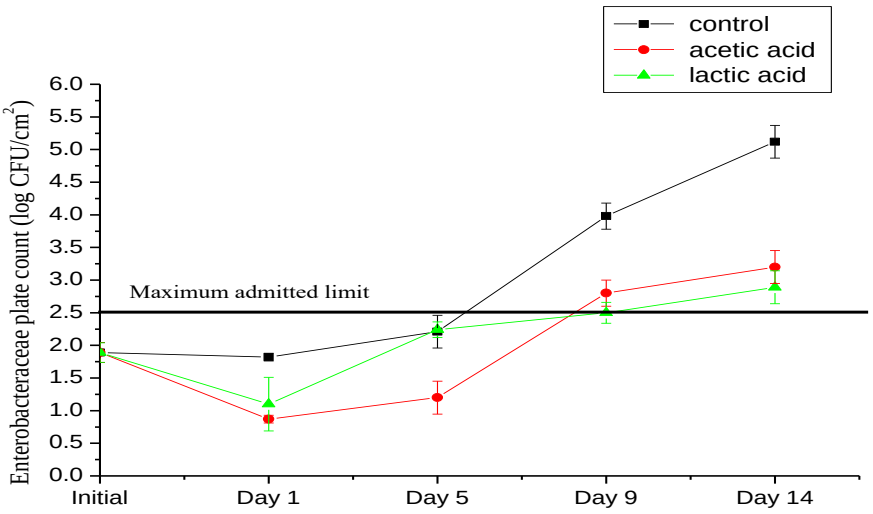

Figure 2. Decontamination effect of 3% organic acid solution regarding *Enterobacteriaceae* load at the surface of bovine meat (n=6)

Following 24 hours since the application of 3% lactic acid solution, initial microbial load of *Aeromonas* spp. decreased from 2.21 ± 0.15 log CFU/cm^2, to 0.89 ± 0.14 log CFU/cm^2. Starting with the 5th day, *Aeromonas* spp. was totally inhibited. Acetic acid solution produced total inhibition of *Aeromonas* spp. after 24 hours since spraying due to their increased susceptibility to acetic acid (Figure 3). Significant differences were recorded when compared acid with lactic acid ($p<0.05$).

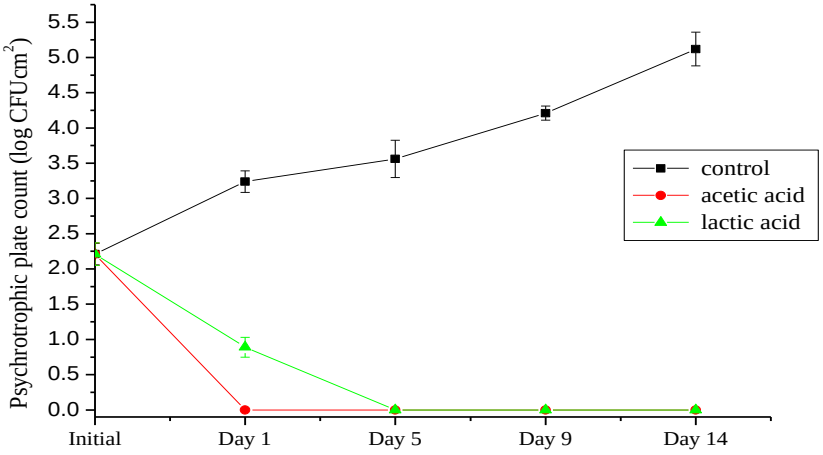

Figure 3. Decontamination effect of 3% organic acid solutions regarding *Aeromonas* spp. at the surface of bovine meat (n=6)

In case of the pseudomonads, an ascending trend regarding microbial load was noticed, ranged from 2.41 ± 0.15 log CFU/cm^2 to 5.6 ± 0.35 log CFU/cm^2 in the final day of the experiment. After the application of 3% acetic acid solution, *Pseudomonas* spp. initial load decreased to 1.96 ± 0.28 log CFU/cm^2, afterwards having an ascending evolution until the last day of the experiment, when they reached 4.02 ± 0.34 log CFU/cm^2. A similar evolution was also noticed for the sample treated with 3% lactic acid (Figure 4). Both solutions of organic acids used caused a decrease of pseudomonads load after 24 hours (1.5 log CFU/cm^2 in case of 3% acetic acid). Afterwards, the results showed a more obvious effect regarding reduction of *Pseudomonas* spp. in case of lactic acid, but no differences were noticed between organic acid solution ($p>0.05$).

With regard to *Yersinia* spp. load, we recorded a constant evolution from an initial load of 3.12 ± 0.20 log CFU/cm^2 to 5.12 ± 0.15 log CFU/cm^2 on 14th day of the experiment (Figure 120). 24 hours after application of the acetic acid solution, *Yersinia* spp. load decreased to 2.31 ± 0.16 log CFU/cm^2, following then by a moderate ascending trend until day 14, when they reached 3.89 ± 0.19 log CFU/cm^2. A similar evolution was established in case of the lactic acid solution, with a more pronounced decrease of the initial germ load, at the end of the experiment (3.56 ± 0.19 log FCU/cm^2).

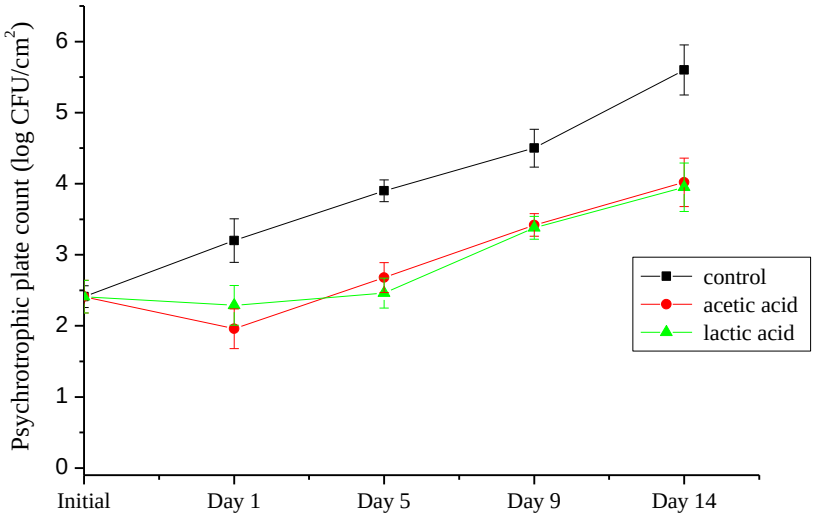

Figure 4. Decontamination effect of 3% organic acid solutions regarding *Pseudomonas* spp. at the surface of bovine meat (n=6)

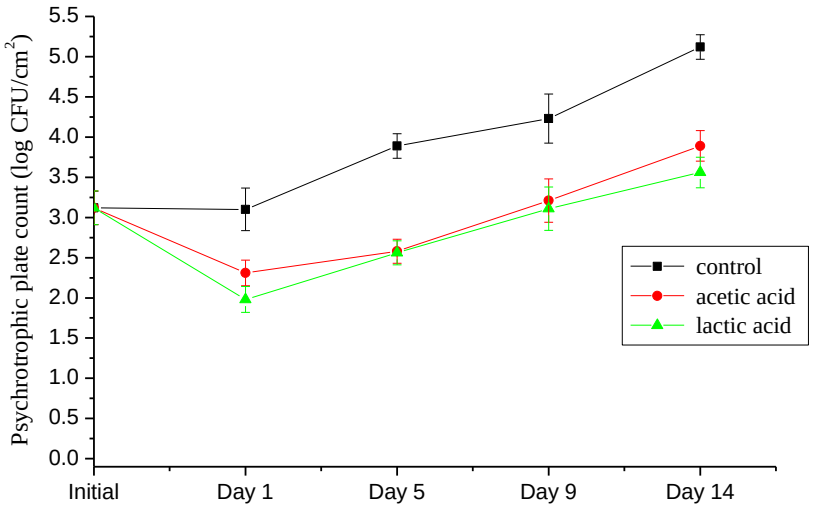

Figure 5. Decontamination effect of 3% organic acid solutions regarding *Yersinia* spp. at the surface of bovine meat (n=6)

Although the antimicrobial effect of the organic acid solutions used to reduce total psychrotrophs, *Enterobacteriaceae*, as well as *Aeromonas* spp., *Pseudomonas* spp. and *Yersinia* spp. load, distinct significant differences were obtained only for aeromonads ($p \leq 0.05$), in which both acetic acid and lactic acid determined total inhibition. These results can be explained by the fact that after the microbial reduction, a variable lag phase (1-5 days), specific for different bacteria followed, to allow some microorganisms to adapt to the new environmental conditions. Similar results were reported by, Alakomi *et al.* (2000), who observed that *Pseudomonas aeruginosa* load from the bovine carcasses surface was reduced from 2.0 ± 0.1 log CFU/cm^2 to 0.23 ± 0.1 log CFU/cm^2 as a result of 2-3% lactic acid spray application. Prasai *et al.* (1991), using lactic acid solutions at different concentrations, from 0.75 to 2.5%, observed that 1.25% lactic acid reduced the number of *Enterobacteriaceae* by 1.0 log CFU/cm^2.

Conclusions

The most sensitive psychrophic bacteria regarding the decontamination effect of lactic and acetic acid were *Aeromonas* spp. Lactic and acetic acid solution shown an obvious residual antimicrobial effect during the shelf life of bovine carcasses, when compared with control ($p<0.05$). Although acetic acid has more pronounced residual antimicrobial effect, we recommend using 3% lactic acid because it is a natural metabolite of muscle tissue and does not induce organoleptic changes in meat compared to acetic acid. The use of these methods of decontamination of carcasses should be considered as complementary measures to ensure hygienic quality and meat, and must be integrated within HACCP systems.

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Lactococcus lactis – an important probiotic of a healthy digestive system

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Abstract

The scientific investigations revealed in this investigation has the purpose the identification of the microbial culture of Lactococcus lactis, considered as a probiotic in the aspects of consumption different milk products as well as the mechanisms of action of probiotics.There were realized bacterioscopic and bacteriological researches by performing microbial preparations and passages on culture media, using different simple and complex methods of investigation, in order to determine the presence and diversity of the saprophytes and pathogens microorganisms. Simultaneously, there were evaluated the aspects of development the culture of Lactococcus lactis on simple and complex identification mediums, the morphology of microorganisms on images natively visualized smears and microbial colonies. It is revealed the importance of this microorganism on the beneficial effects of the human and animal body, representing one of the most important microorganisms from the milk industry, being considered as a probiotic which expresses the balancing of the intestinal flora. In this aspect was proved, that bacteria Lactococcus lactis represents not only the safest and free of risks probiotic which helps the digestion, but also the simplest and natural solution to maintain the health and increase the longevity.

Key words: *Lactococcus lactis, Colonies, Sowings, Culture mediums.*

Introduction

Lactococcus lactis bacteria is considered to be a probiotic, because of its benefic efects. The benefic efects are expressed through the balancing of the intestinal flora, acting against the harmful bacteria from the intestine and preventing the formation of free radicals in the organism. A multitude of infections can be prevented, if it is maintained an equilibrium of the intestinal flora and also if benefic bacteria are provided to the human and animal organism, such as the Latococcus probiotic [1; 5].

According to the scientific investigations, in the last years attempts are made to completely remove antibiotics from the animal feed, from prophylaxis and even from the therapy of some infections, especially digestive. Instead of antibiotics that often destroy both pathogenic and normal flora, without selectivity, or sometimes only destroy normal flora, the pathogen being more resistant, it is indicated the use of probiotics containing bacteria from Lactobacillus and Streptococcus genres– for probiotics containing vegetative forms of bacteria and Bacillus subtilis for probiotics used to increase the bacterial spores. Some probiotics may contain only yeasts or mixes of different genres and yeasts [3; 6].

It is remarkable that probiotics have a direct impact on the digestive system. Thus, the form in which they are ingested in the body is very important, because if their structure is changed too much, they can have adverse effects. It is important to replace the probiotics naturally, and the lactococcus bacterium that reaches the body, to have the possibility to produce proteins for a good functioning of the digestive system [2; 4].

For centuries it has been state, that by the consumption of acidic dairy products health improves, which would increase human longevity. Because the lactococcus bacteria can produce lactic acid, it is one of the most important microorganisms from the dairy industry. It is used for

the production of milk, kefir, yoghurt, cheese and butter. It is also used for fermenting many vegetables, such as pickles, where lactococcus bacteria has a special role.

There are currently numerous studies and data about the practical results of the use of probiotics in animals feed, confirming the advantages of using them. As possible benefits of using probiotics, we can mention improving digestion and stimulating growth, preventing and controlling enteritis, as well as respiratory, bacterial, viral and tumoral diseases [7].

From this point of view, the main objective of this study is to identify the bacterium Lactococcus lactis by the characteristics of microbiological aspects and to reveal the importance of this microorganism as a probiotic in the beneficial effects of the human and animal organism, representing the secret of a healthy digestive system.

