UNIVERSITY
OF AGRONOMICAL SCIENCES
AND VETERINARY
MEDICINE - BUCHAREST
FACULTY OF VETERINARY MEDICINE

SCIENTIFIC WORKS

C SERIES

VETERINARY MEDICINE

VOLUME LVI (1)

2010

RECOGNIZED SCIENTIFIC WORKS
by
CNCSIS – Cod 48B+

BUCHAREST

University of Agronomical Medical Sciences and Veterinary Medicine- Bucharest
SCIENTIFIC WORKS C SERIES
Print ISSN 1222-5304
Electronic ISSN 2067 – 3663
www.fmvb.ro/lucraristiintifice/editia-2010
Volume LVI, 2010

Dane Rom Graphics srl

Computerized tehnoredactation: Emilia Ciobotaru, Tudor Nicolae , Furnaris Florin

To be cited: University of Agronomical Medical Sciences and Veterinary Medicine – Bucharest, **SCIENTIFIC WORKS, C SERIES, volume LVI (1)**

Manuscript submission. Published by:
Dane Rom Graphics srl, str. Cornului nr. 33, sector 6, Bucuresti,Romania
Phone 004-021-2209010
e-mail: mircea _ posa @yahoo.com

The volume includes Scientific Works of the SYMPOZION “Contributions of scientific research to the progress of veterinary medicine”. – 18th-19th November 2010, Bucharest, receved until 30 September 2010

Scientific works, C series LVI(1), 2010
ISSN 1222-5304

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

INVESTIGATIONS ON THE IMMUNOLOGICAL PROFILE, BY POTENTATION OF IMMUNE RESPONSE IN RABITS, USING IMMUNOMODULATORS

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Key words: immunological profile, leporidae, immune response.

SUMMARY

Immunological responses of the body was followed ,as a result of potentiation with unspecific immunomodulators, to the immune response induced by vaccination against the rabbit haemorrhagic disease (specific immunomodulation) in rabbits reared in semi-intensive system. There were tested 30 rabbits in the form of three lots from the age of 180 days.
Lot 1 was used only as a witness being subjected to vaccination against rabbit haemorrhagic disease. In group 2, the animals received vitamin E and selenium, Romselevit product using a dose of 0.1 ml / kg. In group 3,animals received vitamin E and selenium using Romselevit product, the double dose. The two experimental groups were subjected to vaccination against the rabbit haemorrhagic disease.
The results obtained confirm the existence of an immunomodulatory action after treatment with vitamin E and selenium in normal doses, with positive influence on the immune status in rabbits. WBC counts presented at the end of the experiment, a striking decrease in statistical terms, in the group that received E and selenium, and the results confirmed the existence of an immunomodulatory action after treatment with vitamin E and selenium in normal doses, with positive influence on immune status in rabbits with high dose, compared with other groups. Immunostimulation by vitamin E and selenium increased the percentage of lymphocytes, in inverse proportion to the percentage of neutrophils, which fell from the same batch, the effect is increasing the percentage of antibodies against the rabbit haemorrhagic disease.

Animal organism can increase resistance by using immunomodulating products (Amici A. et. all, 2000). They may act specifically to produce different effects (destruction of pathogens, or blocking their activities); in this category fit vaccines, immune sera and even antibiotics (Gonzales, S.R.,et. all, 1998; Ijaiga, 2000,). Intensity of immune response may be increased in non-specific way(Rivera J.D., et. Duff G.C, 2003) for a particular type of aggression, using a diverse range of cellular structures, organic or inorganic substances. It was well demonstrated the participation of mineral elements to the proper functioning of the immune system - is standing out above all the observations showing the participation of compounds of selenium, iron, copper and zinc. Bodies deficiencies in these elements have imunodeficite complex at cellular and humoral levels (Uko O.J et. all, 2000). Within the research it has been aimed the immunological

responses of the body, after potentiation with non-specific substances acting on the immune response induced by vaccination against the rabbit haemorrhagic disease (specific immunomodulation), in rabbits reared in semi-intessive system.

1.MATERIALS AND METHOD

There were tested 30 rabbits in the form of three groups (1, 2, 3), from the age of 180 days; testing was performed on animals reared in semi-intensiv system.

Lot 1 was used only as a witness being subjected to vaccination against rabbit haemorrhagic disease. In group 2 ,the animals received vitamin E and selenium using the product Romselevit inoculated sc at a dose as the experimental scheme (table 1). In group 3,the animals received vitamin E and selenium using the product Romselevit inoculated sc in double dose, according to the experimental scheme (Table 1).

Also the two experimental groups were subjected to vaccination against the rabbit haemorrhagic disease.

The experiment was conducted over a period of 45 days, during which three samples were made of blood.

Table 1

Experimental Scheme

Stage	Batch	Day	Vitamin E + Se	Vaccination	Sampling blood
Stage 1	1	Day 1	-	0,5ml sc/anim	*
		Day 3	-		-
	2	Day 1	0,1ml sc/kg	0,5 ml sc/anim	*
		Day 3	0,1ml sc/kg		-
	3	Day 1	0,25 ml sc/kg	0,5 ml sc/anim	*
		Day 3	0,25 ml sc/kg		-
Stage 2	1	Day 15	-	-	*
		Day 17	-		-
	2	Day 15	0,1ml sc/kg	-	*
		Day 17	0,1ml sc/kg		-
	3	Day 15	0,25 ml sc/kg	-	*
		Day 17	0,25 ml sc/kg		-

Stage 3	**1**	**Day 45**	**-**	**-**	*****
	2	**Day 45**	**-**	**-**	*****
	3	**Day 45**	**-**	**-**	*****

*** = Group who carried out blood sampling.**

Quantified parameters
1. The total number of leukocytes;
2. Percentage of - Lymphocytes;
- Neutrophyls;
- Eosinophyls;

Blood examinations were conducted by electronic means in an analyzer Coulter - Counter CBC - 5. Data were statistically processed.

2. RESULTS AND DISCUSSIONS

After statistical processing of the leukocyte count there was a significant decrease in statistical terms, the third blood collection , in group 3, receiving the double dose E and selenium ($p < 0.05$), their number being of 7.77 ± 2.77 thousands/mm3, compared to first blood collection where it was obtained a total of 8.90 ± 3.93 thousands/mm3 leukocytes (Table 2, Chart1). For this constant no other changes were found statistically significant.

Table 2

- **WBC count – absolute values**

Batch	Stages		
	I	II	III
1	8,53±3,94	7,99±3,66	8,10±4,94
2	9,03±5,09	8,03±2,84	8,16±4,38
3	8,90±3,93	8,11±4,12	7,77±2,77*

* - (Mean + standard deviation)
* = Significant difference;

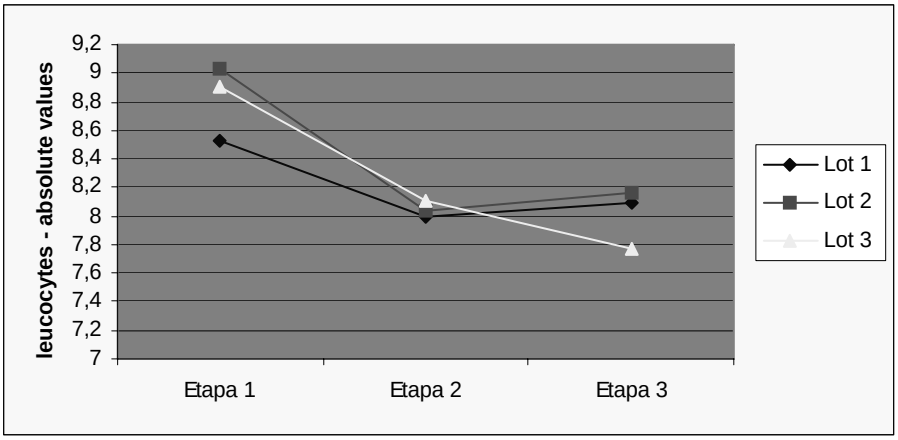

Chart 1 - Graphical representation of leucocytes - absolute values

Table 3

*** Percentage of lymphocytes**

Batch	***Stages***		
	I	II	III
1	55,61±0,81	54,14±1,89	52,27±2,26
2	53,33±0,7	62,11±0,81***	59,13±1,14**
3	55,37±1,06	60,2±0,61**	56,54±0,32

* (Mean + standard deviation)

** = Significant difference separately, *** = highly significant difference.

In Table 3 and Chart 2 is shown the percentage of lymphocytes, which occurred statistical changes only in group 2, which increased significantly distinct ($p < 0.01$) after blood collection II (62.11 ± 0. 81%), compared to blood collection I (53.33 ± 0.7%).

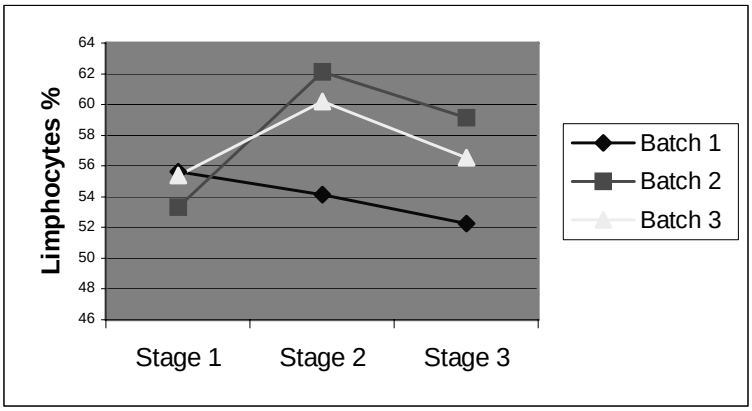

Chart 2 - Graphical representation of percentage of lymphocytes

In contrast to the percentage of lymphocytes, the percentage of neutrophils (table 4, graph 3) changed in group 2 and 3, decreasing significantly distinct ($p < 0.01$) at the second blood collection compared to first blood collection.

Table 4

The percentage of neutrophyls *

Batch	Stages		
	I	II	III
1	40,83±2,28	44,27±5,74	43,05±6,58
2	42,85±2,04	34,17±2,69**	37,43±3,26
3	44,07±2,83	35,17±2,46**	35,73±12,09

* (Mean + standard deviation)
* = Significant difference, ** = significant distinct difference;

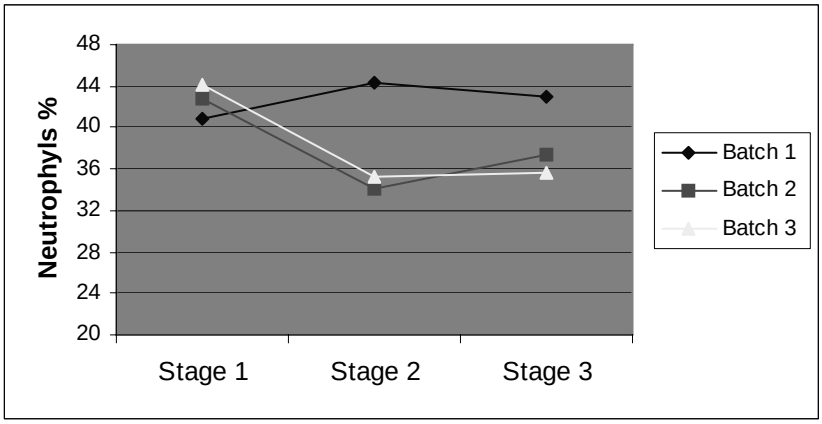

Chart 3 - Graphical representation of the percentage of neutrophyls

3. CONCLUSIONS

3.1. Testing was performed in a semi-intensive growth system, animals were subjected to normal feeding conditions and microclimate throughout the experiment.

3.2. WBC counts (absolute values) presented at the end of the experiment, a striking decrease in statistical terms,in the group who received high-dose E and selenium compared with other groups.

3.3. Immunostimulation by vitamin E and selenium increased the percentage of lymphocytes, in inverse proportion to the percentage of neutrophils, which fell in the same group, the effect being the increasing percentage of antibodies against the rabbit haemorrhagic disease.

3.4. The results obtained confirm the existence of an immunomodulatory action after treatment with vitamin E and selenium in normal doses, with positive influence on immune status in rabbits.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

EPISOD OF PRRS - EPIDEMIOLOGICAL AND CLINICO-LESIONAL ASPECTS

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Keywords: porcine reproductive and respiratory syndrom, epidemiology, morpho-clinical aspects

Summary

Pig farmers have still serious problems with respiratory conditions contained in the so-called "porcine respiratory disease complex (PRDC), which is an interaction between viruses, bacteria, suppressed immune system and stress. PRDC has multiple etiology, which may include bacteria (Mycoplasma hyopneumoniae, Pasteurella multocida, Actinobacillus pleuropneumoniae) and viruses (Aujeszky virus, virus, porcine reproductive and respiratory syndrome (SRRP), influenza virus, transmissible gastroenteritis virus and respiratory coronavirus). The paper deals with epidemiological and clinical aspects lesion in an episode of porcine reproductive and respiratory syndrome (SRRP) wich has recently evolved in pigs exploited intensively.

Porcine reproductive and respiratory syndrome of pigs (SRRP) is still in actuality over 20 years of its beginning and still produces great economic losses in the large growth of pigs, both in the European continent and in the world. The economic consequences of infection are different from country to country. In Europe, losses ranging on average between half and two piglet per sow per year.

The paper deals with epidemiological features, clinical and lesional aspects in an episode of porcine reproductive and respiratory syndrome (SRRP) wich has recently evolved in pigs exploited intensively in south-eastern teritory of Romania, near the Danube river.

1. MATERIAL AND METHODS

Epidemiological observations were made in two units of pig husbandry industry in the period 1 January 2009 to 31 December 2009. The company is divided into two separate farms situated at a distance of about 30 km apart. The first farm, called "The Farm", operating on a complex infrastructure of 35,000 pigs. The second farm, called "Farm B" operates on a complex infrastructure with a capacity of 150,000 pigs. Biological material is purchased from a company in Romania, providing the basis for obtaining genetic hybrid PIC. In the " Farm A" to get parents and the "Farm B" to obtain commercial animals.

Both farms use artificial insemination, having own centers for artificial insemination.

Each farm is held on closed circuit principle farms with a breeding area (pregnancy), the maternity sector, youth sector and farm sector for pig fat. In the gestation sector of "Farm B" gilts comes from "Farm A" and those of "Farm A" from the company PIC.

Prophylactic quarantine is assured in "Farm B" by a separate shelter for animals coming from "Farm A" and in "Farm A", by one shelter removed from technological circuit with direct access from outside .

Immunoprophylaxis actions applies from prophylactic quarantine period, where, in addition to collection of blood samples, animals are vaccinated with Farrow Sure (rujet, Parvo-virus, leptospirosis), Litterguard (E. coli , anaerobic), Begonia Aujeski (Aujeski disease) and disinfestation with Ivercen (Ivomec). Since october 2009, in "Farm B" applies to vaccination against PRRS (PRRS Porcilis - lyophilised live vaccine), with booster every four months.

This paper aimed to conduct an epidemiological study in a SRRP outbreak occurred in 2009, bringing together data from herd movement (introduction of new animals, births, birth rate, prolificacy, morbidity and mortality) with the clinical course of the outbreak of PRRS in flocks under study and analyze the results of specific actions undertaken during the period analyzed.

2. RESULTS AND DISCUSSIONS

In the “Farm A”, the animals were entries in January 2009, 11 boars and ends March 3 boars and 165 gilts (Table nr.1). Here, onset was observed in sows and gilts that showed apathy, loss of appetite, fever and weakness. The disease had a high diffusivity and had included all categories of pigs on the farm in a relatively short time (less than one month). One of the possible sources of infection could be the group of 11 boars introduced into the unit in January.

In the "Farm B", following the pace and number of animals entering the farm, we found that in March and in June there were two entries of boars outside the company, which went through the "Farm A". In August, was the first entry of gilts held by the "Farm A" (606 head), and in September, had came 236 gilts from the same source, in October 96, and finally, 342 heads in November. (Table nr.2). In "Farm B", the episode of PRRS was manifested only by excessive growth of abortions at sows and gilts. During the period in which there were large numbers of abortions in the pregnancy ,by clinical examination was observed

daily alteration of appetite and temperature. During development of these abortions was not clear deterioration of health status at category of boars, piglets and youth.

By serological tests conducted in this period was excluded from the development of diseases such as bacterial or fungal / toxic, the only pathogen isolated was PRRS virus.

Analyzing data recorded, abortions reveals a large increase in September at sows (2.14% more than 0.11% in December) and increased less significantly at gilts (0,91% in June more than 0% lower value, in December). (Graph nr.1 and Fig, nr.1).

Clinical signs in piglets aged 5-14 days after calving, were expressed by the general status changes, apathy, low viability, pale skin and mucous membranes, eyelid edema, dyspnea, unproductive cough, and occasionally, diarrhea.

Piglets mortality curve (Graph nr.2) registered a sharp decline from May, starting from 55.1% in April, to 20.3% in May, for June to be close to the parameters technology.

Piglets corpses had lung lesions such as pulmonary congestion, edema and atelectasis and, sometimes, catarrhal enteritis and gastric dilatation.

At youth category, decreasing the losses by death, from one month to another, is much slower because the piglets arrived in this category have been tared from birth by bacterial infections (Salmonella choleraesuis, Pasteurella multocida, Streptococcus suis) amid transient immunosuppression caused by PRRS virus. The mortality increased from 3.63% to 13.66% (Graph nr.3).

The corpses from this category revealed skin redness, cyanosis ears (Fig. nr.2), and hirsutism /hipotrepsie, presence of lesions in various stages of bronchopneumonia (Fig. nr.3), lymph nodes enlarged and haemorrhagic.

Fat pigs were infected, it seems, by the respiratory route (following entries recorded in March, 165 gilts and three boars), because the preventive quarantine hall is located near the halls of fat pigs.

This category has shown loss of appetite, general condition changed, ear cyanosis, respiratory disorders (Fig. nr.4) and markedly increased mortality, 8.38% in April from the previous month when mortality was only 2.19 % (Chart nr.4).

In "Farm B" during the outbreak of SRRP losses were not significant for any of the categories of pigs.

3. CONCLUSIONS

3.1. PRRS virus infection in pigs analyzed was made possible by the introduction of animals from outside unit

3.2. location of preventive quarantine area does not eliminate the possibility of transmitting infection by the respiratory route

3.3. PRRS virus dissemination in pigs from "The Farm" was favored by the state of confusion created by the initial suspicion of an onset of transmissible gastroenteritis (TGE) with controled infection applicated directed to pregnant sows;

3.4. Mortality peaked in April and May, higher values (at least double) in "The Farm" than "Farm B" in all categories (infant, youth and fat pigs)

3.5. Greater losses recorded in "The Farm" were justified by the actual status as free by PRRS infection when was entering the herd;

3.6. Although implemented later, immunoprophylaxis program for PRRS significantly decreased the number of abortions in "Farm B";

3.7. Bacteriological examinations conducted during the development of PRRS episode,had showed increased incidence of bacterial infections associated (secondary) with Salmonella choleraesuis, Pasteurella multocida, Streptococcus suis.

3.8. an reorganization of circuits between the two farms, with strict biosecurity rules, will must take under control a epidemiological situation in the farms, including the occurrence of PRRS virus in one of the farms.

Table nr.1

Entries by purchasing in the „Farm A”

month	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Gilts	0	0	165	0	0	0	0	0	0	0	0	165
Boars	11	0	3	0	0	5	0	0	0	0	0	0

Table nr. 2

Entries by purchasing in the „Farm B”

month	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Gilts		0	0	0	0	0	0	606	236	96	342	0
Boars		0	11	0	0	10	0	0	0	0	0	0

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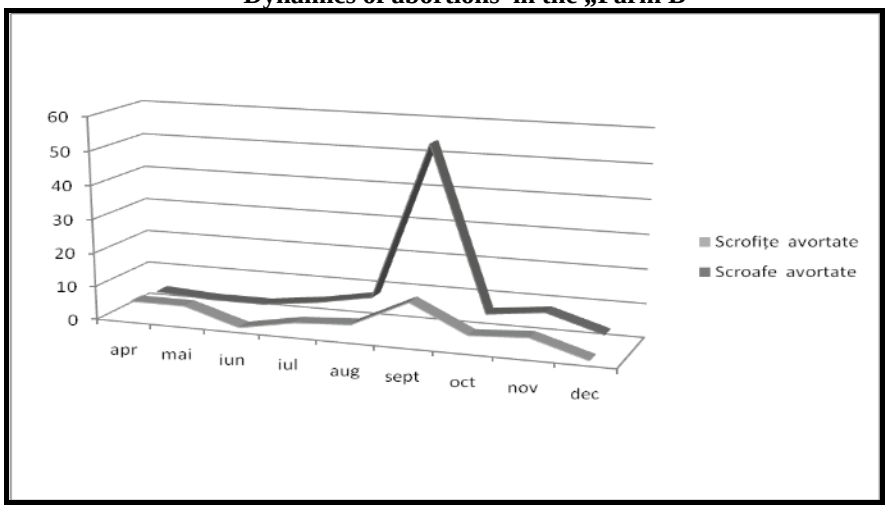
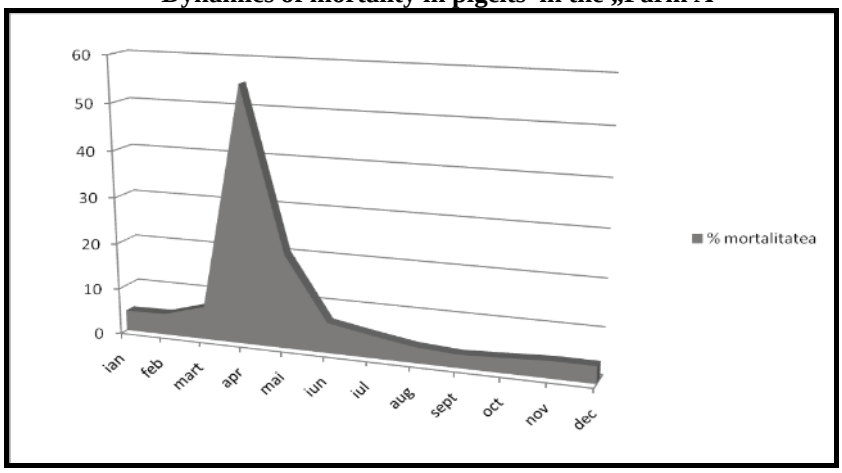
Graph nr.1

Dynamics of abortions in the „Farm B”



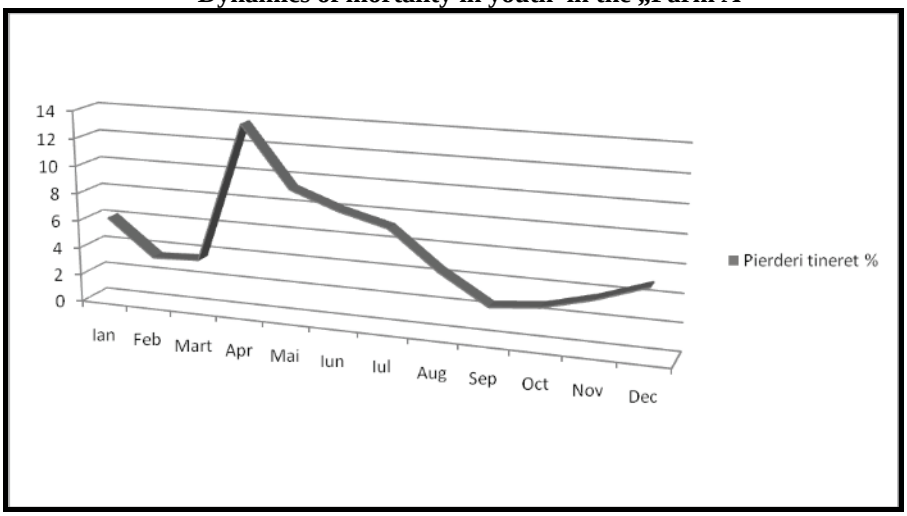
Graph nr. 2

Dynamics of mortality in pigelts in the „Farm A”



Graph nr.3

Dynamics of mortality in youth in the „Farm A”



Graph nr.4

Dynamics of mortality in fat pigs in the „Farm A”

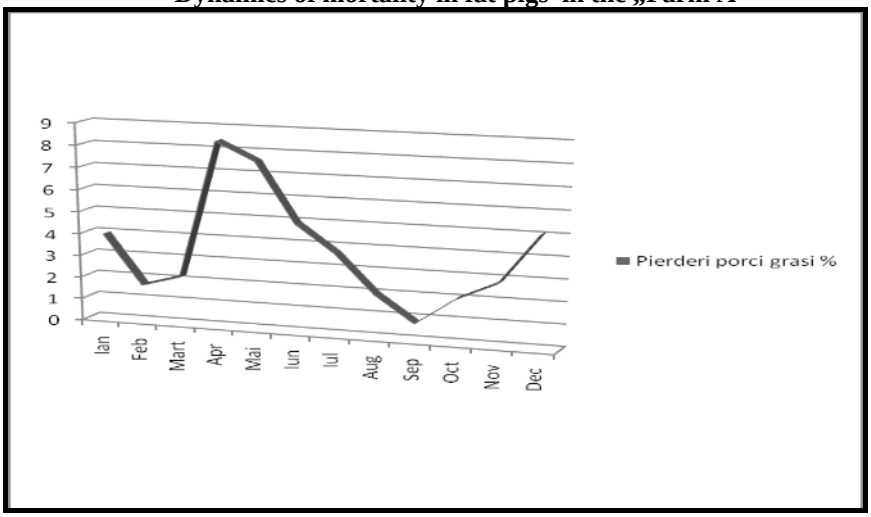


Fig. nr.1

Abortion at 102 days of gestation

Fig. nr.2

Skin redness and ears cyanosis

Fig. nr.3

Lesions in various stages of bronchopneumonia

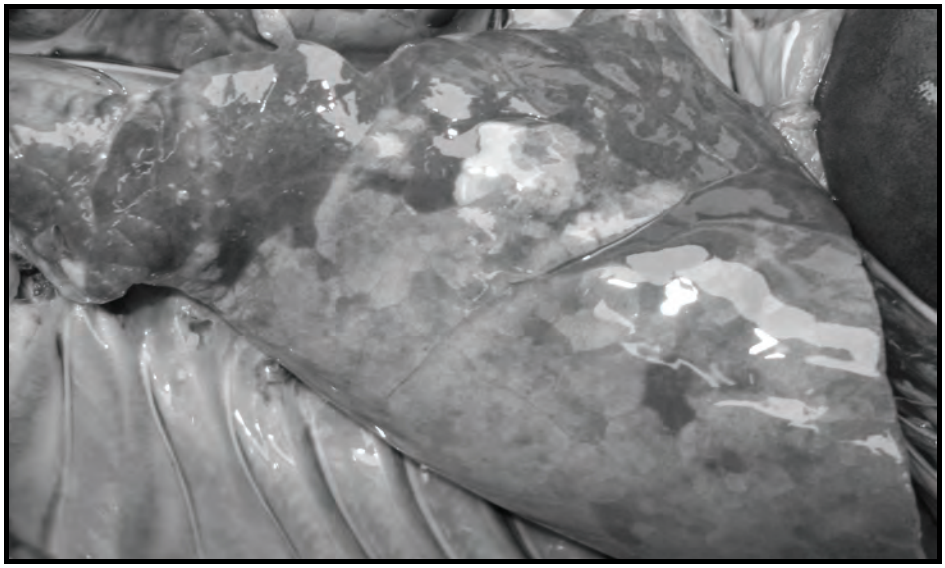


Fig. nr.4

Ear cyanosis and respiratory disorders in fat pigs

Scientific works, C series LVI(1), 2010
ISSN 1222-5304

A COMPARISON BETWEEN THE TOTAL GERMS NUMBER (TGN) ISOLATED FROM THE MEAT OF THE FOOD SNAILS COLLECTED FROM THREE DIFFERENT AREAS

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Keywords: total germs number, food snail, *Helix pomatia*

SUMMARY

Lately, in our country, the food snail Helix pomatia is much appreciated. Many people began to raise this specie in organized farms. The snails are processed or exported. Helix pomatia as a food snail can be collected from different areas: gardens, forests and farms, so our purpose was to see if there is a difference between the total germs number isolated from their meet, according to their growing area. The methods we used are: the serial decimal dilutions method and the inoculation on several media. High values of CFU/g were found in the meat of the snails collected from all the areas, due to their ecology. The values of the CFU/g isolated from the meat of the farm snails were lower than the values of the CFU/g isolated from the garden and the forest snails. This fact may be related to the fact that in farms, the snails are raised in a controlled environment, with more hygienic conditions.