Material and method

For performing the investigations were used diary products. The investigations were realized in the laboratory of microbiology and immunology of the Faculty of Veterinary Medicine of the State Agrarian University of Moldova.

In order to identify the micro-organisms from the food product, there were performed bacterioscopic investigations by performing smears, by simple method of coloring using the methylene blue dye, fixing to the spirit flame, microscopy, objective 90, immersion.

Bacteriological investigations were carried out by performing the passages on Petri plate culture media and in tubes, using the simple agar nutrient media, broth and differentiation: Endo Levin.

The plates were incubated thermostat at 37 °C during 24-48 hours. Later, were visualized the developmental characteristics of microbial cultures were studied by studying microbial colonies (edges, color, consistency, relief, etc.). There were highlighted, the character of the development of microorganisms in broth liquid medium, by visualization the microorganisms development characteristics in liquid media (turbidity, sediment, consistency, smell, etc.).From the identified primary cultures characteristic to Lactococcus lactis bacteria, there were performed repeated passages in order to identify pure cultures simultaneously, following the same scheme by performing smears and seedings.

Results and discussions

The detailed analysis of the conducted researches allowed us to isolate under laboratory conditions by bacteriostomy and bacteriology investigations the Lactococcus lactis bacterium.

The determinations carried out on the samples examined by bacterioscopy allowed us to reveal under the results determined by performing smears from native samples and from microbial cultures.The smears were carried out on sterile blades, degreased by bacteriological ansa near the flame of the burning spirit lamp. A drop of milk evenly spread into a thin layer on the blade.After drying the smear at room temperature, the smears were fixed by physical method to the flame of the spirit lamp, colored with the methylene blue dye and examined on the microscope immersion system. On the examined preparations, the microorganism presents spherical form, Gram-positive, unsporulated, unciliated, chain-shaped germs.

The action mechanism of the Lactococcus lactis bacterium is expressed by its action in the digestive tract (gastrointestinal tract). As long as it is situated here, the body takes advantage of its beneficial effects over a long period of time. When the Lactococcus lactis bacterium is added in the composition of milk, it creates energy molecules (ATP) from latose using enzymes. The lactic acid is a product of ATP. The lactic acid prevents the proliferation of microbes. Because of these

facts, Lactococcus lactis bacterium is a natural solution for maintaining our health, being found especially in the digestive tract and vagina. Nisin is produced from milk with the help of Lactococcus bacteria. Also known as bacteriocin, it is a polycyclic peptide. It is made up of 34 amino acids and is commonly used in the food industry due to its antibacterial properties.

Table 1. The effects of probiotics consumtion from acid dairy products

Type of effect	**How it influences the health**
Physiological effects	Antagonist effects on pathogens. Production of bacteriocins
Action on the digestive system	Prevention of intestinal disturbances. Stimulation of intestinal immunity
Alterations of intestinal microflora.	Recovery of intestinal microflora.
Effects on diarrheal disease.	Prevention and treatment of some types of diarrheal diseases.
Systemic effects	Enhancement of immunity. Reducing the blood pressure. Reducing the incidence of some types of cancer. Reducing the cholesterol.

Nisin is capable to stop the bacteria proliferation. Therefore, the lactococcus bacterium is very effective in treating various gastrointestinal affections. Recently, lactococcus bacterium became popular because it is the first live genetically modified organism used in the treatment of different diseases. Not only that this bacterium does not present risks, but is also is recommended, because beside the fact that it produces lactic acid, it also generates the Nisin, very effective against microbes or pathogens.The performed bacteriological investigations confirmes the isolation and identification of the Lactobacillus lactis culture in the kefir food product. The sowings were performed by bacteriological ansa on the culture media of agar, broth, Endo, Levine, next to the spirit lamp, after which they were incubated in the thermostat (Figure 1).

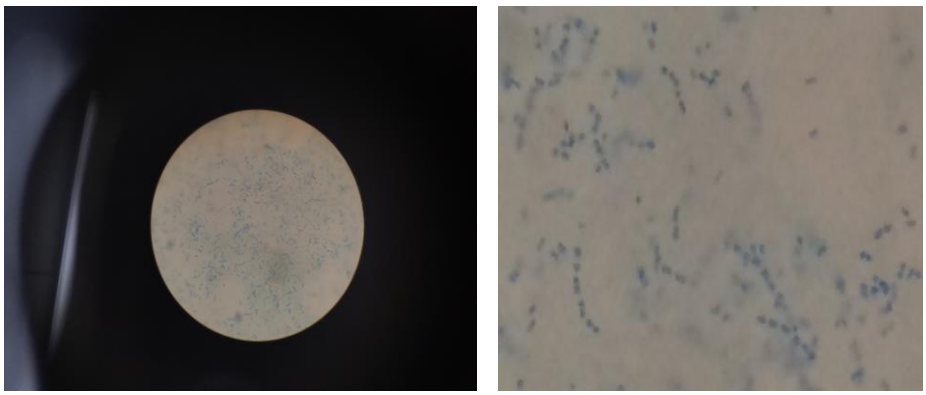

Figure 1. The Lactococcus lactis microscopy

The colonies of microorganisms were visualized in simple broth and agar mediums. On these mediums, lactic bacteria presented microbial cultures by forming a deposit with floconous aspect, slightly abundant, the medium remaining clear (Figure 2). On the agar medium, the characteristic

cultures presented round shapes of "S" type, with edges and smooth surface, being small, regular, semi-transparent, unpigmented.

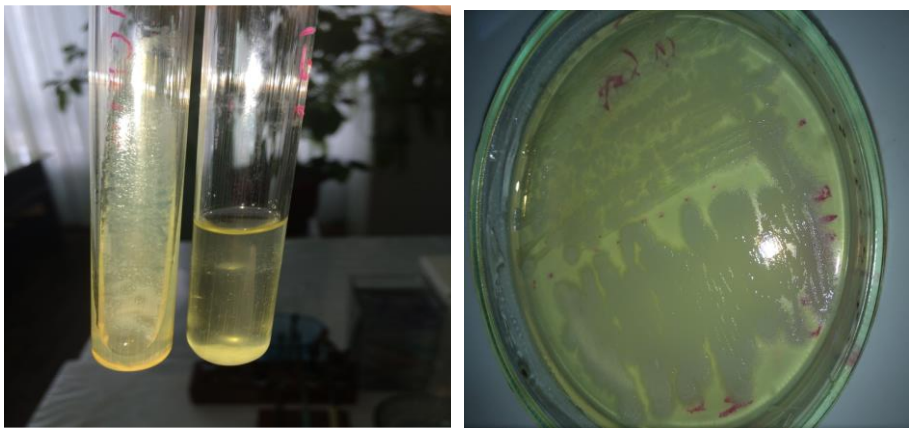

Figure 2. Cultivation of Lactococcus lactis

In this aspect the bibliographic researches reveal, that the acid dairy products are obtained by fermenting milk under the action of lactic bacteria cultures, these are fermenting lactose with the formation of lactic acid, which determines the increase of the acidity of milk; causing its coagulation. The acid dairy products, by lactic acid which is contained in them, prevent the development of harmful microflora in the intestines, helping to prevent and even to treat gastrointestinal diseases.

Table 2. Microorganisms used as probiotics

Genus	Species and subspecies
Lactobacillus	Lactobacillus acidophilus, L.plantarum, L.casei, L.delbrueckii, L.fermentum
Lactococcus	Lactococcus lactis subsp.lactis, L.lactis subsp.cremoris.
Bifidobacterium	Bifidobacterium bifidum, B.longum, B.breve
Streptococcus	Streptococcus thermophilus
Enterococcus	Enterococcus faecalisl; E.faecium

In the same time, unde the action of lactic bacteria, the proteic substances from milk, suffer chemical transformations being decomposed into simpler substances becoming soft, easily digested by the body and thus easily assimilable.Therefore, these products are characterized by a special nutritional value, containing all the nutritive elements of milk in an easily assimilable form. Acid dairy products are also characterized by their quality to conserve longer than milk which is an important economic advantage.

It should be noted that the lactic acid bacteria, which reach the intestine are developing and creating a beneficial medium for the organism health, because their development does not allow the development of other types of bacteria with diverse degrees of pathogenicity. Lactic acid bacteria may also be called „digestive tract sanitars".

The immunity of the organism depends in high proportion, of 50-60%, on the health condition of the colon.

The recent researches has shown that certain components of acidic dairy products, namely conjugated lindeic acid, dairy sphingolipids and probiotic cultures and metabolites can reduce the risk of colon cancer. In vitro studies and on experimental animals have highlighted the internal mechanisms by which these components of acidic dairy products ensures protection against colon carcinogenesis.

Human clinical studies, including recent studies on the use of rations rich in acidic dairy diet products ensures support for reducing colon cancer when the diet is rich in such products. At the same time, the administration of bifidobacteria ensures the production of antibodies for the experimental animals.

We consider this information useful because Lactococcus is a digestive bacterium that can not survive outside the intestinal tract. It has been shown that the bacterium lactococcus lactis is not only the safest and risk-free probiotic that helps digestion, but also the simplest and most natural solution for good health. It is no coincidence that fermented foods are consumed for hundreds of years, which means that the bacterium lactococcus lactis is in our body for centuries. Unfortunately, it is very difficult to find a dietary supplement that can replace probiotics in our body.

Conclusions

1. In the process of growth and development of the animal organism, the most commonly used bacterial strains for the preparation of probiotics are: L. lactis, Lactobacillus acidophilus, L. bulgaricus, L. casei, Bifidobacterium etc.
2. The benefic action of Lactococcus lactis for the animal or human organism is the mechanism of action on the reduction of pathogenic microorganisms, the production of useful substances (amino acids, vitamins, enzymes, etc.), the neutralization of toxins (E. coli enterotoxins) and stimulation of organism immunity.
3. In order to manifest its maximum efficiency, it is recommended that probiotics to be administrated to animals immediately after birth, during periods of stress, and also immediately or simultaneously with the mass therapeutic treatments.

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Immunological investigations regarding the cuantification of cellular populations of the new born calves

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Abstract

The scientific investigations reflected in this study have the purpose of quantification of the cellular populations at the new-born calves in different periods of age. There were determined in dynamics the number of the lymphocytes indices, T and B cells with populations. These data indicates the principal effector role in the immune system activity, as effector of the immune reactions mediated cellularly and humorally, capable to sinthesize some lymphokynes etc. There were determined the increasing of the concentration level of the T and B lymphocytes with populations, which justifies the early instalation of the cellular and humoral specific resistance of the immune status and receiving the antibodies through colostrum in the neonatal period, as result of the instalation of the colostral immunity. The research results demonstrated, that the new-born calves are born with well organized cellular lypmhocytic immune system, represented by the corection of the immune homeostasis of the organism.

Key words: *Immunocompetent cells; Lymphocytes; Cellular immunity; Immune status.*

Introduction

The concept of the immune system represents the capacity of response that differs from one individual to another and causes significant variations throughout the life of each individual or animal. Normally the immune system is an important homeostatic system of the animal body, which determines the state of health and the possibilities of adaptation to various environmental factors. Simultaneously, they manifest a perfect tolerance to their own constituents and react against to those "non-self", in the sense of neutralizing the destruction and elimination from the organism. From this point of view, defining the defense capacity or the real immunological competence of the organism becomes a major component of any medical investigation (Brocaw, A. 2013; Gâjâilă, G. 2013; Cristea, V. et al. 2011; Gâjâilă, G. 2002).

The elements of the immune system - macrophages, lymphocytes - T and B with populations, determine the immunological profile or the immune status and results from the process of complex analysis of elements that are part of the immune system. These immunocompetent cells are developing until the animal is born, after which they intensively begin to function, favoring the development of the immune response during the first days of life of the newborn animal. (Andrieș, L. 2014; Rosen, R. 2008; Andrieş, L., Olinescu, A. 1992).

In its concept, the immune system is one of the most complex from the organism. Its complexity derives from the complicated cellular and molecular network structure that permanently maintains surveillance of organisms. They recognize almost limitless variety of foreign cells and molecules, distinguishing them from the body itself. Therefore, they "remember" each infection, and at the second exposure to the same pathogenic agent, they react much more efficiently.(Siloşi, I. 2014; Taşbac, A. 2014; Siloşi, I. 2013).

Remarkably, after Tonegawa (1985), it is the fact that the immune system does all this with a very small "defense budget", using only a part of the organism's genome and resources. Currently, many pathological conditions consecutive to immune deficiencies, represent the most

eloquent confirmation of the need to determine the immunological profile by investigating the immune system.

From this point of view, the main objectives of this study are to perform and interpret the immunological investigations regarding the quantification of cell populations at new born calves in neonatal period.

Material and method

The investigations were conducted in the microbiology laboratory of the Veterinary Medicine Faculty of the State Agrarian University of Moldova. For performing the investigations were used blood samples from the newborn calves up to 30 days old, in order to perform investigations to determine the blood immunological indices. Blood samples were collected from the heparin jugular vein based on the calculation of 0.3 ml of heparin for 10.0 ml of blood, for the purpose of anticoagulation. The samples were used to identify the number of lymphocytes and competent cells with T and B populations.