Food snails *H. pomatia* meat has always been highly valued for its dietetic and nutritive properties. Lately, in our country, many people began to organize farms in which they raise this specie. The snails' meat is usually exported. Snails as food can also be collected from gardens and forests. Still, in our country, the microbiology of the food snails has not been properly studied. Consequently, the aims of the present paper are: to identify the TGN from the meat of the snails collected from farms; to identify the TGN from the meat of the snails collected from their natural environment: gardens and forests; to see if there's a difference between the values of the TGN from the meat of the snails collected from the three different areas.

1. MATERIAL AND METHODS

The research took place in the following steps:

Step 1 – Thirty adult snails from the specie *Helix pomatia* were collected from three different areas: ten from a garden, ten from a forest and ten from a farm.

Step 2 – After removing the shell, the snail foot was decontaminated in alcohol for one minute and then serial decimal dilutions with Brain Heart Infusion broth were prepared, according to the method mentioned by Bondoc I. and Sindilar E.V. (2002). The dilutions were incubated at 37°C, for 24 hours.

Step 3 – Plates with Agar with sheep bood were then inoculated with 10 µl of the 10^6 dilution. The media were incubated at 37°C for 24 hours.

Step 4 – The values were expressed in log CFU/g, using the classical formula.

2. RESULTS AND DISCUSSIONS

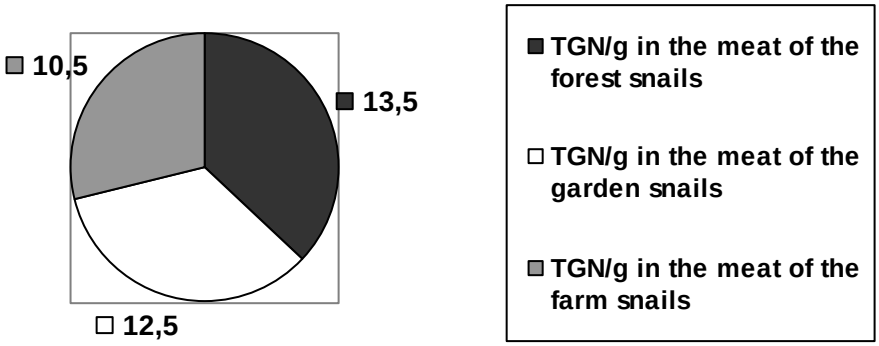
The values obtained from the meat of the snails collected from the three different areas are in table 1.

Table 1.

The values of CFU/g from the meat of the snails collected from three different areas

The snail's number	Areas		
	The CFU/g from the meat of the forest snails ($x10^6$)	**The CFU/g from the meat of the garden snails ($x10^6$)**	**The CFU/g from the meat of the farm snails ($x10^6$)**
1	12	18	7
2	18	20	11
3	9	19	10
4	11	7	12
5	10	11	11
6	8	6	3
7	15	18	9
8	20	10	16
9	20	9	7
10	12	7	19

The arithmetical means were the followings: 13,5 $x10^6$ CFU/g in the meat of the forest snails, 12,5 $x10^6$ CFU/g in the meat of the garden snails and 10,5 $x10^6$ CFU/g in the meat of the farm snails.



Graphic 1.The values of CFU/g from the meat of the snails collected from three different areas

A comparison between the arithmetical means of the CFU/g can be seen in graphic 1.

The results are similar to those mentioned by other authors (Temelli S. et al, 2006).

The relatively high number of germs found in the meat of the snails is due to this specie's ecology. The snails are almost permanently in contact with the soil, from where they get several microorganisms. Other important sources of microorganisms are the plants on which they climb, using them as food, but also as a shelter from the sun.

Another explanation for the high number of CFU/g could be the fact that the snail's foot, the main part used for consumption, is composed not only of muscles, but also of important parts of the digestive and of the genital systems.

The fact that the smallest average value of the CFU/g was found in the meat of the farm snails is due to the fact that they are raised in controlled, hygienic conditions. Analysis of the soil is made regularly, the farm is well railed and the snails are in no contact with any other animals, which could be sources of contamination with microorganisms.

The highest value of the CFU/g found in the meat of the forest snails could be due to the fact that they live in a completely uncontrolled environment and they are permanently in contact with various species of plants and animals and their excrements, a very important source of microorganisms.

The category of snails with the medium average of CFU/g was collected from a garden in the city. Their environment is also a controlled one, regularly cleaned and with no other animals around.

Further investigations are made, in order to identify the bacterial species in the food snail's meat.

3. CONCLUSIONS

3.1. A high number of germs (> 10^6) were found in the meat of the food snails collected from three different areas.

3.2. The number of CFU/g is smaller in the meat of the farm snails, compared to the numbers of CFU/g found in the meat of the snails collected from their natural environment.

3.3. The highest value of CFU/g found in the meat of the forest snails is due to the fact that they live in a completely uncontrolled environment, where they are in permanent contact with various species of plants and animals and their excrements.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

IMPLEMENTATION OF A MASTITIS SURVEILLANCE AND CONTROL SYSTEM IN A DAIRY FARM

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Key words: mastitis, score, keratinisation

SUMMARY

Mastitis continues to be a major cause of economic loss to the national dairy herd and combined with teat injuries it is one of the greatest aggravations to the herdsman management. Mastitis affects the farmer economically in two ways: through direct costs (discarded milk, drugs and veterinary costs) and indirect costs (decreased milk yield during remainder of lactation due to udder damage and subclinical infection, penalties because of icreased cell count, extra labour requirements for treating and nursing, higher culling and replacement rates leading to loss of genetic potential, deaths. The porpose of this study is to evaluate the implementation of a mastitis surveillance and control system in a dairy farm. The infectious pressure was higher in groups 4 and 8, consequences of poor foots (60 and 55% notes of 3+4) and udders hygiene (65 and 25%). The resistance to infectious pressure is variable depending on body condition score and environmental condition; the holstein and red holstein cows (groups 2,3 and 8) had 15, 21 and 15% BCS values below 2,75 wich shows low resistance to the action of infectious agents. Was found failure in milking hygiene and average time for preparing and attaching the last cluster was 50 seconds (higher that limit of 30 seconds). Incidence of subclinical mastitis was higher in groups 2 and 6 (30 and 65%); clinical incidence has cross the limit (3%) in groups 2,3,5,6. This mastitis surveillance and control system can be used successfully in a small or large dairy farm.

Mastitis continues to be a major cause of economic loss to the national dairy herd and combined with teat injuries it is one of the greatest aggravations to the herdsman. Although the incidence of infections caused by *Staphylococus aureus*, *Streptococus agalactiae* and *dysgalactiae* has decreased and the national mastitis cell count has fallen, this has been matched by a rise in the number of cases caused by *Escherichia coli* and *Streptococus uberis*, known as environmental mastitis (Blowey et al, 2000). Mastitis can never be eradicated because environmental infections will always be present and it is also highly unlikely that a single all embracing vaccine will ever be found to supress the multiplicity of types of infection involved (Blowey et al, 2010)

Much of the information needed to reduce the incidence of mastitis has been available for the last 30 years. Reaserch work during the Mastitis Field Experiment at the NIRD in the 1960 formed the basis of the important mastitis control measures used today including next five

point: treating and recording all clinical cases, dipping teats in desinfectant after every milking, dry cow therapy at the end of lactation, culling chronic mastitis cases, regular milking machine maintenance

Mastitis affects the farmer economically in two ways: through direct costs (discarded milk, drugs and veterinary costs) and indirect costs (decreased milk yield during remainder of lactation due to udder damage and subclinical infection, penalties because of icreased cell count, extra labour requirements for treating and nursing, higher culling and replacement rates leading to loss of genetic potential, deaths (Crocker, 2009).

The costs of a clinical case of mastitis have been quantified: it was estimated that the average cost of one case of mastitis was between £60 and an average of £80 is a well-accepted figure for 2000. This work assumed that there were three categories of mastitis: mild, severe and fatal, with an incidence of 70%, 29% and 1% respectively (Hogeveen, 2005).

A Mastitis Surveillance Scheme carried out on 144 herds in England from 1994 to 1996 showed that an average herd of 100 cows would have 43 cases of mastitis each year, defining *a case* of mastitis as one quarter affected on one ocasion.

1.MATERIAL AND METHOD

The study was conducted from June 1, 2010 to June 31, 2010 on a total of 410 cows (six milking groups), breed Holstein Friesian, Red Holstein, Bălțată românească, maintained in loose housing and monitored through a computerized management system of the AfiFarm type.

The research including visits to dairy farm, flow supervision of the milking parlor, animal welfare, environmental conditions, milking machine quality, reports of mastitis incidence, teats lesion, measurement of somatic cell counts (SCC) and total bacterial count (TBC) from bulk tank, etc.

The program included five fields of attention that cause udder health in a dairy farm. For each of them was aimed to achiewe success factors.

1. Infectious pressure-ensuring a hygienic environment, avoid sources of infection.
2. Resistance-adequate food and water, body condition scoring, temperature, ventilation and shelter.

3. Milking-correct procedures, operation and maintenance of milking machine
4. Treatment-detection of infected animals, adapted treatment, dry cows correct procedures, healing or reform cows with problems
5. Evaluation-determining udder health indicators at the farm, SCC, TBC, data recording, monthly and annual reports, reassessment of strategies and protocols for udder health.

For each field of attention we have made observations, reports, records, analyzes. Recording and data analysis was done and by the computerized system AfiMilk (determination of daily average yield, milk conductivity, milking time, milking flow rate)

We took samples and bacteriological tests were performed (total bacterial count, somatic cell count, antibiogrammes) at the specialized laboratory.

2. RESULTS AND DISCUSSION

The research conducted on six groups of milking cows (table 1), from different races, lactation numbers, days in milk, followed five fields of attention.

Table 1

EXPERIMENTAL GROUPS

	2	3	4	5	6	8
Breed	Hf	Hf	baltata	baltata	baltata	Red holstein
N	60	60	76	65	75	67
Lact no	1	2+	3+	3+	3+	1
DIM	150	221	93	168	174	114

Evaluation of infectious pressure-consisted in shelters visits, and had these results: there were complied cows separation (the cows with mastitis were milked at the end of the sesion), shelters daily hygiene, beds desinfection (1/48 hours), clean watered. We found a overcrowding of cows in groups 4 and 8 and inadequate bedding. Was examined foot and udders hygiene in the milking parlor and we note with 1 to 4 score.

Tabel 2

FOOTS HYGIENE EVALUATION

Foot score %	Group						limit
	2	3	4	5	6	8	
1	15	10	10	5	15	10	
2	70	70	30	75	70	35	
3+4	15	20	60	20	15	55	20%

Was exceeded the limit of 20% score 3 and 4 in groups 4 and 8 (60 and 55%), and 20% in groups 3 and 5 (table 2,3). That showed a poor hygiene and a source of infection for the udder with bacterial agents from the environment (*Escherichia coli* and *Streptococus uberis*). The udder hygiene was poor in the sames groups 4 and 8 (65 and 25% scores of 3 and 4; the limit was 10%).

Table 3

UDDER HYGIENE EVALUATION

Udder score %	Group						limit
	2	3	4	5	6	8	
1	45	50	10	5	35	25	
2	45	40	25	75	60	50	
3+4	10	10	65	20	5	25	10%

Evaluation of resistance to infectious pressure found water and food according to nutritional requirements, optimal temperature and ventilation. We apreciated body condition score four each cow (normal limits 2,75-3,25), we found values below 2,75 in groups of Holstein and Red Holstein (15, 21% in group 2 and 3, 15% in group 8) wich shows low resistance to the action of infectious agents and environmental conditions (table 4).

Table 4

BCS EVALUATION

BCS %	Group					
	2	3	4	5	6	8
2,75	15	21	7	3	2	15
2,75-3,25	79	72	73	82	82	78
3,25	6	7	20	15	16	7

Values over 3,25 was found mainly in groups of Baltata romaneasca, with a lower milk production (20% of cows in group 4, 15% in group 5 and 16% in group 6).

Milking procese and miking machine evaluation-was supervised milking procedure, steps and execution times, checking the correct functioning of milking machine. Failure was found in milking hygiene (drying teats with a single service paper), average time for preparing teats and attaching the last cluster was higher:50 seconds towards the limit of 30 seconds (table 5). Milking machine evaluation (2x20, tree type, with alternative pulsation) was to check the integrity of clusters, pulsators, vacuum pump, milk transfer line, washing plant. A dynamic test of vacuum fluctuation in the plant was performed to find irregular fluctuation such as liner slip, low level of vacuum (mastitis risk), high level of vacuum (hiperkeratosis). One single brocken pulsator has been found and was changed.

Table 5

AVERAGE TIME FOR MILKING PROCEDURES

Steps	Start preparing	Final preparing		First cluster atach	Last cluster atach	limit
Average time	0 s	41 s	4 s	45 s	50 s	
Total time	41 s			5 s		
	50 s					30s

The evaluation of cows udder from milking group (table 6); the results was a high percentage of asymmetrical udders in local breed groups 4,5,6 (38,37 and 53%), udders under shank (52, 54 and 53%), high level of compromised quarters in groups 5 and 6 (8 and 15%) wich are difficult to the machine milking

Table 6

Udders evaluation

%	Group					
	2	3	4	5	6	8
Asymmetrical udders	13	26	38	37	53	10
Compromised quarters	5	4	5	8	15	4
Under shank	5	8	52	54	53	8

Proper functioning of milking machine was controlled by checking the level of teats and galactofor channel opening keratinisation. The results showed a medium degree keratinisation (score 2 and 3) with overcome the limits of 10 and 25% in all groups; maximum levels was in groups 5 an 6 (50 and 45%). Keratinisation was consequences of high level of vacuum and milking time, large number of days in milk.

Table 7

TEATS KERATINISATION DEGREE

Teats score %	Group						limit
	2	3	4	5	6	8	
1	49	53	30	34	28	63	
2	33	32	52	50	40	31	25%
3	12	11	15	14	28	5	10%
4	6	4	3	2	4	1	6%

Treatment–the subclinical mastitis incidence was higher (65%-grp 6, 30%-grp2: SCC 400000) using R-mastitest, and clinical incidence (8%-grp6, 5%-grp 2,3). Evolution of SCC and TBC from bulk tank

Table 8

MASTITIS INCIDENCE

R-mastitest	Group						limit
	2	3	4	5	6	8	
Subclinical mastitis %	30	25	23	22	65	23	10%
Clinical mastitis %	5	5	3	4	8	2	3%

Table 9

SCC AND TBC EVOLUTION IN BULK TANK

	SCC x1000	TBC x1000
IANUARIE	429	244
FEBRUARIE	378	112
MARTIE	470	231
APRILIE	373	292
MAI	289	95
IUNIE	313	131

showed improvement of these parameters in the last months. Treatment of cows and drying was done correctly.

The evaluation-we fixed the farm health indicators: SCC 250000, TBC 100000, clinical mastitis 50 cases/100 cows/year, subclinical

mastitis 10% cases/year (these indicators will be checked monthly), correction of errors in the milking parlor, improved the shelters hygiene, setting a new treatment protocol, reforming the animals with high SCC and recurrent mastitis.

3. CONCLUSIONS

3.1 The infectious pressure was higher in groups 4 and 8, consequences of poor foots (60 and 55% notes of 3+4) and udders hygiene (65 and 25%);

3.2 The resistance to infectious pressure is variable depending on body condition score and environmental condition; the holstein and red holstein cows (groups 2,3 and 8) had 15, 21 and 15% BCS values below 2,75 wich shows low resistance to the action of infectious agents;

3.3 Was found failure in milking hygiene and average time for preparing and attaching the last cluster was 50 seconds (higher that limit of 30 seconds);

3.4 Incidence of subclinical mastitis was higher in groups 2 and 6 (30 and 65%); clinical incidence has cross the limit (3%) in groups 2,3,5,6;

3.5 This mastitis surveillance and control system can be used successfully in a small or large dairy farm.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

CONTRIBUTION IN *PASTEURELLA SPP.* DIAGNOSTIC AND TREATMENT ON RABBIT

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Key words: rabbit, *Pasteurella sp*., diagnostic, acute respiratory syndrome, treatment.

SUMMARY

The aim of this study was the isolation and the identification of the microbial agents involved in a rabbit's respiratory acute syndrome in an animal facility from an institute that uses rabbits for diagnostic serum product, for the control of biomedical products and for research. The diseases were started in a conventional area, at the beginning of the cold season and in 2-3 days 60% of the animals were involved. The most important clinical signs were the respiratory disturbing and the multiple edemas of the ears. From dying rabbits were taken bacteriological exam samples from nasal mucous, trachea, lung, heart, liver, bone and blood and for histopathological exam samples were taken from nasal mucous, trachea, lung, heart and liver. At bacterioscopic exam was put in evidence Gram positive cocci and Gram negative cocobacilli. For the isolation and identification of the microorganisms we are use, the cultivation on usual and special culture for *pasteurella, staphylococcus* and *streptococcus*. It was isolated in pure culture *Pasteurella multocida* from bones and *Staphylococcus aureus* from nasal mucous of rabbits and both bacteria from 3. The pathogenity was tested on NMRI mice by intraperitoneal inoculation of 0,5 ml suspension per mouse. The individual treatment was done with injection enrofloxacin and the treatment of all effective was done with amoxicillin in water. It was also made a bivalent auto-vaccin with the two microbiane strains isolated.

The acute respiratory syndrome and the enteritis represent in Romania an important cause from rabbit's morbidity and mortality in farm and also in individual breeding, an especially in spring and autumn (Rebreanu, 1983).

In rabbits breeding appear frequently respiratory disturbance caused by Gram negative bacteria from *Pasteurellaceae* family, alone or/and in association with *staphylococcus* or with *streptococcus* (Di-Giacomo et al., 1983; Potecea, 2002). Also in same time with the respiratory disturbance could evaluated and digestive disturbance.

The isolations and the identification of these bacteria don't put special problems for a laboratory with a medium endowment and by the effectuation of the antibiogram on selected the suitable substances for the treatment (Popa, 2004; Holt *et al*., 1994; Turcu, 2007).

Was made research for establish the diagnostic and for the application the efficient measure in treatement.

1. MATERIALS AND METHODS

In an animal facility with a 230 New Zeeland rabbits (60 adults and 170 young) sudden were start a disease.

The clinical signs were the respiratory disturbing, the multiple edemas of ears, eyelids, and lips, necrosis of ear extremities and purulent blepharitis. Some of rabbits had purulent abscesses perianal and interdigital. In two days the disease affected 60% from animals, and the mortality was over 15% from number of all rabbits.

From 15 rabbits was taken nasal tampon, at 25 bodies was made necropsy examination in laboratory and from 22 bodies were effectuated microbiological tests from trachea, lung, liver and spinal marrow on usual and special culture for *pasteurella, micoplasma*, *streptococcus* and s*taphylococcus*.

Also was taken samples for histopathological exams from lung, heart, liver and spleen.

For antibiogram we used 24 hour microbial culture, nutritive agar, and nutritive agar with serum and bio-disc with penicillin, amoxicillin, gentamicin, spectomicin, oxitetraciclin, erythromycin, enrofloxacin and cefaclor.

The pathogenity was tested on NMRI mice by intraperitoneal inoculation of 0,5 ml suspension per mouse.

2. RESULTS AND DISCUSSION

The sickest rabbits were presents severe respiratory signs like rhinitis, sinusitis, mucopurulent nasal exudates and dyspnea (figure 1); 75% of rabbits were observed with multiple edemas of ears, eyes, nose, and necrosis of ear extremities (figure 2,3,4,5), otitis media and subcutaneous abscesses.

Fig.1 – Rabbit with rhinitis

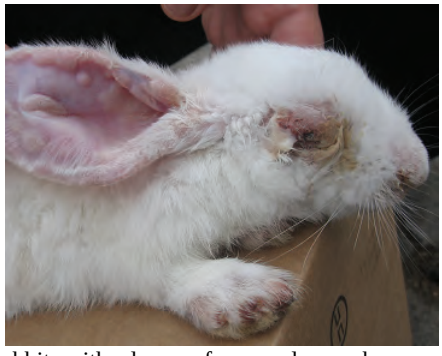

Fig.2–Rabbits with edemas of ears and eyes abcesses

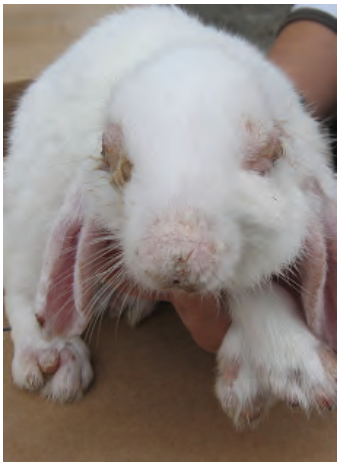

Fig. 3 – Rabbit with edemas of nose and subcutaneous abcesses

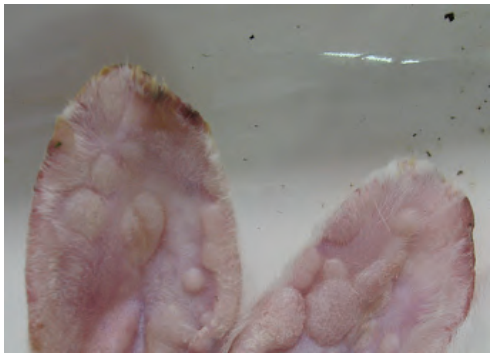

Fig. 4 – Multiple edemas of ears

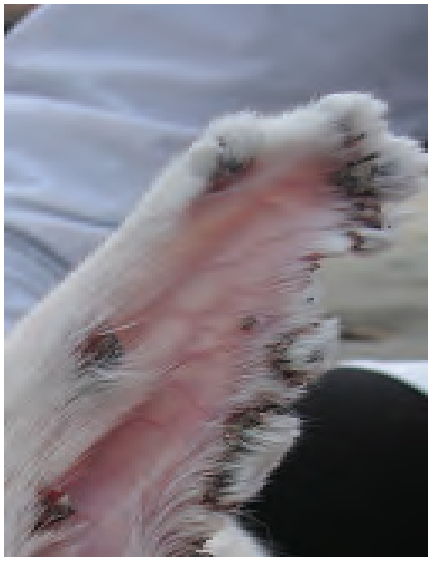

Fig. 5 – Multiple necroses of ears

After 5 days of evolution the mortality was increased at 30 % from all rabbits.

Necropsy examination was performed on 25 dead rabbits. At necropsy was observed the congestion and hemorrhage in the anterior respiratory way and seroasa pericarditis.

In the lung, consolidation, atelectasia and abscesses were observed. In some rabbits the predominant features was fibrinopurulent pleuritis

and pericarditis. Acute hepatic necrosis and splenomegalia was seen in association with pneumonia.

Histopathological exam samples were taken from nasal mucous, trachea, lung, heart and liver. At histopathological exams from lung was observed catarrhal – exudative alveolites with wall sclerosis (fig.6), pneumocites degeneration, catarrhal bronchiolitis with hyperplasia (fig. 7) and metaplasia of bronchioles cells.

Severe granular dystrophies on big area in the liver and myocarditis were observed.

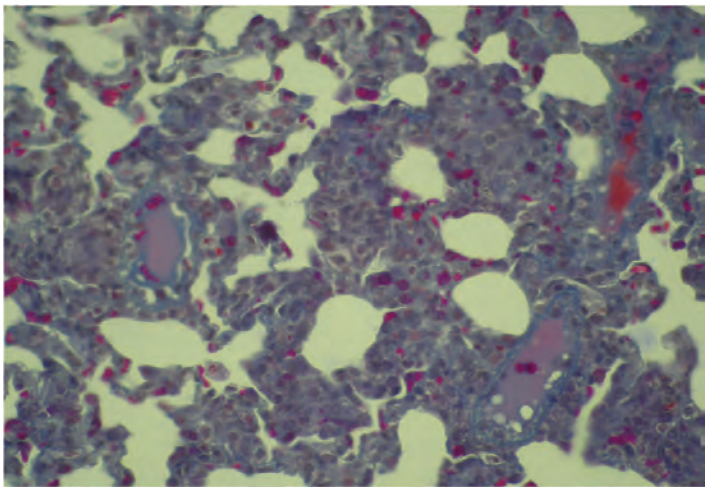

Fig. 6 - Rabbits lung: Catarrhal – exudative alveolites with wall sclerosis and her degeneration; Mallory tricromic staining; 40x.

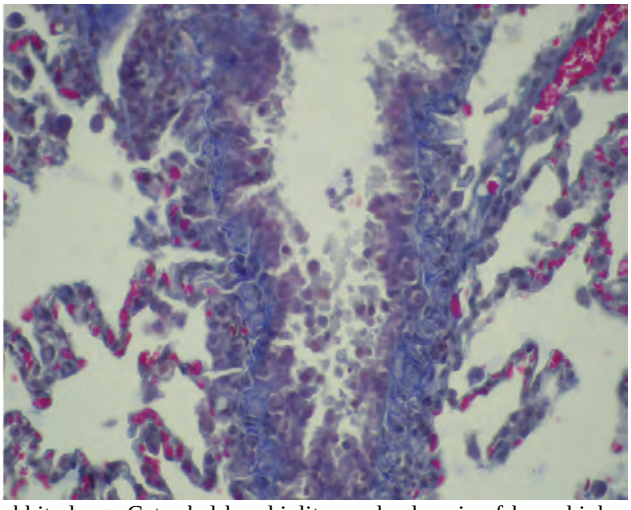

Fig. 7 - Rabbits lung: Catarrhal brochiolites and sclerosis of bronchiolar wall. ; Mallory tricromic staining; 40x.