The separation of T and B lymphocytes was performed by the spontaneous robotic method. The mechanisms of circulating immune complexes stated entering in contact with heterologous erythrocytes by formation of rosette-like complexes, resulting from couplings between the major erythrocyte surface antigens and lymphocyte receptors. Besides these "immune" rosettes, lymphocytes form with red cells "non-immune" rosettes, resulting from the interaction of antigenic determinants of erythrocytes with other lymphocyte membrane receptors than those involved in the immune recognition of effectors. Simultaneously, from heparinized blood were performed smears, which were fixed with methyl alcohol and colored during 8-10 minutes by the Romanowsky-Giemsa method.

Immunocompetent cells with populations were detected by the lymphocyte layer as a result of the use of 17% Ficol solution for cell separation in a density gradient of 1,077 g / cm. Separation was done in neutral glass tubes. In this scope, 2.0 ml of physiological solution was introduced into centrifuge tubes, and centrifuged at 1500 rpm during 45 minutes. At the limit of plasma and erythrocytes was observed a white lymphocyte ring. The lymphocyte ring was harvested with Pasteur pipette and resuspended by repeated centrifugation, after which was listed the number of cells in the Goriaev room.

T-lymphocytes were assessed by the presence of specific receptors to the red cells of ram, using the E-ROC spontaneous rosette reaction, but the B lymphocytes by the presence of the receptors of the active third component of the complement in the complementary mouse red cell rosetting reaction EAC- ROC. Cell populations have been detected: T-helper, T-total, T-helper, T-killer, lymphocytes B. T cell lymphocyte relations were evaluated after the mechanism of finding Ig M (T helper) T-suppressor) by fractionating the suspension of monocellular cells by the theophylline test method in order to detect the subpopulation of T-helper lymphocytes.

T-killer lymphocytes were detected by enumeration of bull red cells, remaining not injured after 4 hours of incubation in effector cell suspension. After the suspension of the fixed cellular components and the rosetting reactions, on smear-free blades, smears were made, colored by the the Romanowsky-Giemsa method. The results of the immunological rosetting reaction of the were determined by microscopy (40 x 10).

Results and discussions

As a result of the immunological investigation, regarding the quantification of the cell populations in newborn calves, various indicators were found characteristic to the total number of lymphocytes that vary in different periods of age (figure 1).

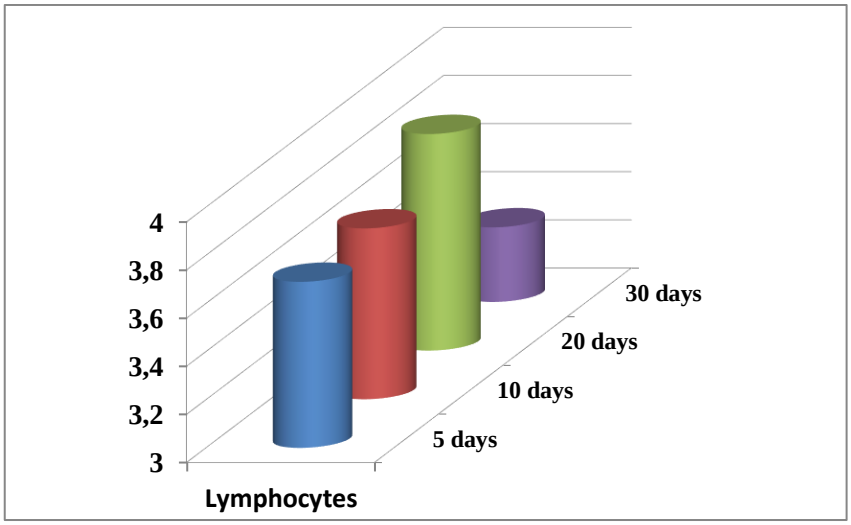

Figure 1. The dynamics of the lymphocytes at the new born calves, %

There was determined and appreciated the number of lymphocytes at the newborn animals, indicating appreciable values at age of 5 and 10 days, constituting 3.69 ± 0.81 and 3.71 ± 0.81 compared to calves aged of 20 and 30 days, constituting 3.90 ± 0.8 and 3.31 ± 0.81. These aspects reveal that the organism is permanently demanded by various factors of the external environment, that come into contact with its own defense mechanism, triggering reactions against what is non-self for the cells of this device. Therefore, these mechanisms determine either a resultant reaction, either neutralization and elimination of them, or an immunological hypersensitivity reaction defined in the true sense of immunology by the concept of immunity.

The Figure 2 reveals the level of T lymphocytes with populations at all researched ages. Thus, at the age of 5 and 10 days, the T-total lymphocytes concentration constituted 14.4 ± 0.075 and 13.4 ± 0.008, compared with calves age of 20 and 30 days, where these indices constituted 16.0 ± 0.08 and 14 , 2 ± 0.08.

In these periods of age were registered significant values of T helper, T suppressors and T killer lymphocytes. Thus, at age of 10 and 20 days, T helper lymphocyte values compared to suppressor T lymphocytes determined 8.10 ± 0.06 and 6.0 ± 0.08. At the age of 20 and 30 days these values determined 8.30 - 0.08 and 7.57 - 0.008, compared to 6.33 ± 0.008 and 6.0 ± 0.08. The study of the T total lymphocytes population compared to T killer lymphocytes revealed significant values in dynamics at newborn calves, which constituted at the age of 5 and 10 days 14.4 ± 0.075 and 13.4 ± 0.008, compared to 14.00 ± 0, 05 and 10.15 ± 0.08. At the age of 20 and 30 days these values determined 16.0 ± 0.08 and 14.2 ± 0.08 compared to 19.45 ± 0.008 and 17.52 ± 0.008.

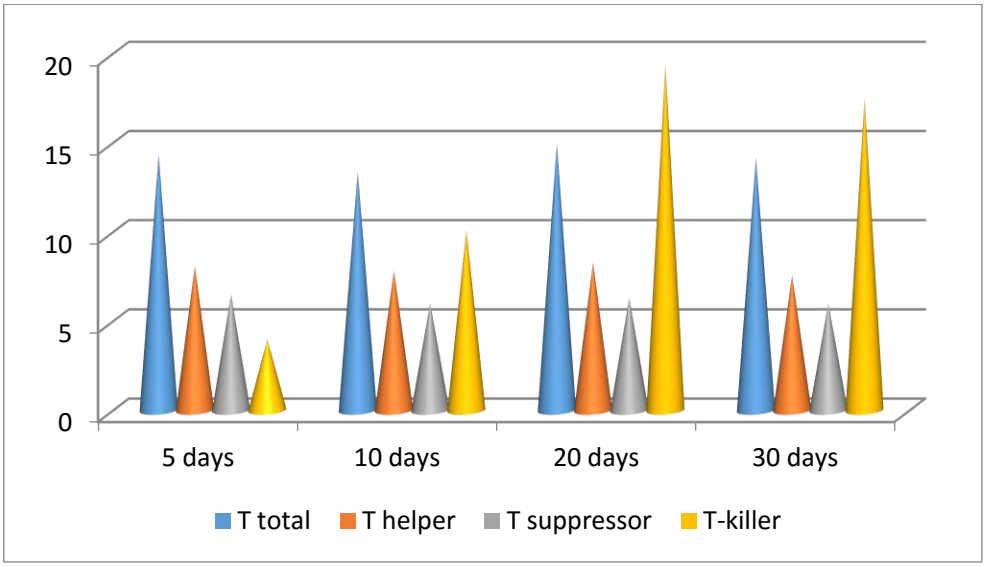

Figure 2. The dynamics of the lymphocytes T with populations at newborn calves depending on clinical status,%

Therefore, the cell activation processes are initiated through mechanisms and complexes characterized by initiation and realization of the functions of the cells involved in the immune response. According to these studies, we can state that the immune system cells are going through the stages of the cell cycle (Figure 3). For these reasons, T cell activation is accomplished by the signals of the antigen and by a costimulatory molecule represented by an IL-1 cytokine. In this aspect, some B lymphocyte activations are triggered as a result of the recognition of the antigen by the BCR molecules. These B cell activation mechanisms induce the realization of synthesis and proliferation of antibodies.

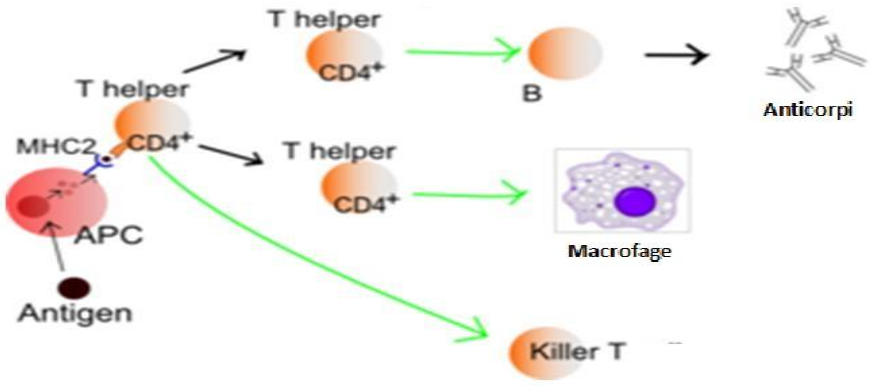

Figure 3. The stages of the cell cycle

Regarding the dynamics of T-killer lymphocytes, are observed significant values at different age ranges at newborn animals. Thus, at the age of 5 and 10 days, the T-killer lymphocyte subpopulation index at newborn calves was 4.00 ± 0.05 and 10.15 ± 0.008. Simultaneously, at the age of 20 and 30 days these values were 19.45 ± 0.008 and 17.52 ± 0.008.

Regarding T killer populations, we can mention the importance of the primary effector role in the cellular and humoral mediated immune system, capable of synthesizing some lymphokines. Therefore, it can be noted that the level of T-killer lymphocyte concentration at the newborn calves at various age periods denotes significant increases due to the activity of these immunocompetent cells, being more significantly expressed from the point of view of immune tolerance and various bacterial infections.

An important role during the investigations determined the separation of lymphocyte populations by the Ficol medium gradient density centrifugation method. The tendency for lymphocyte separation constituted their obtaining and putting in contact with heterologous erythrocytes cells, which later formed rosette complexes, resulting from the coupling of major erythrocytes surface antigens and lymphocytes receptors.

The initiation of these investigations led to the formation of both "immune" and "non-immune" rosettes resulting from the interaction of antigenic determinants of erythrocytes with other lymphocyte membrane receptors than those involved in the immune recognition of erythrocytes. Thus, T lymphocytes present receptors for the erythrocytes of the ram, but B lymphocytes present receptors for mouse erythrocytes. The placement around the lymphocytes of three markers represents a rosette. These reveals receptors for the Fc fragment of the immunoglobulins and respectively, for the C3 component of the complement. For these reasons, we reveal that the mechanism of the immune competence is conditioned by the presence of receptors through which the antigens are recognized.

The most varied cell targets, enveloped by low Ig G concentrations, become sensitive to the cytotoxic effect of certain K-cells (killer) mononuclear cells. This is antibody-dependent cellular cytotoxicity. Therefore, the cells do not have Ig membrane and have lack of T lymphocyte markers.

The mechanism of immune response regulation is based on the immune response, controlled by regulatory systems of a complexity at least equal to those underlying their triggering and expression. In this context, in situations of blocking the regulatory mechanisms, clonal proliferation or immunoglobulin synthesis can no longer be limited, resulting in profound alteration of the immune response, accompanied by the installation and evolution of diseases that usually have a lethal outcome.

Analyzing the dynamics of T and B lymphocytes, it was found that B lymphocytes at the age of 5 and 10 days determined values of 5.85 ± 0.008 and 10.0 ± 0.81, compared to calves age of 20 and 30 days, which constituted 12.0 ± 0.81 and 12.28 ± 0.008. These registered indices reveal an increase in B lymphocytes levels, which justifies the importance of immune resistance specific to immune status and to receipt of antibodies through colostrum during the neonatal period as a result of the installation of colostrum immunity. As a result of activation, the B cells differentiate into plasmocytes and are characterized by the presence of Ig M and Ig G isotypes membrane receptors (Figure 4).

At the same time, according to the performed studies, T lymphocytes are responsible for cellular immunity and express receptors that recognize some peptide sequences from protein antigens. For these reasons, we conclude that the cellular immune response is represented by the T

and B lymphocytes, and therefore the cellular immune response protects the organisms from the aggression of fungi, parasites, viruses and bacteria with intracellular localization.