From 22 dying rabbits were taken bacteriological exam samples from nasal mucous, trachea, lung, heart, liver, bone and blood. At 15 nasal tampons was isolated and identify *Pasteurella multocida*, at 1 samples *Pasteurella multocida* and *Streptoccocus sp*. and at 8 samples *Pasteurella multocida* and *Staphylococcus aureus*.

From 22 samples examinees, at 12 was isolated and identify in trachea, lung and liver *Pasteurella spp*. and at 9 samples from liver and spinal marrow. At 17 bodies was isolated and identification from trachea and lung *Pasteurella multocida* and *Staphylococcus aureus*, and from 2 bodies from the same organs was isolated and identification *Pasteurella multocida* and *Streptococcus sp*.

Pasteurella multocida strain was moderate toxigen, intense edematogen and dermonecrotic and *Staphylococcus aureus* strain was intense hemolitics and weak positive coagulates.

The pathogenity was tested on NMRI mice by intraperitoneal inoculation of 0,5 ml suspension per mouse. At the group inoculated with the association between *Pasteurella multocida* and *Staphylococcus aureus* the mortality was 50% .

At antibiogram test *Pasteurella multocida* was sensitive to enrofloxacin, streptomycin, ciprofloxacin, amoxicillin and gentamicin and *Staphylococcus aureus* was sensitive to penicillin, enrofloxacin, amoxicillin, ciprofloxacin, erythromycin and oxitetraciclin.

The individual treatment was done with injection enrofloxacin and the treatment of all effective was done with amoxicillin in water. After 3 days from the beginning of the treatments the mortality was stopped and after 8 days the respiratory syndrome was stopped.

It was also made a bivalent autovaccin with the two microbiane strains isolated (*Pasteurella multocida* and *Staphylococcus aureus*) and at two weeks after the last administration of antimicrobial medication it was made the vaccination of the entire effective.

After repeating the vaccination at two weeks there hadn’t been any report of new cases of disease.

3. CONCLUSION

3.1. An intense pathogenic strain of *Pasteurella spp*. was isolated from rabbits with acute respiratory syndrome.

3.2.From those 22 nasal pads harvested from rabbits, 22 samples contained *Pasteurella spp.,* 2 samples contained both *Pasteurella spp.* and *Streptoccocus spp.* and 17 samples contained both *Pasteurella spp.* and *Staphylococcus aureus.*

3.3. The isolated strains were sensitive to amoxicillin and enrofloxacin.

3.4 After 3 days from the beginning of the treatments the mortality was stopped.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

KINEMATIC MOTION ANALYSIS OF THE FORELIMBS IN HEALTHY DOGS

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Key words: dog, forelimb, kinematic analysis, gait.

SUMMARY

The aim of this study was to establish the most effective method of kinematic analysis in determining kinematic patterns of the thoracic limb joints in large breed dogs.
For this purpose two systems were used: first - APAS system - is based on video recording of passive reflective markers movements, markers that are fixed on reference points of subject' limbs (videography) and the second is based on measuring the propagation time of an ultrasonic pulse. Although Zebris cell offers good results for humans' gait, irrespectively of assessed subject disease, in dog's case permitted only the general data registering. APAS system proved to be a reliable technique which permits 3D kinematic assessment of dogs' gait.

The need of using sophisticated methods that allow an objective gait analysis results from the complexity of the act itself and the need of simultaneous assessment for several events during a single cycle of steps in a three dimensional space. Although through kinetic analysis of locomotion could be made an objective and reproducible description of lameness, assessment of some parameters, like vertical force peak and vertical impulse, reveals only compensatory changes in body mass distribution on the limbs (Bockstahler et al., 2008a). The method involves adding force that act strictly in stance phase (joint function assessment is not possible), so its value is diminished (Bockstahler et al., 2008a) and there is need for kinematic analysis of gait for assessing this parameter.

Most studies conducted in dogs described kinematic of flexion and extension movements for various joints (Bruggeman 2002; DeCamp et al., 1993; DeCamp 1997; Vilensky et al., 1997).

1. MATERIALS AND METHODS

Kinematic analysis of gait was performed using two analysis systems in Kinematic Analysis Laboratory of Polytechnic University Timisoara.

There were assessed eight common breed dogs with an average size of 55.33 cm and an average weight of 27.16 kg.

The first system used to describe the kinematics of the thoracic limb joints in dogs is Zebris system (Fig. 1). The system is based on emission, reception, capture, and processing of ultrasound information, being commonly used for analysis of human gait. The system is based on measurement of the propagation time of an ultrasonic pulse, the signal come from the both pars of the biped (right and left) being simultaneous quantified. The operating principle is that of triangulations, each sensor having attached three microphones that receive ultrasonic signal emitted by the speakers of the Zebris CMA-HS unit. To record the movement of forelimb joints, in each dog active markers were applied on both thoracic limbs, at half of the distance between the point of scapulohumeral and elbow joints from the left and then right side. After that, the dogs were connected by wires to Zebris CMA-HS unit. The markers were applied on the limbs with the dog standing. After active marker fixation, points needed for obtaining the geometric model of the limb were electronic marked with the help of an electronic pointer. Those points are: greater tubercule of the humerus, epicondyles of the humerus, ulnar styloid process, carpo-radial bone, carpal accesory bone, dorsal face of distal phalanx.

Coordinates of reference points are automatically determined based on the location of active markers throughout the reading.

After electronic marking, dogs where walked with constant speed in test space for a length of 6 meters (Fig. 1). Recordings in which the speed was variable or dogs deviated from direction where eliminated.

Ariel Performance Analysis System (Fig. 2), the second system used, is based on the video recording (videography) of passive reflective markers movement, markers that are fixed on limbs' reference points and that are not connected by cables to the measurement system. Markers were located in places designated as anatomical reference points, as follows: spina scapularis, great tubercule and lateral epicondyle of the humerus, ulnar styloid process, and lateral distal aspect of the fifth metacarpal bone. Joint angle is obtained by setting in the first phase the two vectors in three-dimensional axes, based on reflective markers position. Establishment of thoracic limb joint angles is based on data registration during a single cycle between two maximum flexions of the elbow joint.

Fig. 1. Zebris System

Fig. 2. APAS System

2. RESULTS AND DISCUSSIONS

Based on records obtained through the Zebris system, intervals considered representative for each subject were selected and review reports were generated, the data being presented as mean ± standard deviation.

Unlike the study conducted by Schaefer et al., 1998, in which there were no significant differences between movements of the same biped joints recorded, in our study we records both asymmetry between limbs (left, right) of the same subject and between subjects.

The smallest angles were recorded for adductor movement of the elbow and for flexion / extension of the shoulder, and the highest for the shoulder and autopodium rotation.

Registered parameters did not correspond to physiological aspects of gait symmetry (uniform amplitude and joint angles). Because of animals' deviation from route, Zebris unit microphones have received ultrasonic signals emitted by active markers improperly, and the resulting graphics do not describe the subjects' gait.

These findings are probably due to several disruptive factors intervention, like connection between the markers fixed on the dogs' limbs and the measuring system, which hampered the movement of subjects, and also ultrasonic emission frequency (100 Hz) of the system that interfered audible frequency range of animals, which has values between 64 Hz and 24 kHz, providing an irritative component.

After processing the data obtained from the dogs with APAS system, the following kinematic variables were graphically expressed for thoracic limb joints: displacement, angular velocity and acceleration.

Carpal joint is characterized by an extension movement during the stance phase. The fast flexion starts at the end of the stance phase, being

followed by a quick extension in the early stance phase. There was a single peak of maximum flexion during the early phase of suspension. These results are similar with those obtained in walk by Hottinger et al., 1996 for large breed dogs and at trot by DeCamp et al., 1993 for Greyhound breed.

In a kinematic study conducted by Gosi et al., 2005 on the Flat-Coated Retriever authors compared the chart of radioulnar carpal joint motion with a rounded line whose range of motion is between 80˚and 90˚ during flexion and 230˚-240˚ during extension. This aspect of the curve was obtained also in the present study, being registered differences in the ranges of motion - between 200˚ and 210˚ for extension, respectively 90-100˚ for flexion.

Unlike the data collected in this study performed on common breed dogs, in that the maximal flexion was registered in the early phase of suspension, Bockstahler et al., 2008b reported for Malinois dog the appearance of this movement in the middle phase of suspension.

Both in present study and in Arnold et al., 2005 experiment, the largest range of motion for the thoracic limb joints was recorded for radioulnar carpal joint.

The velocity and acceleration curves have two peaks (minimal and maximal). No significant differences were recorded in velocity and acceleration curves - both are maintained at a constant level by the end of stance phase, when reach the minimum, and then increase gradually. If the velocity reaches the maximum in the middle phase of the suspensions, the highest acceleration was recorded during the early phase of suspension.

Gosi et al., 2005 found on the velocity curve obtained from the Flat-Coated Retriever values close to zero during flexion and support phase, with a relative maximum in the suspension phase and a relative minimum at the end stance phase.

We also noted values near zero for velocity during the stance phase (in extension) and a relative maximal value in the middle of the suspension phase.

Elbow joint exhibits a short period of flexion in early stance phase, followed by extension. In the end of this phase is initiated a fast flexion followed by quick extension during the suspension phase. Two peaks of maximal extension were registered, both in tardy moments of the stance and suspension phases. The presence of the two peaks of maximal extension was observed also by Hottinger et al., 1996 in walk and by DeCamp et al., 1993 in Greyhound trot, but one of the peak antecede stance phase, and the other being registered during this phase.

Comparing to the data reported by Clements et al., 2005, in our experiment was obtained slightly bigger amplitude for first peak of maximal extension registered in the end of stance phase.

Gosi et al., 2005 reported in Flat-Coated Retriever occurrence of the maximum extension at the beginning stance phase, and the second at the end of this. After recording this peak, values tends to decrease rapidly with 20 to 25˚, reaching the minimum value that is positioned at one-third the distance between the peak sites.

In this study, after reaching the first peak of the maximum extension at the end of the suspension phase, the curve reduces slowly with 10 to 15˚, reaching the minimum during the early stance phase.

The pattern obtained in this study on common breed dogs is similar with that obtained by Bockstahler et al., 2008b for Malinois dogs: slow flexion of the elbow joint when limb reaches the ground, followed by extension during stance phase. However, maximum extension was achieved at the transition between the two phases of step (presence of a single peak of maximum extension).

Velocity curve obtained in common breed dogs was characterized by slightly reducing of the velocity at the beginning stance phase, a slightly upward trajectory over the stance phase (velocity being 100˚/s), followed by reduction and appearance of the first peak of the minimum value (-500˚/s) at the suspension phase initiation. During the suspension phase, velocity increased, reaching maximum peak (600˚/s) at its middle, with a subsequent reduction.

Clements et al., (2005) found similar data on the velocity curve obtained during the movement to step treadmill (speed 2.0 m/s) of the Labrador Retriever dogs - presence of a single peak speed both in flexion and extension. If the maximum angular velocity of flexion was associated with the early phase of the suspension of the limb, the maximum extension angular velocity was recorded, unlike our study, at the end of the suspension phase (the last period of the suspension phase).

Similar data were reported also by Gosi et al., 2005, which were detected on velocity diagram obtained both in walking and trot for Flat-Coated Retriever dogs the existence of two peaks (positive value for extension and negative value for flexion) whose values were between 400˚/s - 600˚/s.

Scapulohumeral joint exhibits a gently flexion during stance phase, which attained maximal value in their terminal period, followed by initiation of extension in the end of stance phase. A single maximal extension peak was observed, which was registered in the end of

suspension phase. Similar results were obtained by Hottinger et al., 1996. DeCamp et al., 1993 observed two extension peaks in Greyhound trot, one maximal extension that antecede stance phase, and one with lower intensity during this phase.

Unlike the present study, Bockstahler et al., 2008b recorded in Malinois dogs the appearance of the maximal extension peak expansion at the onset of stance phase, followed by slight reduction of movement during stance and fast flexion, which reaches the maximum in the middle of suspension phase.

In common breed dogs, velocity had a single peak of minimal value (-75˚/s), during early stance phase. Maximal value was attained in tardy stance phase (75˚/s). In the suspension phase velocity was constant at the beginning and had an ascending curve during the remainder suspension.

Acceleration reached maximum amplitude at mid-stance phase (150˚/s) ant minimal value at the end of stance phase (-100˚/s), in the remaining stages preserving at a constant level (near zero).

Comparing to data obtained in this study, Bockstahler et al., 2008b found on the angular velocity curve for Malinois dogs a constant velocity for most of the stance phase and at the onset of suspension.

Globally, joint movements are similar in both types of gait and the difference between our results, obtained for normal walk, and those reported by other researchers for dogs` trot can be a resultant of bigger amplitude of trot movements, applicable for all joints.

Body conformation uniformity of common dogs used in this study and morphological differences between them and Flat-Coated Retriever whose kinematic data were compared may represent a first source of variability of data obtained. Also, discrepancies between studies may be due to different technologies for recording and analyzing data that were used.

3. CONCLUSIONS

3.1. Results obtained in healthy dogs gait analysis with Zebris system are irregular and inconclusive.

3.2. Although Zebris cell offers good results for humans' gait, irrespectively of assessed subject disease, in dog's case permitted only the general data registering.