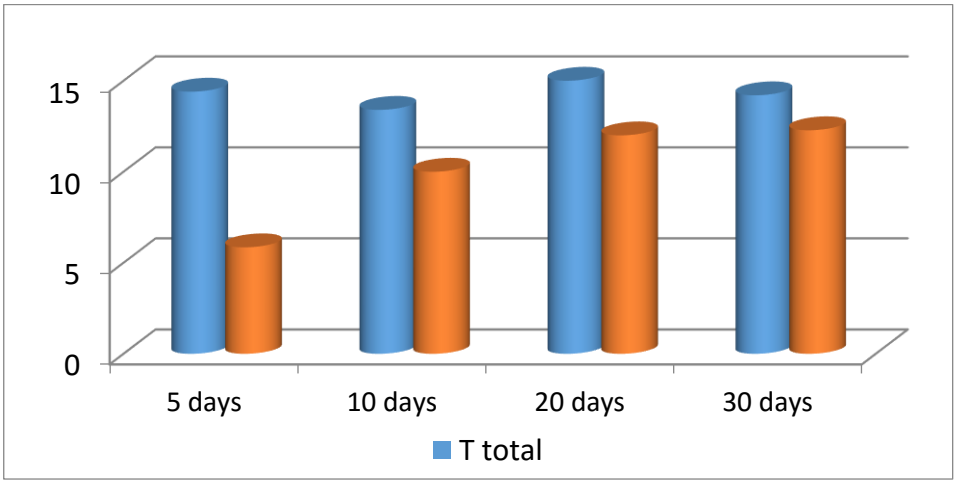

Figure 4. The dynamics of T and B lymphocytes at the newborn calves depending on clinical status,%

The dynamics of T and B lymphocytes indices in various age groups reveal important values in the comparative aspect of T and B lymphocytes. Thus, the level of T and B lymphocytes took values of 14.4 ± 0.075 and 5.85 ± 0.008 at the age of 5 days, compared to the age of 10 days where these indices constituted 13.4 ± 0.008 and 10.0 ± 0.81. Simultaneously, at the age of 20 and 30 days, the T and B lymphocytes values also determined significant characteristic values of 16.0 ± 0.08 and 12.0 ± 0.81, compared to 14.2 ± 0.08 and 12.28 ± 0.008

According to studies performed under the investigation of calves cell populations at birth up to the age of 30 day, there are some peculiarities in the appearance and development of cellular and humoral immunity. Thus, we fully support the idea that the main factor of cellular immunity is the T and B lymphocytes with the respective populations, which determine the immune reactions of the organism.

Based on these considerations, the evaluation of the cellular indices at all ranges of age, starting on the 5th day of life and up to the 30th day, stated significant increases of T and B lymphocytes. These findings allow us to conclude on the fact that the immunological reactivity of the newborn organism and the adaptation to changes in the environment conditions and in particular the action of the pathogens are taking place. In addition to these processes, cellular defense mechanisms against bacteria are performed by effector cells with phagocytic functions (macrophages, neutrophils, etc.). Through various cell mediated mechanisms, the macrophages suffer a process of activation realized under the base of lymphokines secreted by T lymphocytes. They denote the importance of macrophages in triggering and controlling cellular responses that will activate B lymphocytes in order to synthesis antibodies.

Thus, we fully support the study that newborn calves are born with a well-formed T and B lymphocyte system. From this point of view, the ability of young animals to develop immunological responses is dependent on the link to the presence of the immune cell system and the development of the specific immunoglobulins.

In this context, from the presented analyzes, it can be mentioned that it is important to know for the future, the methods of specific prophylaxis of diseases of animal youth of any species. We consider, that these investigations require establishment of an optimal age, doses, inoculation ways, and other parameters determined by the immune response capacity of the animal.

Conclusions

The main factor of the cellular immunity is represented by the T and B lymphocytes with the respective populations, which determine the immune reactions of the organism. These immunocompetent cells determine the activity of cellular systems, which subsequently promote lymphoid cell immunocompetence and regulate other systems with cellular implication.

The evaluation of the mechanisms of the immune system formation at the newborn animal organism offers the possibility to follow the evolution of cellular and humoral responses that maintain immune homeostasis of the body, cellular and humoral protective factors considered the principal factors in the regulation of the immune system.

There were registered increases in the level of subpopulations of T lymphocytes and B lymphocytes in the different age periods by 1.19; 1.21 and 1.26 times.

The performed study under the immunological investigations on quantification of calves cell populations from birth to the age of 30 days determined significant values in appearance and development of cellular and humoral immunity.

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The indices of microflora diversity of chickens saled in the poultry market from Chisinau

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Abstract

The investigations have as an aim to analyze the diversity of the microflora circulating in chickens sold in Chisinau poultry market and to identify the risk factors of contamination with pathogens. As research material were used the chicken flocks which were delivered for the sale from various poultry farms as well as from private and individual breeders from different districts of the country. Lavages of biological material for investigation were collected from the transport units which delivered chickens to the poultry market, from the cells where the chickens were held for transportation, from the floors, walls and equipment where chickens are sold. Insemination was performed on ordinary, special and differential nutrient media. After 48 hours of incubation in thermostat at + 37º C was established a trend of increasing number of colonies of the microorganisms and fungals on Petri dish where the inseminations were carried from the lavages sampling from the transport units as well from cells for birds transportation. As results on nutrient media were present isolated colonies of Streptococcus, Staphylococcus, E. coli, fungals. From the colonies of microorganisms have been prepared smears were stained by the Gram method and examined on the biological microscope (ob. 10x40), which confirmed the presence of microbial flora associated with various forms as mentioned above. The antibiograma test established the highest sensitivity of the isolated microflora was proved to florfinicol, trimethoprim and ciproflocsacin. Investigations have shown that in the poultry market in Chisinau are present several types of conditioned pathogen microorganisms from different units of birds with intensive and extensive production. This represents a serious risk of spreading bacterial infections at commercial and individual units as well. It was found that the most important vectors of transmission through direct inevitable contact on the territory of the poultry market can be the transportation units that transport the birds.

Key words*. Birds, lavages, contamination, microorganisms, the culture medium*

Inrtoduction

The domestic chicken occupies a special place in science and society. It is the most common domesticated animal and the most important and distributed production of food animal in the world. Chickens are an important food animal but can also be responsible for public health problems such as Salmonella. Actually is important to maximize the growth of birds without compromising its ability to resist to the infectious diseases.

Poultry manure can contain a variety of pathogens. Some are host-adapted; therefore, do not represent a health risk for humans. Others can produce infection in humans. The more common zoonotic pathogens in manure include *Escherichia coli 0157:H7, Campylobacter, Salmonella, Cryptosporidium parvum,* and *Giardia lamblia*. The level of risk to humans depends upon a number of factors that dictate how readily the microorganisms are transported through the environment and how long they remain infectious, as well as the numbers of microbes and their infectious doses. It is estimated that most (60%-80%) poultry routinely receive antimicrobials. Antimicrobials may be administered to treat and prevent diseases and outbreaks, or at sub-therapeutic levels to promote animal growth and feed efficiency [2,4].

There are many poultry diseases transmissible to human, among them avian Colibacillosis and avian Salmonellosis are the prime concerns. But the detailed information about avian Colibacillosis and avian salmonellosis in connection to the public health concerns are not available yet in one place. Avian *Salmonella* infections are important as both a cause of clinical disease in poultry and as a source of food-borne transmission of disease to humans. Under the family of Enterobacteriaceae, the genus *Salmonella* is a facultative intracellular pathogen causing localized or systemic infections; as well as a chronic asymptomatic carrier state. The etiological agent of fowl typhoid and pullorum disease is *Salmonella enterica* subsp. *enterica* serovar *Gallinarum*, which is divided into two distinct biovars under the serogroup D1, *Gallinarum* and *Pullorum*, which are denoted as *S. gallinarum* and *S. pullorum, respectively*. Age wise prevalence of avian salmonellosis showed highest infection rate in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens [1,3].

Chickens may be infected early by vertical transmission either from an infected ovary, oviduct or from the infected eggs during the passage through the cloacal faeces from infected or carrier hens. The birds survive from clinical disease. When infected in young stage they may show few signs of infection but become carriers. In adult carriers the reproductive organs are the predilection sites that often lead to the infection of ovarian follicles and as a result transovarian transmission of the disease occurs. The bacteria are passed out through the faeces and lateral spread takes place through the fecal contaminated feeds, transport units, water, litter, commercial pleases etc. [2].

Crowding, malnutrition, transportation, concentration of large number of birds in limited spaces, in specially poultry markets and other stressful conditions as well as unsanitary surroundings can increase the risk of appearance of infectious diseases with high rates of morbidity and mortality in poultry flocks. Taking into consideration the above aim, our scientific direction was to establish some microbial indices of the chickens sold in the poultry market in Chisinau.

Material and methods

The research was conducted at the Chisinau poultry market. As research material served chicken flocks which were delivered for sale from various poultry farms as well as from private and individual breeders from different districts of the country. Lavages of biological material for investigation was collected from transport units which deliver chickens to poultry market, from cells for transporting chickens, from the floors, walls and equipment from the hall where chickens are sold. Microbiological insemination were performed on ordinary, special and differential nutrient media as agar medium, bismuth sulfate agar, medium Endo, medium Saburo and Levin, SSA(Salmonella Shigela Agar). The samples was examined at Republican Veterinary Diagnostic Center, mun. Chisinau and in the laboratory of Microbiology, department Clinics II, Faculty of Veterinary Medicine and Animal Science, SAUM. An index of investigation was to establish the following microbiological criteria: morphological parameters of the colonies that have grown on nutrient media, microscopic variety of microorganisms and sensitivity of bacterial microflora to certain antibiotics.

Results and discussion

The research was conducted in the poultry market of mun. Chisinau where usually are sold chickens and birds of varied species and ages. The birds are being delivered from various poultry farms with intensive and extensive production situated in different districts of the country. Even

though the poultry market entrance has a sanitary filter and the vehicles entering into the market go through a sanitary disinfection barrier, the cells with birds have a specific microflora. Also the movement of microflora is made possible from a flock to another through the air, traders, buyers, and the baskets with birds remaining unsold at the end of the day which are delivered back to the poultry units.

On the figure 1 is shown the aspect of poultry market from mun. Chisinau with the basic construction elements (metal pillars, roof, the sides are covered with wetal mesh and partially transparent glass. Inside of the poultry market (access and the cars which delivering birds of different species and ages from different regions of the country.

Fig. 1 Interior aspect of poultry market

To monitor microbial circulation and its diversity on poultry market the lavages were taken from the cars which delivering poultry for sale on the market (fig. 2), from the cells which are maintained poultry stock in the market (fig. 3), from the market interior walls, floor and other equipment (fig. 4). From the lavages were made insemination on nutrient media and with subsequent study of microbial colonies. The results of this study are shown in figures 5-9. In case when the insemination was performed from the lavages which were collected from interior parts of the market hall (floor, walls, etc.) on medium bismuth sulfite agar have developed colonies of *Salmonella* placed on Petri plate placed unevenly, with dark-gray color and with spherical or oval forms and various sizes (fig. 5). In case when the insemination was performed from the lavages collected from the interior space and equipment of the vehicle for poultry delivery, the colonies have developed very intensive, in a big number, with light-brown color, small and medium sizes, placed over the all surface of Petri dishes, from both types of the lavages which was collected from the interior of transport units and from equipment for birds transportation (fig.6).

Fig. 2 Sample collection from the transport units

Fig. 3 Sample collection from the poultry equipment

Fig. 4 Sample collection from the walls

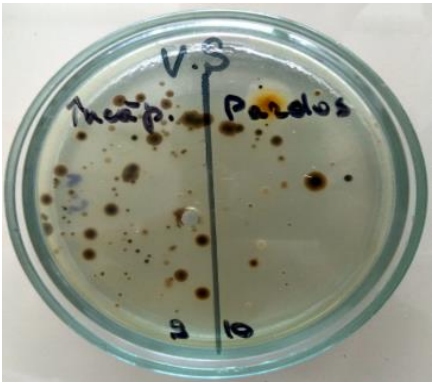

Fig. 5 Microbial colonies on bismuth sulfite agar (Lavages from the hall supports)

Fig.6 Microbial colonies on bismuth sulfite agar (Lavages from the transport units)

Fig. 7 Colonies of *Salmonella*; Salmonella Shigela Agar (Lavages from the transport units)

In case when the insemination was performed from the lavages which were collected from interior parts of the market hall (floor, walls, etc.) on medium Salmonella Shigela Agar (fig. 7), the

colonies of microorganisms have been developed quite intensive, occupying all of the surface of the plate Petri, predominantly with small-size round form colonies and ping-red color.

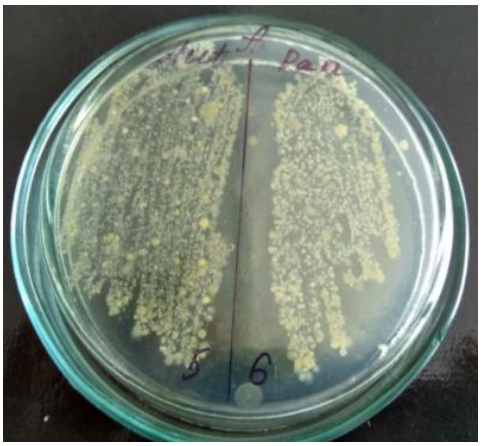

Fig. 8 Colonies of *Streptococus* on Agar medium (Lavages from the transport units)

Fig.9 Colonies of *fungals* on Saburo medium (Lavages from the hall supports)

An intensive growth of *Staphylococcus* and *Streptococcus* colonies was established in case of insemination on agar medium when the lavages were performed from hall equipment and transport units. Colonies were a green color with round and shape forms and with massive number (fig.8). Simultaneously were made insemination on Saburo medium (fig. 9). On this imagines there are a intensive growth of different types of fungi, with regular shape, oval or diffuse forms and with brown, green and gray colors.

Antibiogramma was conducted using microbial colonies isolated from hall supports and transport units. As a result was established that the highest sensitivity of microflora was to florfinecol and trimethoprim with zone of growth inhibition of microbial flora at 22 mm to 17 mm respectively (fig.10 and 11).

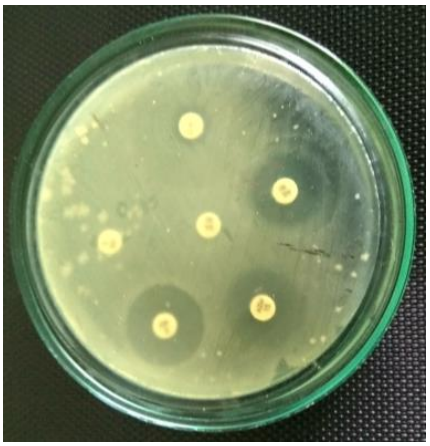

Fig.10 Antibiograma (hall microflora)

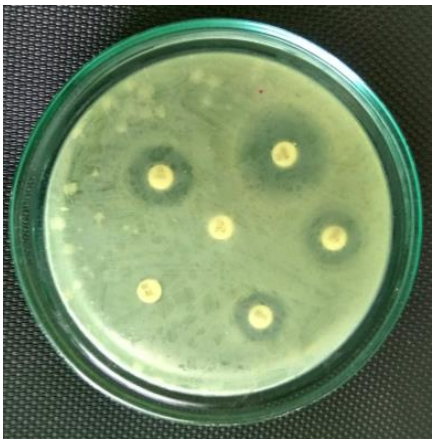

Fig. 11 Antibiograma (transport units microflora)

From microbial colonies were prepared smears by Gram method. On the fig. 12 are presented the *Salmonella* and *E. coli* microorganisms. Its appears in rods form with oval ends, blue

color, placed in separate piles. In fig. 13 are represented *Staphylococcus* and *Streptococcus* which are colored blue, are placed in the field of the microscope in the form of a chain or in heaps.

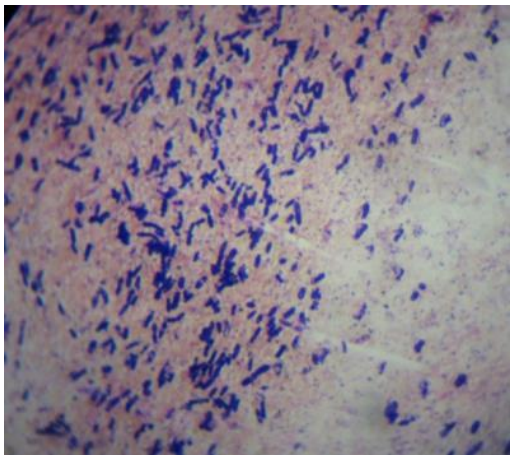

Fig. 12 *Salmonella* and *E. coli*
(Lavages from the transport units)

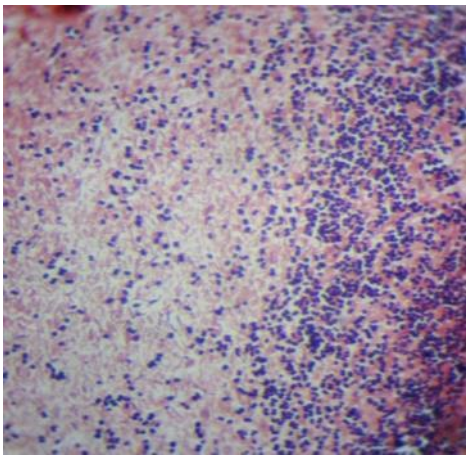

Fig. 13 Colonies of Streptococcus and Staphylococcus
(Lavages from the hall supports)

Conclusions

1. As a result of microbiological monitoring was established that the poultry market remains a diverse background of microorganisms which are represented mainly with *Streptococcus, Staphylococcus, E. coli, Salmonella* and *fungals* which persist inside of the market hall as well as in the transport units.
2. Poultry market can be an important vector of accumulation and spread of pathogenic microorganisms through transport units, poultry inventory or through direct contact between poultry flocks.

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Histopathological and ultrastructure studies in lung of hypoxic bucks

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Abstract

Hypoxia is the result of an imbalance between oxygen supply and demand tissues. This lead to different histopathological changes in lung of bucks at high altitude. The aim of this study is to determine the histological and ultra-structural changes at different ages in high altitude and compare these changes to that happened in low altitude.Sex groups of male rabbits were classified according to age and physiological status young, adult and senile in High and Low altitude. The lung were collected for histological, histochemical and ultra-structure (TEM), examination. Lung of bucks at high altitude revealed histopathological alternation especially in senile one. The lung tissue showed hypertrophy of the alveolar cells and thickening of the alveolar septa and wall. Narrowing of the some alveoli and compensatory emphysema. Intravascular hemolysis in pulmonary blood and infiltration. On the other hand, none of these changes were observed in lung of low altitude exception of slight changes correlated to the aging process.

Introduction

The lower respiratory airway begins with bifurcation of the trachea into two bronchi, one leading to each lung. Both main bronchi further subdivide into their respective lung subsmenental lobar. Further branching into bronchioles occurs, with airway trees ending in terminal bronchioles. Terminal bronchioles are connected to alveolar ducts, where gas exchange took place (Ramchandani et al ,2001; Murray,2010).

The atmosphere pressure and air density decreases. In spite of this decrease in air pressure, the mixture of respiratory gases is the same at sea level (79.04% Nitrogen 20.93% ,Oxygen, 0.30% carbon dioxide) (Senate,2009). Barometric and partial pressures of the available gases in air reduce as the altitude increases. This reduction in partial pressure of oxygen (oxygen pressure at sea level and at altitude 300 meters is 159 and 110 mmHg, respectively) at high altitude causes depletion in oxygen absorption by cells and as a result, the respiration rate increase at high altitude (Jacobson.,2005 and West 2006).

Hypoxia causes a remodeling of the pulmonary vasculature and increases pulmonary vascular resistance (leading to pulmonary hypertension) in humans, rats, and mice (Tucker , et al 1975 and Klinger, et al., 1993).

Materials and methods

Tow group of bucks used in this study ,one group consist of 30 bucks representing Highhabitant was collected from Al-Safa rabbits farm in Taif Governorates [at 1,800 meter height, temperature (19-24 °C), atmospheric pressure (365-367 atm) and(Relative humidity)(31-30 %)] .another control group ofequal number collected from sea level places.In each sample, different stages of bucks life were represented with equal numbers, namely young (<6month), adult (6<36 month), and senile (≥36month). Rabbits under study from each place were kept for 48 hours before conducting research for observation to selected the clinical healthy bucks for the study. All bucks were kept in the same place of collection and fed on the same type of foods.

Experimental design

1. The Tow group of bucks collected from high and low attitude were classified according to age and physiological statusinto youngage, adult and senile.
2. The lung were collected and preserved in formalin10%,Bouin's solution
3. Small pieces of the lungpreserved in gluteraldhyde 2.5% for (TEM)examination .

Collection of organs

The lung were removed from bucks under ether anesthesia preserved in 10% formalin ,bouin's solution and gluteraldhyde 2.5% . These techniques were done according to (Kiernan, 2008). The samples stained with general and special stains Bancroft et al .,1994)

Results and Discussion

Lung tissues in young age at low altitude showed presence of intra pulmonary bronchus, bronchioles, alveolar duct, alveolar sac and alveoli. The intra pulmonary bronchus lined by respiratory epithelium was surrounded by fibroblastic connective tissue and plates of hyaline cartilage. The alveoli are thin walled sac lined by simple squamous epithelium rest on thin layer of inter alveolar septum (fig 1a). (Brody et al., 1981). The alveoli collected together forming alveolar sac which spread in the lung tissue and surround the bronchioles (fig 1b). .Positive PAS reaction in the alveolar wall and bronchiole epithelium and muscles (fig 1c).(Jeffery and Reid, 1977). Faint alcianophilic reaction was found in the alveolar wall while moderate alcianophilic reaction in the epithelium lining of the bronchioles and strong alcianophilic reaction in the cartilaginous plate that surround the bronchioles (Hal and Ghoshal, 1988).

Ultrastructure of the lung, the bronchial epithelial lining formed of two types of cells, the first one elongated columnar with vesicular nucleus and ciliated. The other cell non ciliated contain electron dense granules Clara cell (fig 1d),similar findings obtained by Plopper, (1983) and Massaro et al (1994) who described Clara cells were found primarily in membranous bronchioles, in which they constitute the majority of the epithelium. They are columnar in shape and bulge somewhat into the air way lumen, the cytoplasm contains prominent electron-dense granules which contain a number of lipids and proteins known as Clara cell-specificprotein that may have a role in the regulation of local inflammatory and immune reactions.In present work it was noticed that pulmonary alveolus is lined with continuous epithelium, consisting of type I cells, these results agree with (Crapo et al.,1982). Type I alveolar cells cover approximately 95% of the alveolar septal surface. The nucleus of this cell type is small and covered by a thin rim of cytoplasm containing few organelles Epithelial Type II cells contain characteristic and secretory structures, called lamellar bodies (fig 1e)., similar results were obtain by Bakewell et al., (1991). Who stated that Type II epithelial cells contain characteristic lamellar inclusions that are the source of surfactant, the substance responsible for modifying alveolar surface tension, and small number are mitotically active and are able to differentiate into epithelial type I cells (Crouch and Moxley 1987).

Regarding to the change in the lung tissue in young bucks at high altitude we observedthickening of alveolar septa and mild congestion of pulmonary blood vessels (fig2a& 2b), this finding was supported by Groves, et al., (1993), who explained the alveoli in the lungs tissue in low hypoxic environment the vaso-constrictive response takes place over most of the lung resulting in a rise pulmonary artery pressure.

Ultrastructure of the lung tissues reveal thatthe ciliated cells in the epithelial covering the bronchi contain large vesicular nucleus, cytoplasm contain mitochondriab (fig2c),. The alveolar

septa contains septal capillaries contain RBCs and neutrophil cell with its characteristic lobulated nucleusthis results was supported by Harada et al., (1980). The alveolar cell type one appeared flat and elongated(fig2d), (Walski, 2005)

Our observation in lung of adult bucks from low altitude revealed that most of the lung is composed of thick walled alveoli this was in agreement with Kurozumi et al., (1994). The alveoli are composed of a single layer of squamous epithelium. The intra alveolar septa contain numerous capillaries with positive PAS reaction in epithelial linning of the terminal bronchiole and alveoli.

Ultrastructure of the lung of adult bucks showed the alveolar cell type two large with large nucleus and abundant cytoplasm containing numerous vesicles. This result was supported by Carroll et al., (1993) in human , the latter stated thatpneumocytes type II become clear in adult age that cell type serves several important functions in the biosynthesis and secretion of surfactant from lamellar bodies, which is its primary function their role in the protective or defense mechanisms of the lung.

Histopathological changes in lung of adult bucks at high altitude**.** The alveolar cell showed metachromatic granules in the alveolar tissue, this result was supported by Schneeberger, (1976) in rabbits**.** Increased C.T. fiber around blood vessels, (Fig.3a) these results agreement with Aubert and Lansdorp (2008) in rats, the thickening of connective tissue develops in the diffusion barrier and fibrotic elements that elongates the diffusion path for oxygen from the alveoli to capillaries. Circumscribed lymphocytic infiltration around terminal bronchial and inter alveolar lymphocytic infiltration (Fig.3a)Hamanaka, (2007) who mentioned that elevations in pro-inflammatory mediators associated with hypoxia.

Congestion of the pulmonary capillaries is observed with intra vascular heamolysis (Fig.3c) West et al., (1995) who described that simulated increasing the pulmonary arterial pressure of rabbits due to oxygen decreased lead to rupture of the endothelia lining the pulmonary capillaries and alveoli

Ultrastructure of the lung the alveolar cell type II hypertrophied and having large number of secretory vesicles, these results was supported by Crap et al., (1982) the later said that the alveolar surfactant is a type of phospholipid secreted by alveolar epithelial type II, cell vesicle extensive morphologic changes in the epithelium, endothelium, and interstitium, have been demonstrated during exposure to low oxygen. The alveolar cells type I flat and lining the most alveolar lumen showed thickening of the septal wall with presence of numerous thin bundle of collagen fiber in the septal wall (Fig.3d)(Peter et al., 1981).