3.3. APAS system proved to be a reliable technique which permits 3D kinematic assessment of dogs' gait.

3.4. Our investigations established the standard pattern of kinematic analysis for large size dogs' normal gait.

ACKNOWLEDGMENTS

This work was supported by CNCSIS – UEFISCSU, project number PNII – IDEI 130/2008

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

HEART RHYTHM DISORDERS IN DOG

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Keywords: dog, regular sinus rhythm, cardiac arrhythmias.

SUMMARY

This study sought to differentiate the physiological disturbances of heart rhythm of their pathological aspects in dogs of different breeds and ages. With a mobile ECG (CARDIOLINE DELTA1 PLUS), electrocardiograms were performed by standard method of the six bipolar limb derivatives. Compared with reference data characteristic regular sinus rhythm were identified aspects of sinus tachycardia (mean heart rate 200 b / min, the presence of TP wave, PQ interval duration <0.06 s, QT duration <0.15 s), issues of sinus bradycardia (average heart rate 50 b / min during the PQ interval > 0.13 s, QT duration > 0.25 s), stopping sinus issues (PP interval duration > 0.12 s). Depending on clinical context, the systolic blood pressure, heart sound intensity changes, the response to carotid sinus massage, the response to vagal blockade by i.v. injection with atropine, could determine the origin of these changes of heart rhythm.

Heart rhythm disturbances are changes in the timeline of myocardial contraction, due either to abnormal bioelectric impulse formation in embryonic heart tissue, or abnormalities in its leadership to working myocardium (Irisawa et al., 1995). All heart rhythm disorders are defined by reference electrocardiogram changes identified in regular sinus rhythm characteristic values of reference (Collet and Bobinnec, 2001). In a healthy animal body, physiological changes are regular sinus rhythm the neurovegetative regulation.

The purpose of this study was to identify and differentiate the physiological aspects of regular sinus rhythm compared to its pathological encountered in some cardiac arrhythmias, based on clinical signs, changes in ECG interpretation and application of techniques of stimulation and vagal inhibition.

1. MATERIALS AND METHODS

The study was conducted in the Clinic Faculty of Veterinary Medicine, Spiru Haret between 2008 - 2009, recorded ECG changes and clinical signs of cardiac arrhythmia in 87 dogs of various breeds, aged between three and 14 years, who presented to us for investigation.

In these dogs were recorded clinical signs induced by decrease in cardiac output and the changes appeared in precordial shock frequency,

heart sounds and pulse. Also were measured with a digital tensiometer (OMRON M3), and were noted blood pressure values.

Electrocardiography were performed using a portable ECG device (CARDIOLINE DELTA1 PLUS) using standard bipolar limb six derivatives and working parameters were: speed 25 mm / sec and amplitude of 10 mm / mV. Determination of cases of cardiac arrhythmia dependent sinus rhythm disorders was made following the interpretation of ECG changes reported routes to regular sinus rhythm characteristics (Martin, 2007).

Technical exploration of rhythm disorders by stimulating vagal aimed achieve compression carotid sinus and vagal inhibition was performed by i.v. injection with atropine 0.025 mg / kg and then were followed qualitative effects of rhythm and heart rate, as the literature shows (Collet and Bobinnec, 2001).

2. RESULTS AND DISCUSSION

After those two criteria, electrical impulse formation and leadership excitation, cardiac arrhythmias are classified into three main groups: sinus rhythm disorders, disorders due to ectopic rhythm, disorders due to the intracardiac conduction system abnormalities.

Of sinus rhythm disturbances that have followed and identified during our study were noted: respiratory sinus arrhythmia, sinus tachycardia, sinus bradycardia, sinus arrest.

Ectopic rhythm category, we identified the following arrhythmias: atrial extrasistola (ESA), ventricular extrasistola (ESV), supraventricular tachycardia.

The category of conduction abnormalities, we identified the following arrhythmias: sino-atrial block grade II, type Möbitz (BSA II), atrio-ventricular block grade II, type II (BAV II).

The techniques of stimulation and vagal inhibition that we used, allowed us to determine the type of abnormal cardiac rhythm caused or neurovegetative dystonia or pathological causes.

Respiratory sinus arrhythmia (figure 1), was characterized by a thinning heart rate during exhalation and an acceleration of its inspiration. Physiological respiratory arrhythmia is due to excitation of vagal nerve endings following distension of pulmonary alveoli from the

end of inspiration (figure 1). Respiratory sinus arrhythmia have met the 43 cases studied arrhythmias.

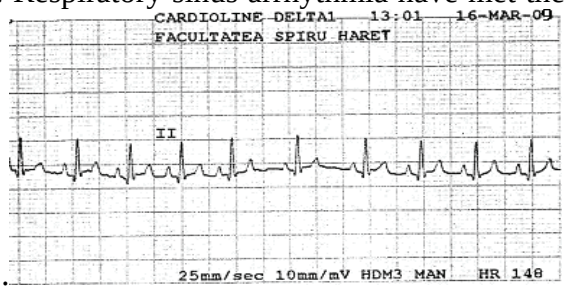

Fig. 1. Respiratory sinus arrhythmia

Many changes occurring on the route of the ECG in terms of succession sequences of P-QRS-T, the duration of R-R cycles and T wave morphology can lead to confusion the respiratory sinus arrhythmia with atrial extrasistola (figure 2).

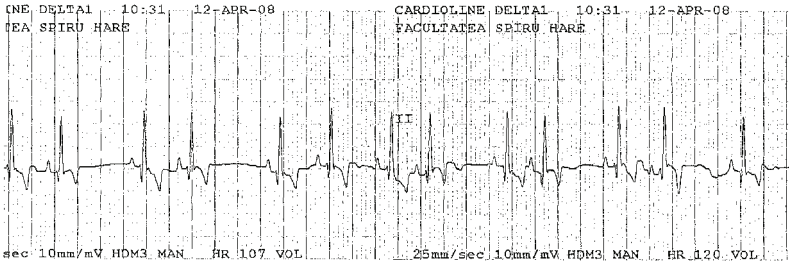

Fig. 2. Atrial extrasistola

Diagnostic criteria for ESA were: irregular heartbeat (second premature beat), the disparity between expiratory phase of respiratory cycle and cardiac cycle frequency thinning, premature P waves exist with different forms of normal P waves and deformed T waves preceding premature beats. Of arrhythmias studied, we met at ESA eight cases and because the state was not accompanied by lipotimie or other clinical signs, was considered due to a neuro-vegetative dystonia.

ESA regular-irregular cycles may be mistaken with ESV, when listening with stethoscope. Ventricular extrasistola (figure 3), we identified a total of 10 cases studied arrhythmias, ECG based on the following features: wide and premature QRS complex (> 0.12 s), with high amplitude and ST-T interval in opposition with QRS. ESV trigeminism type, were produced by a reentry mechanism with R-R' intervals constant. ESV has not been accompanied by major clinical

signs and vagal stimulation resulted in changes refractory periods and thinning accesses the ESV, which showed its sympathetic origin.

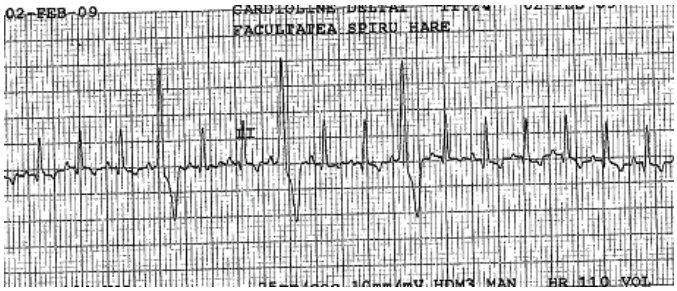

Fig. 3. Ventricular extrasistola

On the following clinical signs: excessive tiredness, breathlessness, increased heart sound, rapid pulse, low blood pressure (mean 10 / 8 mmHg, as a result of ventricular diastolic filling gaps), we identified 18 cases, another form of arrhythmia, tachycardia. Based on specific ECG changes we could differentiate the 10 cases, sinus tachycardia and in 8 cases, supraventricular tachycardia. For sinus tachycardia (figure 4), ECG changes were: sinus character of P-QRS-T sequences, shortening segment PT (electrical diastole), duration of the PQ interval <0.06 s, duration of QT interval <0.15 s, average heart rate was 200 b / min.

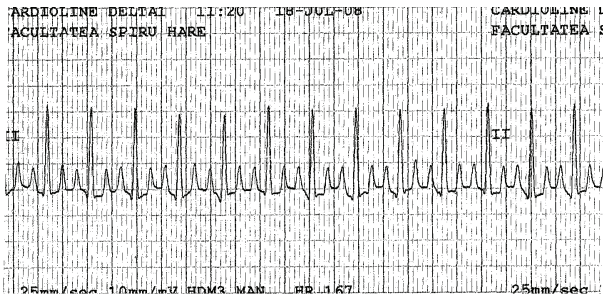

Fig. 4. Sinus tachycardia

Of the 10 cases of sinus tachycardia, six cases had pathological origin, accompanied by clinical signs of conditions such as: hyperthyroidism (one case) and chronic heart failure (five cases). The remaining four cases were detected sympathetic origine after vagal stimulation, which produced a temporary restoration of sinus rhythm but with immediate return to the original frequency. For supraventricular

tachycardia (figure 5) ECG changes were: the tendency of overlapping successive waves T and P, average heart rate 255 bpm.

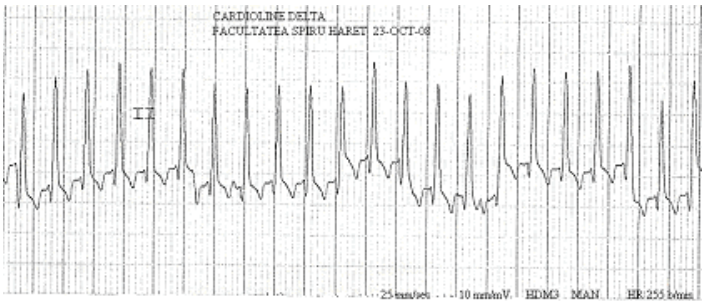

Fig. 5. Supraventricular thaycardia

Supraventricular tachycardia had an abrupt onset following the entry into office a center of ectopic or sinus node area during an atrio-ventricular heart failure. ECG examination revealed a regular sinus rhythm and quickly superimposed P waves and inverted T waves (negative) but the QRS complex remained normal (figure 5). Supraventricular tachycardia was present in the two cases of mitral stenosis and six cases of aortic insufficiency, which led to decreased cardiac output due to a low ejection fraction and low blood pressure. In the supraventricular tachycardia, the labor vagal stimulation by carotid sinus compression for 3-5 seconds, has no effect.

Studied 8 cases of arrhythmias we observed the following clinical signs: departed, conditions of lethargy and even syncope, low tolerance to the effort. Recorded pressure was low, averaging 9 / 5 mmHg consequence of inadequate systolic ejection. Average heart rate was 50 b / min. Of this total, as recorded ECG changes, arrhythmias were differentiated as follows: three cases of sinus bradycardia, two cases of sino-atrial block second degree, type Möbitz, two cases of atrio-ventricular block second degree, type II and one case of sinus arrest. Thus, for sinus bradycardia (figure 6), the ECG showed: the P-QRS-T sequences with sinusal characteristics and identical between them, increased ventricular diastole, the duration of PQ interval > 0.13 s, QT duration > 0.25 s. Of the three cases of sinus bradycardia, two were secondary to toxic conditions (digitalis and beta blockers intoxication), and the third was primitive. Atropine test produced a doubling of value of heart rate without ECG morphology.

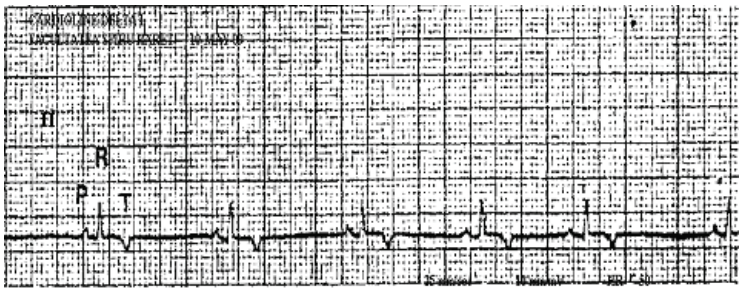

Fig. 6. Sinus bradycardia

Sino-atrial block grade II, Möbitz type (figure 7), revealed by blocking regular management electrocardiographic sino-atrial, sinus rhythm breaks (breaks that led to bradycardia), fixed PP intervals before and after the break (break was a multiple of the basic PP interval).

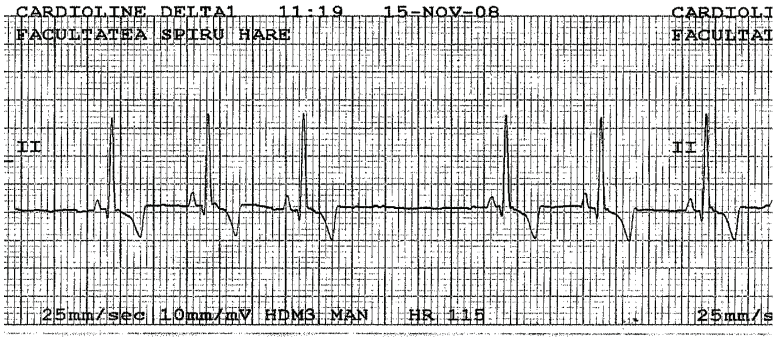

Fig. 7. Sino-atrial block grade II, Möbitz type

Sino-atrial block grade II, Möbitz type, had a vagal origin, as evidenced by atropine test which produced their transient suppression.

Atrio-ventricular block grade II, type II (figure 8) was characterized by the absence of electrocardiographic atrio-ventricular management of sinus impulses (not all P waves were followed by QRS complex). PQ interval duration was constant (Möbitz period), where P waves led to more than blocked.

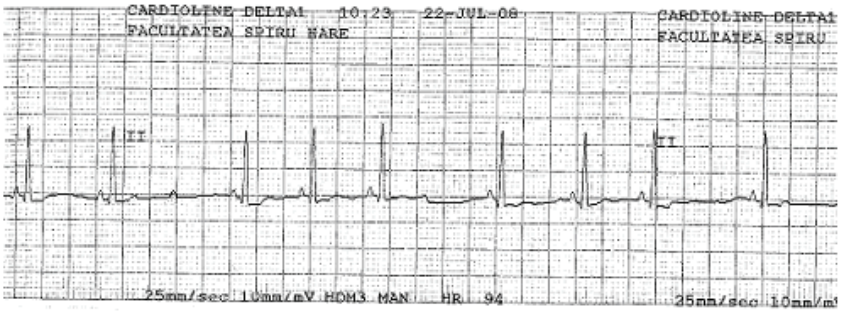

Fig. 8. Atrio-ventricular block grade II, type II

BAV grade II, type II was seen in healthy animals and vagal origin has been evidenced by atropine test, which produced their transient suppression.

We found one case of sinus arrest, that it was characterized by absence of pulse generation in the whole sinus node. The origin of sinus arrest can be a sinus node dysfunction or increased vagal tone. Sinus arrest typically is of pathological (appears as a manifestation of end stage disease caused by sinus node tissue fibrosis), is known as sick sinus syndrome.

ECG showed: sinus nature of P-QRS-T sequences, frequent and irregular sinus pauses, PP long cycles unevenly distributed (figure 9).

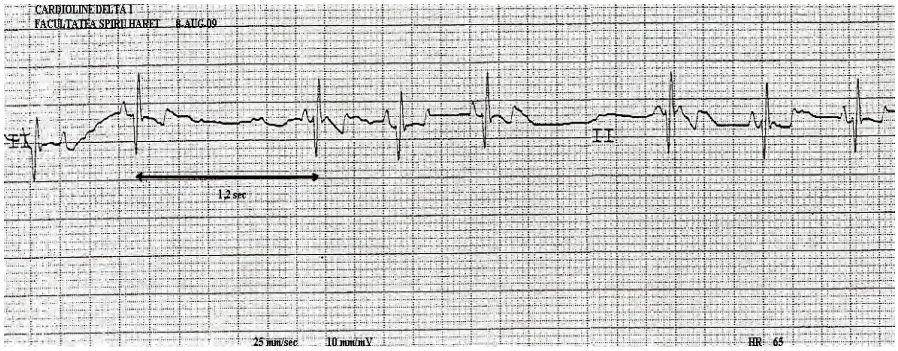

Fig. 9. Sinus arrest

The atropine test had no electrocardiographic response which shows that sinus arrest was pathological origin. In case of sinus arrest were vagal origin, the test would have the effect of atropine, heart rate doubled.

3. CONCLUSIONS

3.1. Using tests of vagal stimulation and inhibition may be a common practice in the differential diagnosis of cardiac arrhythmias.

3.2. Vagal stimulation and inhibition tests can differentiate existing rhythm disorders of the latent rhythm.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

PRRS AND ENZOOTIC PNEUMONIA IN A FATTENING PIG FARM

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Key words: PRRS, *Mycoplasma flocculare*, respiratory diseases, pigs.

SUMMARY

The paper presents the investigations made upon the diagnosis of a mixed outbreak of infection with PRRS virus (PRRSV) and enzootic pneumonia.
By using serological ELISA test it was confirmed the infection with PRRSV, from 10 samples examined, 9 were positive.
From pig lungs with characteristics lesions of enzootic pneumonia there has been isolated and identified a strain of *Mycoplasma spp*, which proved to be *Mycoplasma flocculare* (*M. flocculare*).
There were made cultivations on special culture media for mycoplasmas. Based on cultural and morphological aspects there has been identified a strain of *Mycoplasma spp*. Using Multiplex PCR technique was identified *Mycoplasma flocculare* (*Assuncao* et al., 2005, *Holko* et al., 2004, *Hovind-Hougen* et al., 1991).

In industrial pig breeding farms, respiratory disorders are often caused by combined action of viruses and bacteria. PRRSV is frequently involved in respiratory problems of fattening pigs, being the one that creates fertile ground for the development of bacterial infections (*Loeffen*et et al., 1999).

Enzootic pneumonia, caused by *M. hyopneumoniae*, is a chronic respiratory disease that produces considerable economic losses in the pig industry worldwide. *M. flocculare* was, and is still consider by majority of researchers a non-pathogenical species. Nevertheless, some researchers (*Armstrong and Friis*, 1981) isolated this *Mycoplasma* species from lungs with characteristic lesions of enzootic pneumonia, which could sustain the hypothesis in which *M. flocculare* has a patogenic potential, generating symptoms and gross lesions like *M. hyopneumoniae*.

1. MATERIALS AND METHODS

In a fattening farm, from the west side of Romania there were reported serious respiratory disorders, a low rate of average daily weight gain and low feed conversion efficiency. Breeding farms were vaccinated against *M. hyopneumoniae*.

There were taken 10 samples of sera for testing antibody against PRRSV. Serum samples were tested by immunoenzymatic test ELISA (Herdchek PRRS X3, Idexx Switzerland) according to manufacturer's recommended protocol.

There were investigated lungs with bronchopneumonia lesions. From younger pigs (10-12 week-old of age) from 8 lungs there were made cultivations on PPLO broth and agar supplemented with horse serum 5%. All cultures were incubated for 3-4 weeks at 37ºC, using several types of culture media. The isolated strain was subcultured in 3 tubes, containing liquid media, noted A, B and C.

- tube A contains the strain after 5 days of incubation, noted sample 1,
- tube B contains the strain after 7 days of incubation, noted sample 2,
- tube C contains the strain incubated for 7 days in PPLO broth supplemented with horse serum 5%, noted sample 3.

This strain was identified by Multiplex PCR in Molecular Biology Laboratory from Pasteur Institute Bucharest.

DNA extraction was made with QiAamp DNA mini kit (Qiagen, SUA). DNA samples were obtained by heat lysis.

***Protocol*:**

- Centrifugation of the samples from the 3 tubes for 10 minutes at 13.500 rpm at 4°C;
- Removal of supernatant;
- Over the remaining deposit are added 200 µl of PBS (phosphate buffered saline), 20 µl of proteinase K solution and 200 µl of buffer AL (Lysis buffer);
- Mix by vortex for 15 seconds;
- Incubation in water bath for 15 minutes at 56°C;
- 200 µl ethanol are added in an Eppendorf tube,
- Mix by vortex for 15 seconds;
- Transfer in purification columns;
- Centrifugation at 13.500 rpm for 2 minutes at 25°C;
- Removal of supernatant;
- 500 µl of Buffer AW 1 (Wash buffer 1) are added in purification columns;
- Centrifugation at 13.500 rpm for 2 minutes at 25°C;

- 500 µl of Buffer AW 2 (Wash buffer 2) are added in purification columns;
- Centrifugation at 13.500 rpm for 5 minutes at 25°C;
- Removal of supernatant;
- Centrifugation at 13.500 rpm for 2 minutes at 25°C;
- 500 µl of Buffer AE (Elution buffer) are added in purification columns;
- Transfer in Eppendorf tubes;
- Incubation for 5 minutes at room temperature;
- Centrifugation at 13.500 rpm for 2 minutes at 25°C.

Amplification

Amplification was performed with illustra^{TM} PuReTaq^{TM} Ready-To-Go^{TM} PCR Beads (*GE Healthcare* 27-9557-01), using Virgilia Popa 2007 program.

Mix preparation

In an Eppendorf tube it is prepared the mainly mix/master mix containing the following primers: *M. hyopneumoniae* F, *M. hyorhinis* F, *M. flocculare* F, *M. spp* R, *M. hyopneumoniae* R, and $MgCl_2$ and nuclease free water. The final volume consists of 24 µl mainly mix per sample.

The sample (24 µl from the mainly mix) and 1 µl from bacterial lysed are added in Amersham Biosciences amplification minitubes (200 µl) which contains the beads (enzyme. dNTP's and reaction buffer).

In the control tube 1 µl of nuclease free water is added.

Amplification

One cycle (5 min at 94^{o}C), thirty cycles (15 s at 94^{o}C; 15 s at 54,6^{o}C; 1 min 68^{o}C), one cycle (10 min at 68^{o}C) and 4^{o}C – forever (until electrophoresis control) were run on a Gene Amp PCR 9600 System Thermal cycler (Perkin Elmer) using Virgilia Popa 2007 program.

Amplicons control was achieved through electrophoresis in 1.5% agarose gel, 1x TBE buffer migration (Tris borate-EDTA ethylenediaminetetraacetic acid), migration an hour, at 120 V, 60 mA.

Calculation of results are carried out digitally using UnScanIt program (Silk Sci. Ink), after taking the computer imaging through Easy Herolab RH system (ImageWin2PC program).

Part of the research presented in this paper was processed in the „Bacterial Infectious Diseases Research Laboratory" from Faculty of Veterinary Medicine Timisoara.

2. RESULTS AND DISCUSSIONS

Serological investigations carried out using ELISA kit have shown the presence of PRRSV infection in 9 of the 10 sera examined as shown in table 1.

Table 1

Results obtained at samples ELISA testing

Sample no	OD	S/P	Result
1	0,263	0,542	Pos
2	0,634	1,481	Pos
3	1,209	2,937	Pos
4	0,779	1,846	Pos
5	0,733	1,729	Pos
6	1,047	2,527	Pos
7	1,221	2,965	Pos
8	0,607	1,413	Pos
9	0,327	0,704	Pos
10	0,172	0,311	Neg

The isolated strain was identified as *Mycoplasma spp*. based on cultural and morphologic aspects.

On PPLO agar, fried-egg microcolonies were observed. Filamentous and bizarre forms were observed in smears from cultures. These issues are mentioned in literature by other authors (*Quinn* et al., 1994, *Stakenborg* et al. 2005).

At Multiplex PCR technique, performed for genomic typing of *M.hyopneumoniae*, *M. hyorhinis* and/or *M. flocculare*, there were obtained positive results for *M. flocculare* and negative for the other two *Mycoplasma* species (fig. 1).

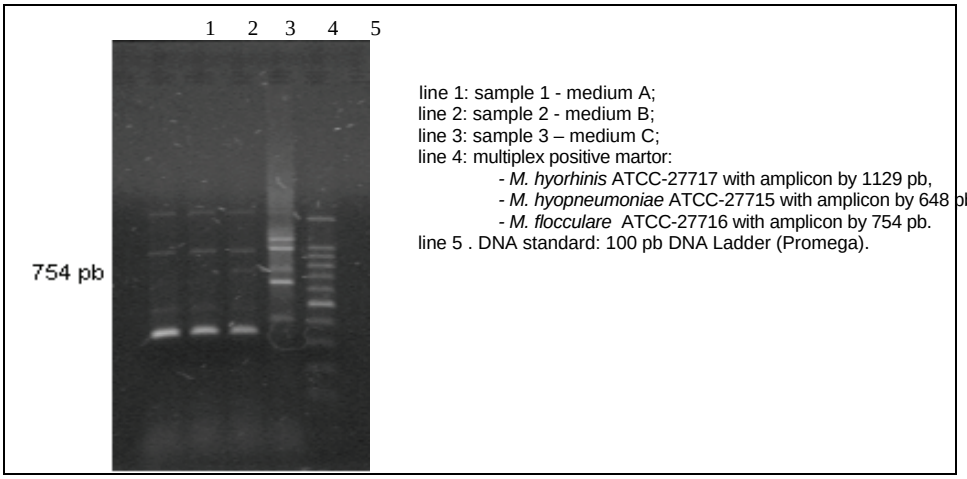

Fig. 1. Electrophoresis-gel post PCR

All samples were generated unspecific amplicons for *M. hyopneumoniae*. It is recorded the amplicon by 754 pb specific for *M. flocculare* only in one tube (sample 3 – medium C), in which was PPLO broth supplemented with horse serum 5%. For the other two we consider that in these tubes was insufficient DNA for testing.

3. CONCLUSIONS

3.1. A mixed infection with PRRS virus and enzootic pneumonia was diagnosed in a pig fattening farm.

3.2. Serological examination demonstrated the presence of PRRSV infection in nine of the 10 sera examined.

3.3. Using Multiplex PCR technique there were obtained positive results at one sample for *M. flocculare*. Besides all unspecific amplicons for *M. hyopneumoniae* for all three samples, 754 pb specific amplicon for *M. flocculare* was record only in a sample, cultivated in PPLO broth supplemented with horse serum 5%.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

PRELIMINARY STUDY OF TOXOPLASMIC INFECTION IN DOMESTIC PIGS FROM TIMIS COUNTY

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Key words: *Toxoplasma gondii,* pigs, Timis County

SUMMARY

Seroprevalence of toxoplasmosis in domestic pigs was investigated in 32 localities (CSV) from Timis County. Serum samples from 1600 pigs were examined by ELISA (enzyme-linked immunosorbent assay). For this study 50 pigs were randomly selected from each CSV.
The prevalence rates of *Toxoplasma gondii* infection in domestic pigs was 21.93% in the Timis County, with variations between 0% and 80%.