Lung of senile bucks at low altitude showed thatthe alveolar epithelial lining was thickened and lung parenchyma is expanded. There were packs of collagen fibers in the interstitium, similar observation by Alder et al., (2008) in aging rat. The secondary bronchiole showed hypertrophy of the its lining epithelium .The intra pulmonary blood vessels was congested in addition to intravascular haemolysis in some of them. The alveoli showed rupture in its wall and collected together forming large size alveoli (Jean, 2005).

Ultrastructure of the lung in senile bucks showed most of the pneumocytes type I and II sparsely degranulated cytoplasm and vacualation of some cells, the intralveolar septum thickening (Walski et al., 2009).

Lung at senile age at high altitude showedcongestion of the septal capillary is observed and bleeding around the vessels in the inter-alveolar septa andNumerous inflammatory cells were occupied the inter-alveolar septa, (Fig. 4a&4b)Frank et al., (2008).Harris, (1986) Parker et al., (1998) said that hypoxic pulmonary vasoconstriction becomes an encumbrance to survival at high

altitude and adaptation by natural selection would be expected to be accompanied by a diminution or elimination of the vasoconstrictor response. Faramoushi et al., (2012) argued that at high altitudes where air pressure decreases gradually, expiration becomes easier. Moreover, due to the plasticity tissues of lungs, its capacity at high altitude also follows the Boyle-Marriott law (inverse proportional relationship between the absolute pressure and volume of a gas (Sonna, 2002 and Brenner, 2011).

Hypertrophy of the alveolar cells and thickening of the alveolar septa and wall of terminal bronchiole. Which lead to narrowing or collapse of the alveoli and compensatory emphysema in the neighboring area (4c&4d), this finding was supported by Verbeken et al., (1989) who said that in humans alveolar ducts increase in diameter and alveoli become wider, also his enlargement is remarkably homogeneous as opposed to the irregular distribution of airspace enlargement in emphysema.Significant thickening and remodeling of the alveolar, collagen deposition has also been reported in emphysema in man and in experimental animals, he collagen changes observed deposition and remodeling in the pathogenesis emphysema (Cardoso et al., 1993, Lang et al., 1994 and Finlay et al., 1996).

Ultrastructure of the lung reveals that the epithelial lining of the bronchiole lined by two types of cells, the first one numerous columnar ciliated cells containing large vesicular nucleus beside the other cell organells such as mitochondria (4e) similar finding was obtained by Paul, (1994), who reported that increased mitochondrial density increases the ability of the cell to utilize the available oxygen. Measures of mitochondrial density provide a guide to the capacity of the tissue for aerobic metabolism. A lower density of mitochondria implies a lower capacity for oxidative breakdown of molecules.

The pneumocytes type II are were and contain electron dens granules and the luminal surface not contain cilia, also thickening of the septa due to cellular reaction, the most reacting cell are monocyte which appear large with abundant light dens cytoplasm obliterating the lumen of septal capillaries (4f)(Fox et al., 1980 and Daniels 2003). the latermentioned that when the lung tissues experience shortages of oxygen and blood, the normal metabolism of the alveolar epithelial type II cells is disturbed.

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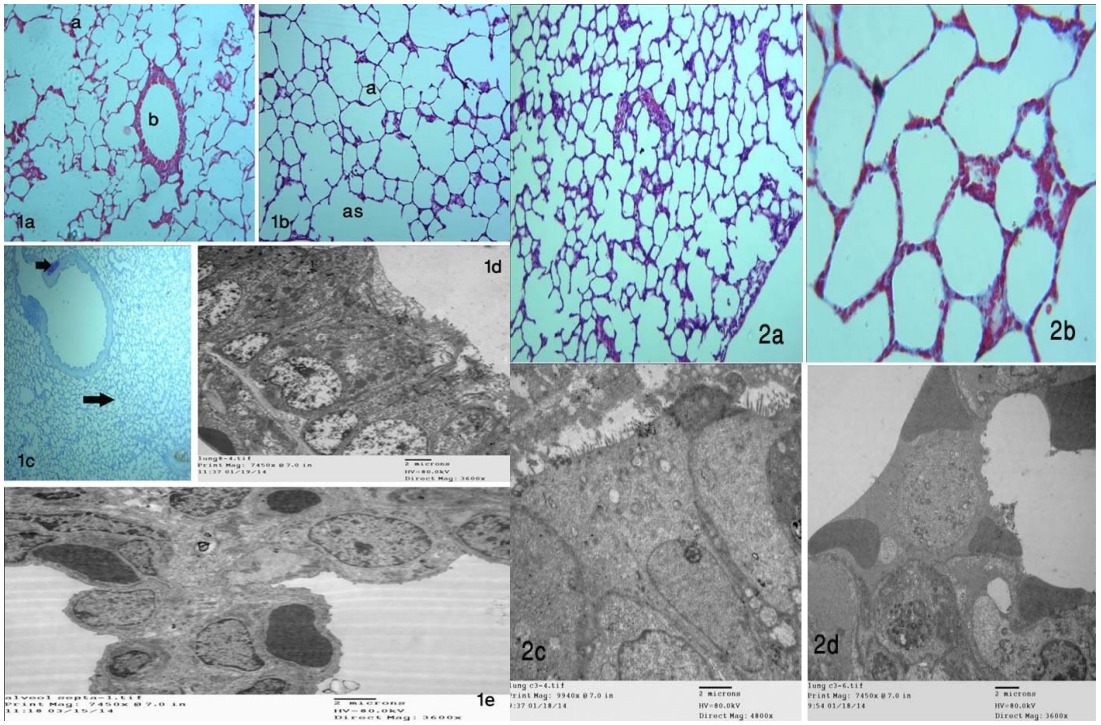

Fig. 1a, lung of buck from low attitude showed alveoli (a) and terminal terminal bronchioles (b) lined with a columnar ciliated epithelium (masontrichrom X10).In 1b, normal alveoli (a) and alveolar sac (as) (H&EX10).In1c, showing positive PAS reaction alveolar wall and bronchial epithelium (arrow)(PAS stain X 40).In1d, TEM of bronchiole showing the simple columnar cell and Clara cell(x3600). .In 1e,TEM showing pneumocystis type towcontaining numerous secretory vesiclesand pneumocystis typeone closely related to the blood capillaries(x3600).

Fig. 2a,lung ofyoung bucksshowedthickening of the alveolar septa and narrowing of alveolar sac (Massontrichrom X40).In 2b, mild congestion of septalcapillaries. In 2c, TEM of bucks lung showing the bronchial ciliatedepithelium contain large vesicular nucleus, the lumen of the bronchi contain mucinous secretion(x3600). In 2 d,TEM of alveolar tissue showing the alveolar septa contain septal capillaries contain RBCs and neutrophil(x3600) .

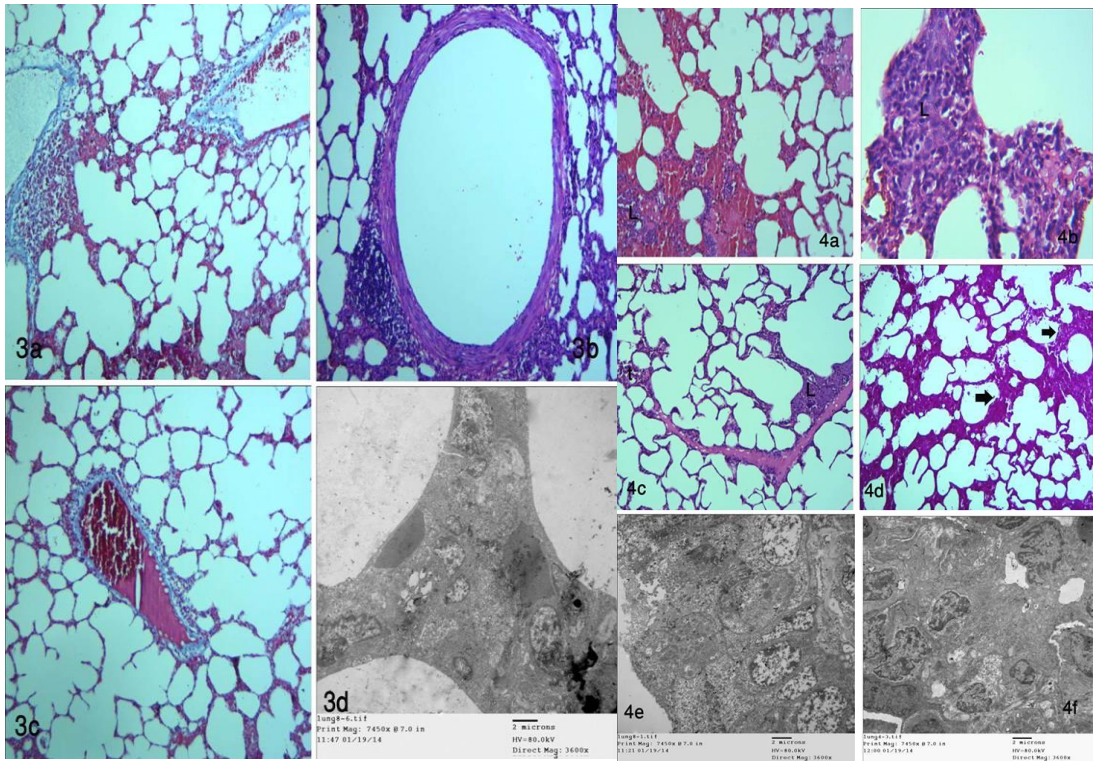

Fig.3a,lung of adult bucks showedincrease CT fiber around blood vessels and thickening in inter alveolar wall (Masson trichrom X40).1n 3 b, circumscribed lymphocytic infiltration around terminal bronchil and inter alveolar (H&E X40). In3 c, showed intra vascular hemolysis with increase the inter alveolar CT (Massontrichrom X40).In 2 d,TEM of septal wallshowed presence of numerous thin bundle of collagen fiber in the septal wall (x3600).

Fig. 4a,,lung of senile buckshowed hemorrhagelocalized the inter alveolar septa (H&E X40).1n 4 b,showed numerous inflammatory cell that occupied the inter alveolar septa and obscured its structure(H&E X40).1n 4 c,lung showed inter alveolar edema with emphysema of some alveolar sacs (H&E X40) .showed intra vascular hemolysis of pulmonary blood vessels(Masson trichro X10).In 4d , strong positive PAS reaction in the epithelial lining alveolar sac(PAS stain X40).In 4e,TEM showed septal capillaries contain RBCs and neutrophil .In4f, collapse of the alveolar lumen and cellular reaction ,The most reacting cell are monocyte .A numerous pneumocystis type two containing secretory vesicles .Also numerous small collagenous fiber in the septal(x3600).

Considerations regarding the morpho-topography of certain lymph nodes in the thoracic cavity in rabbit

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Abstract

In order to conduct the research on the morphotopography of the lymph nodes in the thoracic cavity a number of eight rabbits bodies were used. In the thoracic cavity these lymphnodes can be observed macroscopically, especially after injecting the coloring substance. Investigations on the thoracic cavity lymph nodes were performed so as to preserve their physiological rapports with nearby formations. The parietal and visceral lymph nodes were investigated. Particularities that appear in the cases of the dorsal thoracic lymph nodes, the ventral thoracic lymph nodes, the mediastinal lymph nodes and the tracheobronchial lymph nodes were described. The dorsal thoracic lymph nodes are represented by a fusiform cranial thoracic aortic lymph nodes, with a diameter of approximately 5 mm. The ventral thoracic lymph nodes are represented just by the cranial sternal lymph nodes. The cranial mediastinal lymph nodes appear developed, while the middle mediastinal lymph nodes appear reduced in size. The tracheobronchial lymph node is represented by a distinct lymph node packet and by some reduced lymph nodes at the level of the hilum and the pulmonary parenchyma.

Key words: rabbit, lymph nodes, coloring substance.

Introduction

The specialist literature contains insufficient data on morphological topography of cavitary lymph nodes in leporidae (Barone, 1996; Coțofan, 1975; Harkness et al., 1995).

Due to the fact that these mammalian species are raised in captivity, it is necessary to complete the existing data in the literature. Raising rabbits is practiced both for meat consumption and for capitalizing fur.

We consider that the results obtained from this study constitute a material support that guides the veterinarian to diagnose specific diseases of these animals (Predoi et al., 1997; Predoi et al., 2001).

Materials and methods

The research was conducted on a total of eight leporidae corpses. Taxonomically, Leporidae belong to the Lagomorpha Order, Glires Superoerder, Leporidae Family. The animals were heterogeneous in age (adults), the corpses lacked morpho-pathological lesions that indicate diseases.

The work method was injecting the coloring substance (China ink dye 40%), pre-filtered with a sealed filter paper on a Berzelius glass. For the substance filtering was used physiological saline serum, the dilution used was 1/1 This ink dye was used because it penetrates very well the lymphatic structures. The injection was done slowly to allow the penetration of the dye to the lymphatic structures from the injection site but also proximal to it. Sterile syringes with atraumatic needles were used. The injection dose was 0.3 ml of the coloring solution. The elevation points for injection are the ventral cervical region, dorsal thoracic region, trachea, scapular region. The route of administration was subcutaneous and intratracheal.

Dissection was performed 24 hours after injection. The cutis was removed, the superficial formations were observed and the thoracic laparatomy was performed for highlighting the cavitary lymph nodes: parietal and visceral and the lymphatic vessels. The dissection was made until to the limit of visibility using the Nikon stereo microscope. For the opening of the chest cavity, the proximal and distal ribs were cut at the level of the chondro-sternal joint. In the thoracic cavity was examined the trachea, the esophagus, the base of the heart, the vasculo-nervous formations, lymphatic structures.

Results and discussions

The leporidae chest cavity is relatively short, and the diaphragm convexity appears disposed in a transverse plane passing through the IV diaphragm intercostal space.

The lymph nodes appear embedded in a relatively small amount of adipose tissue. From the parietal lymphocenters of the thoracic cavity, the dorsal thoracic lymph centrer and the ventral thoracic lymph center were highlighted. In the visceral lymph centrers were highlighted the mediastinal lymph node and the tracheo-bronchial lymph center.

Within the dorsal thoracic lymph center, only the cranial thoracic-aortic lymph node constantly evidenced, with fusiform aspect and dimensions of approximately 5 mm diameter. The afferent lymphatic vessels collect the lymph from the parietal and mediastinal pleura and from the ”ceiling” of the chest cavity. Eferent lymphatic vessels are tributary to mediastinal lymph nodes. (Fig. 1)

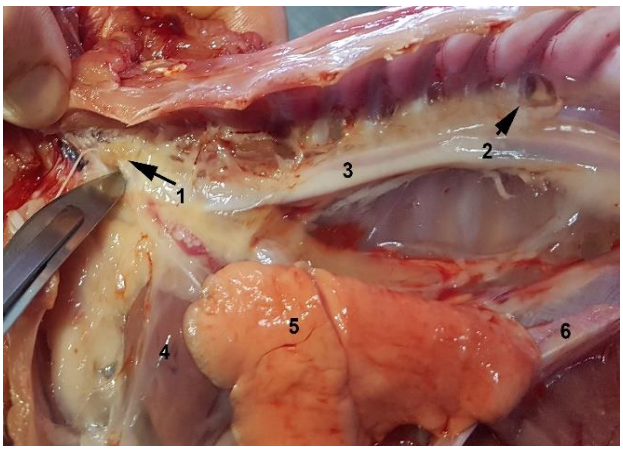

Fig. 1 Dorsal thoracic lymph center

1 – Cranial lymphnodular group ; 2 – Caudal lymphnodular group ; 3 – The aorta artery ; 4 – Heart ; 5 - Left lung ; 6 – Caudal cava vein.

The topography of thoraco-ventral lymph nodes is bilateral symmetric. The **cranial sternal lymph nodes** have been highlighted, which are present in all cases in the number of 3-4 lymphnodular units. (Fig. 2) The form of these lymph nodes is ovoid. The afferent lymphatic vessels collect the lymph from the pericardium, trachea and esophagus. The efferent lymph vessels discharge into the thoracic duct. These lymph nodes appear disposed at the first sternebrae rib, at the dorsal face of the first costo-chondral joint between the first sternebrae rib and second

sternabrae rib.. The length of these lymph nodes is approximately 10 mm. No caudal sternal lymph nodes were identified.

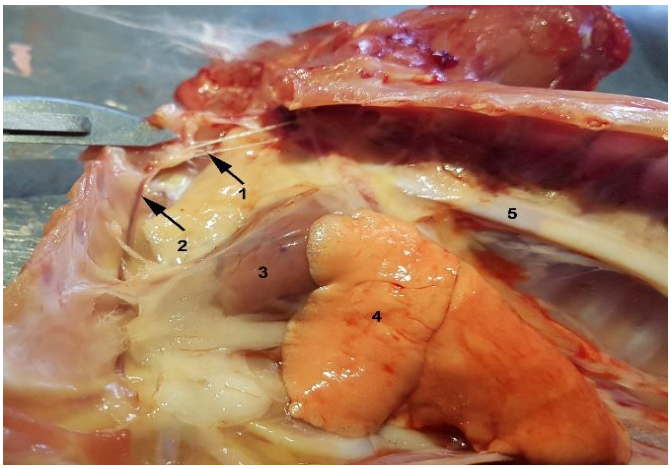

Fig. 2 Cranial sternum lymph nodes
1 – Cranial sternum lymph nodes ; 2 – Internal thoracic artery (cranial sternal lymph nodes located at the origin of the artery); 3 – The hearth ; 4 – The lung ; 5 – The aorta artery. .

Tracheobronchial lymph nodes appear represented as a package made up from two to three lymphatic formations, oval in appearance, with dimensions of about 3 mm. This lymph package is surrounded by the pulmonary parenchyma. In the pulmonary parenchyma as well as near the pulmonary hilum, some lymphatic nodes could be identified, though reduced in size. The afferent lymphatic vessels collect lymph from the pericardium, a visceral pleura. (Fig. 3) The efferent lympatic vessels are tributary to the cranial and middle mediastinal lymph nodes.

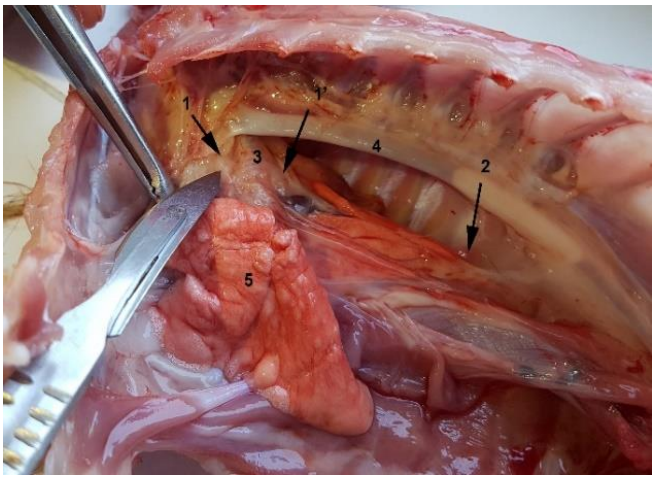

Fig. 3 Visceral lymph nodes of the chest cavity
1 – Left tracheo-bronchial lymph node ; 1' Right tracheo-bronchial lymph node– ;
2 – caudal mediastinal lymph node; 3 – trachea; 4 – The aorta artery; 5 – Left lung

The cranial mediastinal lymph nodes appear well developed, while the middle mediastinal lymph nodes are reduced in size. The cranial mediastinal lymph nodes are located at the origin of the brachio-cephalic artery. These lymph nodes are ovoidal or spherical, and they generally appear as 2-3 formations. The middle mediastinal lymph nodes are placed between the aorta and the base of the heart, two lymph node units with dimensions of about 2 mm. The caudal mediastinal lymph nodes are placed between the base of the heart and the diaphragm. Three to four such lymph nodes were highlighted, with dimensions of about 1,5 mm, in two out of the eight investigated cases. The afferent lymphatic vessels of the mediastinal lymph nodes emerge from the cranial side of the pericardic sac, the heart and the middle mediastinal lymph nodes.

Conclusions

Tracheobronchial lymph nodes appear lymph represented as a package made up of two to three formations limfnodulare appearance ovoid, with dimensions of about 3 mm.

The ventral thoracic lymph center is represented only by the cranial sternal lymph nodes whose topography is bilateral symmetric. The dimension is about 10 mm. They appear at the first sternebrae rib, at the dorsal face of the first costo-chondral joint, at the level of the first and second sternebrae rib.

The tracheo-bronchial lymph center appears as a lymphnodular pack, consisting of two or three lymphatic units, surrounded by pulmonary parenchyma. Some lymph formations are at the level of the hile and lung parenchyma.

The medial mediastinal lymph nodes are reduced, located between the aorta artery and the base of the heart.

The medial mediastinal lymph nodes are reduced in size, and located between the arta and the base of the heart.

The caudal mediastinal lymph nodes are inconsistent (in two cases of the eight investigated).

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Detailed morphological description of pre diaphragmatic digestive system of chinchillas in relation with its clinical significance

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Abstract

In recent years, chinchillas are receiving increasing interest both as pets and experimental model and more recently as commercial importance; and hence, increased attention in terms of medical and veterinary care. Using as research method gross dissection, this study conducted on ten chinchillas, provides a detailed anatomical description of the components of the prediaphragmatic digestive tract in relation with their clinical importance and significance.The results of the present research demonstrate the presence of numerous morphological particularities with strong clinical implications. The small opening of the oral cavity and a large tongue leads to the impossibility of orotracheal intubation in the absence of adequate equipment The elodont, aradicular, hypsodont and monophyodont dentition have a low degree of wear, leading to the appearance of dental diseases. Foreign bodies often block the narrow oesophagus, but hypersalivation makes the difference between blockage and respiratory problems. A strong gastroesophageal barrier prevents the emesis act. The study results are important for both practitioners and researchers in management and care of chinchillas.

Keywords*: morphology, digestive system, chinchilla, clinical signs*

Introduction

In the past few years, the specialization of veterinarians in the field of pet animal medicine has gained more ground along with the medicine dedicated to the animals for rent. In fact, this component is the basis of many practitioners in cabinets and veterinary clinics. Knowledge in the field is useful both for researchers in research institutes that use small animals as an experimental model (Stan 2015), as well as for the general public, which possess these disrespectful cuddles, wholly deserved the name of "exotic species" within pet animals. This leads to a motivated need for a proper knowledge of the anatomy of these species, in order to meet the requirements of treating the many diseases for which they are presented to the veterinarian. Often, owners exceeding financial value seek the highest quality of care, the same level of care provided to dogs and cats presented in the veterinary practices. This research presents the morphological particularities of the prediafragmatic digestive system which are relevant in expressing the clinical symptoms of different diseases.

Materials and methods

The prediagragmatic digestive components of ten chinchillas of varying ages and weights (1-3.5 years, 350-560 g) provided from the chinchillas breeding farm for fur, were examined. All animals received care according to the rules contained in the "Guide for care and Use of Laboratory Animals". The study was approved by the Bioethics Committee of the University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca. Euthanasia was performed by administration of an overdose of isoflurane (AErrane, Baxter, USA) in all animals. Gross dissection was performed and the morphological particularities of prediaphragmatic digestive system have been identified. The scientific literature was reviewed and the clinical expression of a symptom due to an anatomic particularity was documented. Terms were used in accordance with NAV 2012.

Results and discussions

The oral cavity of the chinchilla was small and narrow, relative to the size of the chinchilla head that appeared relatively higher compared to the size of the body. Above the upper lip the presence of many, but tiny, resistant hair, of approx. 1-3cm long were noted. The interior of the oral cavity was examined after the two bilateral incisions were performed. The hard palate (*Palatum durum*) of the oral cavity presented 4-6 transversal ridges (*Rugae palatinae*), disposed only in the rostral region of the angle of the jaw with cranial orientation of the first ridges, while the last ridges showed caudal orientation (Fig. 1).

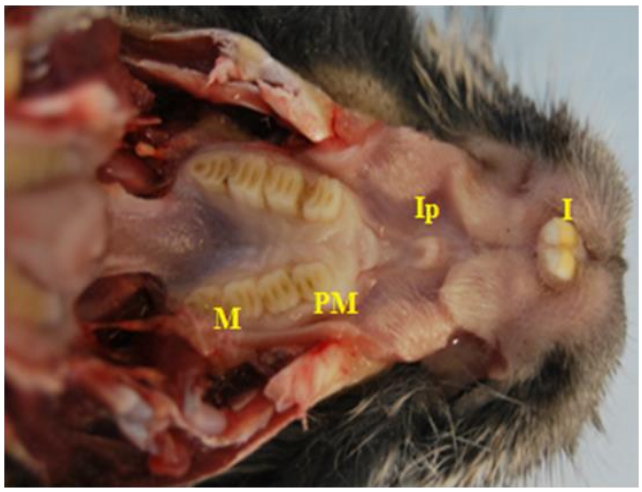

Fig. 1 The hard palate and the presence and divergent orientation of palatine crests, appearance of the upper incisors and cheek teeth. Noted a well represented incisive papilla – Ip, and a large

The soft palace (*Palatum molle*) was smooth and glossy. At the level of the soft palate the presence of a palatal ostium (*Isthmus faucium*) that made the oropharynx's communication with the pharynx was noted (Fig. 2). The tongue presented in the posterior third, a pair of well-represented papillae (*Papillae vallatae*) (Fig. 2). The dentition at the examined chinchilla had the same teeth arrangement on the jaw arcade and the mandibular arcade with the following dental formula: 2 x 1/1 (incisors), 0/0 (canines), 1/1 (premolar), 3/3 (molars), in a total of 20 teeth. The length of dental crowns and the absence of roots were noted in all teeth. The characteristic features of permanent teeth were as follows: enamel of the same colour, increased resistance, proximity between premolars and molars. The incisors were sharp, chisel-shaped, and normally yellowish orange. The upper incisor teeth showed a right angle indentation while the lower incisor teeth showed double radius of curvature that of the maxillary incisors. An obvious anisognathism was noted. A wide diastema was observed between incisors and premolars, both on superior and inferior jaw. Differentiation between the premolar and molar teeth has not been achieved due to the strong resemblance and lack of interdental spaces. The occlusal surface of the premolars and molars appeared broad, tough, and uneven, showing a succession of enamel grooves and grooves (Fig. 3). Also, the occlusal surface of premolar and molar teeth appeared almost horizontally. Both upper and lower dental arches had a divergent pattern with caudal opening, the upper arch being narrower than the inferior arch. The examined chinchilla jaw appeared narrower than the mandible. The

temporomandibular joint allowed minimal lateral movements and wide backward movements due to the presence of obvious mandibular fossils in which mandibular condyles glide in the caudal direction under the action of masseter muscles.

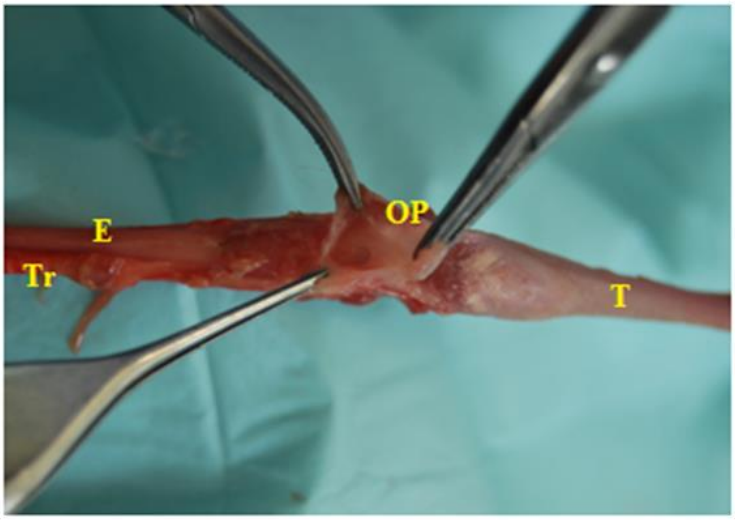

Fig. 2 The ostium palatinale –OP and the two well represented papillae vallate – arrows. T – tongue; E – esophagus; Tr – trachea

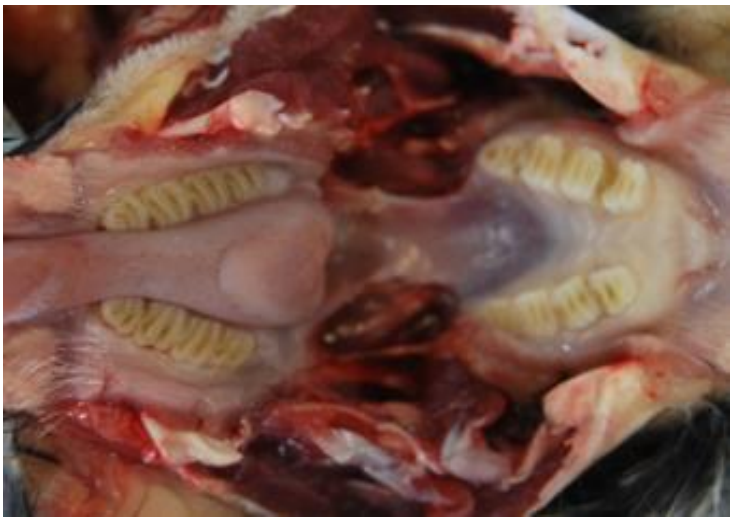

Fig. 3 The large occlusal surface of the cheek teeth and divergent aboral orientation of the upper and lower arcade

The oesophagus (*Esophagus*) presents the same three common segments: cervical (*Pars cervicalis*), thoracic (*Pars thoracica*) and a very short abdominal segment (*Pars abdominalis*). Starting from the pharyngeal-esophageal orifice, the cervical segment of the esophagus was disposed dorsally from the trachea, in the median plane of the neck having a length of approx. 10-15mm (Fig. 4). Prior to entering the mediastinum, the esophagus was positioned to the left of the

trachea, thus entering into the mediastinum at the level of the first rib pairs, and then re-positioned dorsally to the trachea. The next oesophagus report was with the left bronchus which it was passed, then with the base of the heart, the level at which the oesophagus crossed the aortic arch. In the caudal mediastinum, the esophagus was placed disposed between the two lungs, being covered with pleural effusions. The mediastinal segment of the oesophagus did not show any changes related to its caliber. The passage of the oesophagus through the diaphragm was performed between the two diaphragm medial posts, at the level of the phrenic center. Immediately after the passage through the diaphragm, the oesophagus was covered by the peritoneal serosa, derived from the gastro-phrenic ligament.. The abdominal segment was very short, about 2-3 mm. At the level of the dorsal surface of the liver, the oesophagus made an notch on the liver surface and then entered the stomach through a well-represented cardiac ostium (*Ostium cardiacum*).

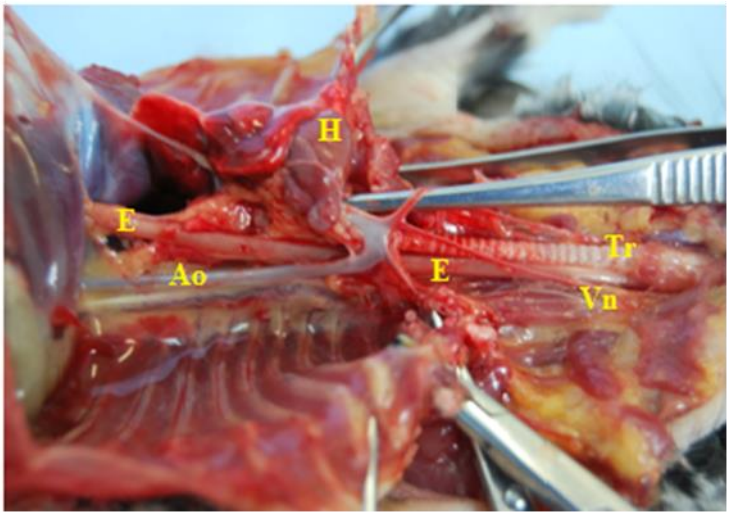

Fig. 3 The oesophagus - E. In cervical segment it runs dorsal to the trachea – T, in the midline being accompanied by the branches of vagus nerve – Vn, and common carotid arterys . In cranial mediastinum the esophagus is placed on the left to the trachea, crossing the aortic arch. H – heart; Ao - aorta

In past years, there were few publications on Rodents and Lagomorphs; currently there are many books, journals, and articles about these species. However, most approach of various systems briefly describes the anatomy of their components. Moreover, comparative anatomical studies are relatively few. Clinical implications of different morphological particularities are often underestimated and the animals are treated according to the common rule. Working with exotic species is a clinical challenge and only a proper knowledge of the anatomy in order to understand the basis can properly treat these pets. This paper described the detailed anatomy of the prediaphragmatic digestive system in relation with the clinical signs of each component. The digestive system of chinchillas poses several particular characteristics which might influence clinical approach and their veterinary care.

The oral cavity is small and a large elongated tongue covers most of the floor of the mouth and oropharynx. The nasopharynx and orophaynx are separated by the soft palate, which communicates with the oral cavity through the palatal ostium. The palatal ostium (previously named the interpharyngeal ostium – *Ostium intrapharyngeum*) is the only connection between the

oropharynx and pharynxes, making the chinchillas obligate nose breathers (Quesenberry et al., 2004). This feature is common in rabbits, guinea pigs and the majority of rodents (Hoefer and Crossely 2002; Stan 2015). This feature is unique among rodent and is important when a surgical procedure is performed which needs endotracheal intubation (Brewer and Cruise 1994). Intubation is difficult due to the small glottis, long tongue, narrow oropharynx and laryngospasm (Brewer and Cruise 1994; Donnelly 2004).

The presence of a pair of vallate pappilae on the caudal dorsal surface of the tonque was described by the Crossley and Miguelez too and is similar to that is found in insectivores and armadillos.

Morphological and in terms of dental formula, the teeth of Rodents and Lagomorphs differ, but, functional similarities are obvious (Stan 2104). The dental anatomy and physiology of the herbivorous rodents has evolved for efficient prehension and chewing of their natural diet. The combination of fibrous structure and its abrasive feature requires prolonged chewing which leads to a permanent wear of the teeth. The ability of the rabbits and chinchillas to adapt to the type of abrasive diet is given by the continuous growth of the teeth, of the so called the elodont dentition (Brewer, 2006; Bohemer and Crosley, 2009). This continuous growth of the teeth is due to the fact that in both species, even if they are part of different orders, the root of the tooth is missing, more specifically, the teeth have an open root where the germinative layer of the tooth is laying (Fischer 2010).

Some authors use the term aradicular hypsodont, thus indicating that the teeth have a long crown, are continually erupting, and have open roots (Wiggs and Lobprice, 1995). However, a differentiation can be made between the Lagomorps and Rodents in that the rabbits have a diphyodont type dentition, characterized by the successive development of the deciduous teeth followed by the permanent ones (Crossley and Miquelez 2001). This feature is contradicted by some authors who claim that the rabbits are monophyodont. Deciduous teeth are lost immediately after birth – the incisors, or a month later – the premolars, a matter that is often underestimated or unobserved. In contrast, chinchillas possess a monophyodont dentition, but other species belonging to the Rodentia order (hamsters, mice, rats) have incisors that are continually erupting – (aradicular elodont hypsodont teeth) and the premolars and molars do not erupt, have short crown and closed anatomical roots (anelodont, brachyodont) (Donnely, 2002). Domestic chinchillas, compared to wild ones, have premolars and longer molars due to less abrasive diets, and therefore a reduced weight, which reduces dental attrition and the appearance of dental diseases.

The terminology of dental characteristics in animals is adapted to the dental nomenclature of humans, and is not always appropriate. We affirm this because the teeth of Rodents are covered with enamel, and the components of the tooth are not well differentiated: the crown, the neck, and the root as in the case of humans. These aspects were also reported by Blood 1999, which used the term anatomical crown for the entire tooth, differentiating the overlying and intragingival components. Thus, he defined the supragingival portion as the exposed crown or "clinical crown", while the intragingival segment was defined as the back-up crown or “clinical root". The terms may be confusing, which is why most authors use human terminology, although anatomically none of the species have a root or apex, the root shape being rather cylindrical than conical as in humans.

The tooth enamel is white in rabbits and guinea pigs (Stan 2014), while in chinchillas, hamster and rats it has yellow enamel, given by the dental enamel transparency of this species and iron-base pigment charge. Although there are authors who claim that the incidence of white incisors at chinchillas is a mineral deficiency, there are studies that demonstrate that this feature is not

pathological, but it is the prerogative of the young age (Wiggs and Lobprice 1995, Crossley and Miguelez 2001; Riggs and Mitchell 2009). The enamel is thicker on the vestibular surface of the teeth, hence the chisel-shape of the teeth in Rodents. The incisors are strongly curved in chinchilla as in the rabbits, hamsters and guinea pigs. The growth rate is high, averaging 2mm per week (Shadle 1936), being directly related to the rate of rash and attrition, hence the need for a fibrous diet is obvious.

Also, notable differences have been noted with regard to the occlusal surfaces of the teeth. The shape of the teeth's occlusal surfaces is preserved by the teeth wear phenomenon due to the diet and the chewing movements that rabbits do in the absence of food. Thus, we can say that the rabbit possesses a typical herbivorous occlusal appearance of the teeth, the premolars and the molars being grouped as a functional unit, with a relatively horizontal surface, and transverse enamel grooves adapted to crushing and grinding the fibrous diet (Stan 2014). The ansognathism of chinchillas with the mandible larger than the maxilla, along with the convergent aspect of the dental arches, provides the explanation of the inclination of the occlusal surface of the teeth.

The oesophagus did not show significant differences between the chinchillas and other Rodents. Placed dorsally to the trachea in the cervical segment, to be positioned to the left as it enters the thoracic inlet, had a relatively equal caliber on the entire tract. This aspect is not present in humans and other mammals such as carnivores, which, in addition have striated muscles in the proximal segment and smooth muscle in the distal one, exhibit narrowing of the oesophagus. However, blockage of foreign bodies in the oesophagus is less reported, but is quite common in postpartum chinchillas, which eat their placenta after parturition. Also, small fibrous or cage pieces can obstruct the oesophagus. Symptoms are very similar to acute respiratory illness (dyspnoea, anorexia), sometimes an oesophagus obstruction can be misinterpreted as pneumonia in the absence of a history of hypersalivation

Conclusions

The morphological particularities of the prediafragmatic digestive tract in chinchilla are of major importance in the clinical expression of various diseases of this segment of digestive tract.

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