Toxoplasmosis is one of the most common parasitosis in humans and animals, it being placed on the top three global spread (Dărăbuş, 2006). The cat is the key element in the epidemiology of toxoplasmosis. For toxoplasmosis transmission, a very important role it have raw meat consumption. In pigs, infection occurs by eating kitchen scraps unsterilized or rodents (Tenter, 2000).

Necropsy diagnosis in the slaughterhouse, it is very difficult to done, because very small necrotic lesions are difficult to observe. Serological diagnosis is possible to made in the slaughterhouse, but is not warranted in our economic Country's conditions (Chiţimia, 2007).

Reporting an increased incidence of toxoplasmosis in humans and animals worldwide and the small number of bibliographic data in our Country about *Toxoplasma* infection, motivates our study.

1. MATERIALS AND METHODS

The assessment of the toxoplasmosis prevalence in pigs was conducted in 2008-2009 in Timis County. The household pigs were aged 4 months to 2 years, males and femals. Herds of household pigs comprising between one and seven animals. 50 pigs were randomly selected from each locality (CSV).

Collected blood was left to express serum and it was kept in a freezer until the month of November 2009 when samples were

processed in the laboratory of Parasitology and Parasitic Diseases of Faculty of Veterinary Medicine of Timisoara.

Serum samples were examined by indirect ELISA method using ID Screen Multi-species kit (ID.VET., France) for anti-*Toxoplasma* specific Ig G antibodies, resulting from infection with *Toxoplasma gondii*. Kit can be used for determination of anti-*Toxoplasma* specific Ig G antibodies from sera of ruminants, pigs and cats. We respect technology manufacturing indicates by producer company.

The S/P values above 200% were considered strongly positive, between 50 and 200% samples were considered positive, between 40% and 50% were doubtful, while values below 40% were considered negative.

2. RESULTS AND DISCUSSIONS

Out of 1 600 samples taken from CSV, from Timis County only 351 sera had (21,93%) *Toxoplasma gondii* Ig G antibodies (Fig. 1).

In seven from 32 studied CSV we have not find any seropositive sample. In other 25 studied CSV we identified 3 to 40 positive samples for *Toxoplasma gondii* infection.

We conclude that from 32 studied CSV's, in 25 (78.12%) we have identified positive animals for *Toxoplasma gondii* infection and only in 7 CSV's none of the tested pigs presented *Toxoplasma* antibodies. Not having sufficient information about examined animals can't refer to the distribution of prevalence by age or gender.

For studied County, information obtained are particularly important as they are the first reported data on *Toxoplasma* infection in the area.

These data indicate a high prevalence of diseases in pigs reared in extensive sistem and shows the importance of farm animal contact with feces of cats or with rodents. Household pigs were kept in relatively optimal hygienic conditions, but with access of cats and birds inside the housing. Pigs were fed with not always heat treated kitchen scraps. Concentrated feed with wich the pigs ration is supplemented are stored around the house, so that cats can easily get to them, or even defecate in the feed store. The prevalence of 21.93% obtained by us in the CSV 's, can highlight a real risk of contamination from exposed humans and species of carnivorous animals, by eating poorly cooked pork.

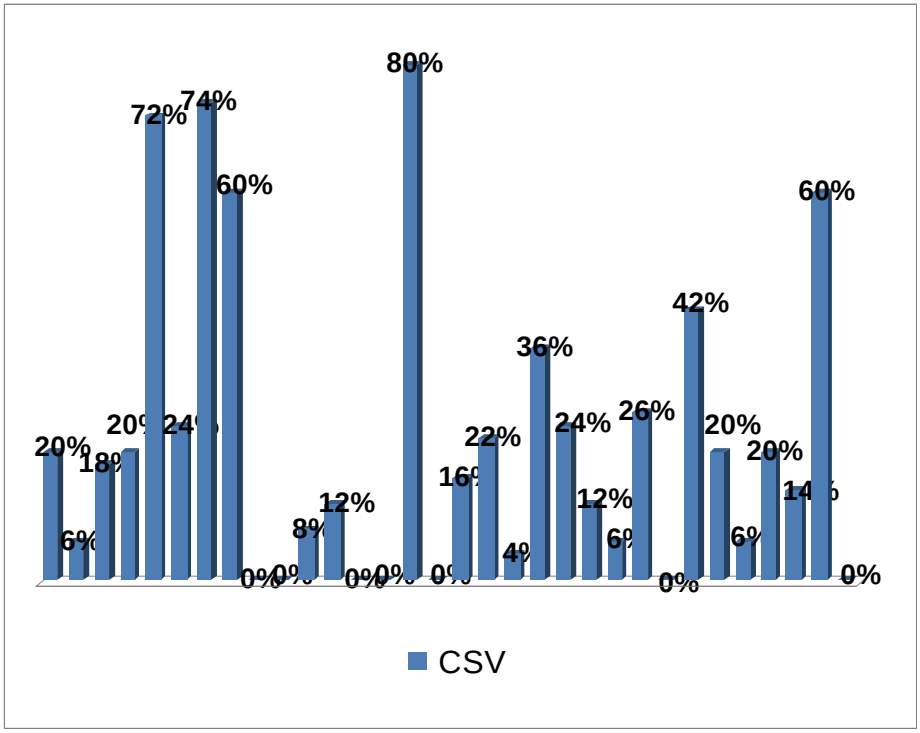

Fig. 1. Prevalence of *Toxoplasma gondii* infection in studied CSV's

The prevalence obtained in CSV's (Table 1) show us, once again, the importance of environmental contact of pigs. In households where the pigs are periodically removed from shelters outside on environment, or where cats and birds have access to animals shelter, the prevalence obtained by us is high, comparable with other data from specialist literature.

This study highlights the absolute need of best practice animal husbandry and food to reduce the risk of transmission of *T. gondii* infection in humans and other animals.

Table 1.

Prevalence of *Toxoplasma gondii* infection in pigs from Timis County

No.	CSV	No. positive samples(%)	No. doubtful samples(%)	The minimum and maximum titres values	Positive titres values
1.	Balinț	10 (20%)	-	14.29-286.70	231.15-286.70
2.	Beba Veche	3 (6%)	-	21.05-	58.69;

				134.81	93.72; 134.81
3.	Belinț	9 (18%)	-	33.41-157.63	77.14-157.63
4.	Biled	10 (20%)	3 (6%)	12.65-199.87	101.25-199.87
5.	Brestovăț	36 (72%)	-	18.35-186.02	129.34-186.02
6.	Buziaş	12 (24%)	-	7.96-189.05	67.08-189.05
7.	Cenad	37 (74%)	-	11.23-157.87	114.35-157.87
8.	Comloşu Mare	30 (60%)	-	20.3-183.96	53.28-183.96
9.	Criciova	0 (0%)	-	12.69-33.29	-
10.	Darova	0 (0%)	1 (2%)	13.85-45.92	-
11.	Denta	4 (8%)	-	13.80-83.21	54.01-83.21
12.	Deta	6 (12%)	-	11.96-83.79	51.68-83.79
13.	Dudeştii Vechi	0 (0%)	-	10.86-24.11	-
14.	Dumbrăvița	0 (0%)	-	9.90-23.51	-
15.	Ghiroda	40 (80%)	-	14.23-197.09	65.78-197.09
16.	Giulvăz	0 (0%)	-	26.55-31.95	-
17.	Jimbolia	8 (16%)	-	8.71-78.21	51.03-78.21
18.	Lenauheim	11 (22%)	-	10.05-103.58	62.09-103.58
19.	Lovrin	2 (4%)	-	14.21-109.17	102.01; 109.17
20.	Lugoj	18 (36%)	-	39.02-146.95	70.32-146.95
21.	Moravița	12 (24%)	4 (8%)	13.91-92.92	62.21-92.95
22.	Moşnița Nouă	6 (12%)	-	14.5-93.2	55.02-93.2
23.	Nădrag	3 (6%)	2 (4%)	11.2-107.37	57.37; 83.15; 107.37
24.	Pişchia	13 (26%)	-	9.45-128.92	63.04-128.92
25.	Sacoşu Turcesc	0 (0%)	-	13.72-35.98	-
26.	Sânmihaiu Român	21 (42%)	-	15.15-286.89	156.64-286.89
27.	Sânnicolau Mare	10 (10%)	-	13.45-145.25	77.29-145.25

28.	Sânpetru Mare	3 (6%)	-	25.19-78.96	52.36; 64.12; 78.96
29.	Teremia Mare	10 (20%)	-	13.91-203.66	156.86-203.66
30.	Tomeşti	7 (14%)	-	9.04-92.38	55.19-92.38
31.	Topolovăţu Mare	30 (60%)	-	15.81-194.75	160.91-194.75
32.	Fabric	0 (0%)	-	8.13-25.3	-
	TOTAL	**351 (21.93%)**	**10 (0.62%)**		

In the world the results are different. Thus, the Netherlands in 2007, has acquired a *Toxoplasma* prevalence of 5.62% in pigs reared in extensive sistem. In households, the prevalence ranged from 4 to 33% (Giessen, 2007). In 1995, in Netherlands, the infection prevalence was 30.9% (Knapen, 1995).

In Italy, the infection prevalence was 16.3%, with the lowest prevalence (7%) at 5-7 months age group and the high values (19%) in pigs older than 24 months (Villari, 2009). In Germany, 5.6% of tested pigs were positive for *Toxoplasma gondii* infection (Schulzig and Fehlhaber, 2005). In Serbia, from 605 examined serum samples, 28.9% were positive to *Toxoplasma* infection (Klun, 2006).

In U.S.A., the prevalence of *Toxoplasma* infection ranged between 27.7 and 88.6% and in Brazil, between 8.54 and 66.67% (Gamble, 2005).

Toxoplasma infection of pigs in Timis County matters both because of neonatal death can occur in pigs, and the possibilities of disease transmission to humans through inadequately cooked meat.

3. CONCLUSIONS

3.1. The prevalence of *Toxoplasma gondii* infection in pigs from CSV in Timis County was 21.93%.

3.2. The seropositivity presented variations between 0 and 80%.

ACKNOWLEDGMENTS

This work was supported by CNCSIS, Bd, grant No. 87/2008 obtained by Ionela Hotea and by CNMP, grant PC No. 51-013/2007.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

THE TREATMENT OF LONG BONE DEFECTS IN DOGS WITH B-TRICALCIUM PHOSPHATE AND COLLAGEN MATRIX LOADED WITH PERIOSTEAL DERIVED CELLS

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Key words: bone defects, periosteum, β-ticalcium phosphate, colagen matrix, dogs

SUMMARY

The literature studies regarding the bone substitutes used in dogs are not many, this study evaluate the healing process in the presence of β-tricalcium phosphate and collagen matrix loaded with periosteal derived cells for repair bone defects.
The bone healing process in all subjects is included in the physiological limits, increased density of the defects during the healing process is the consequence of calcium and hydroxyapatite presence at the mineralization site of the soft callus.

The use of bone grafts increase to stimulate healing of long bone fractures that have failed to heal, to promote healing and to regenerate and replace bone lost (Mata *et al.*, 2010, Petrie, 2010).

The autogenous bone grafts technique requires a labourious procedure, with morbidity risk at the donor site (Wong and Rabie, 2010).

The bone substitute that increase bone formation and improve the healing is requided for the treatment of bone loss. The uses of osteoconductive and osteoinductive bone substitutes are such alternatives or supplementary therapies (Giannoudis *et al.*, 2005, Hakimi *et al.*, 2010).

Alternative methods of skeletal reconstruction include the use of alloplastic materials or synthetic implants (Woo and Koo, 2005).

Collagen is the main organic constituent of bone tissue, has been used in the composition of biomaterials for bone reconstruction, Kim (1998), Yang (2004), cited by Rodrigues Cunha (2008), due to its biological properties such as biocompatibility, biodegradability and bioabsorbability; acts as a support for bone-inducing proteins. These properties are important to facilitate the process of bone regeneration (Rodrigues *et al*., 2008).

Calcium phosphate biocompatibility makes it an important factor in the healing of bone defects (Gao *et al.*, 1997, Gerhart *et al.*, 1993). In an

osseous site, provide an ideal environment for cellular reaction and colonization by osteoblasts. Tricalcium phosphate (TCP) ceramic as a filler of bone defects has yielded encouraging preliminary results. The tissue compatibility, degradability and porosity of TCP are optimally involved in biological function in vivo (Clarke *et al.*, 2007, Gao *et al.*, 1997, Ragni and Lindholm, 1992, Schouten *et al.*, 2010).

Recently, there are many reports on bone engineering using periosteal derived cell as a cell source and various cell delivery vehicles such as a tricalcium phosphate (TCP), hydroxyapatitie (HA), polylactico-glycolic acid (PLGA), collagen matrix (Clarke *et al.*, 2007, Woo and Koo, 2005).

1. MATERIAL AND METHOD

The study was made on 15 dogs divided in 3 groups, of commune breed, both sexes, with ages between 2 and 6 years and body weight between 19 and 34 kg, on which under general anesthesia (acepromazine – ketamine – propofol - izofluran), The surgical approach at the tibial medial surface was classic, medial (Schouten *et al.*, 2010). On one tibia of each dog was made a defect of 3/1 cm, maintaining the periosteum. In the first group (Group A, Control group), the defect was covered with the periosteum, in the second group (Group B) the defect was filled with collagen matrix loaded with periosteal cells and covered with periosteum, in the third group (Group C) the defect was filled with β-tricalcium phosphate and covered with periosteum.

The subjects were monitorised intraoperatory by a standard protocol which include the measure at fixed time intervals (5 minutes), of the blood pressures (systolic, diastolic and average), the oxygen saturation of hemoglobin, the heart rate, the pulse rate and the respiratory rate.

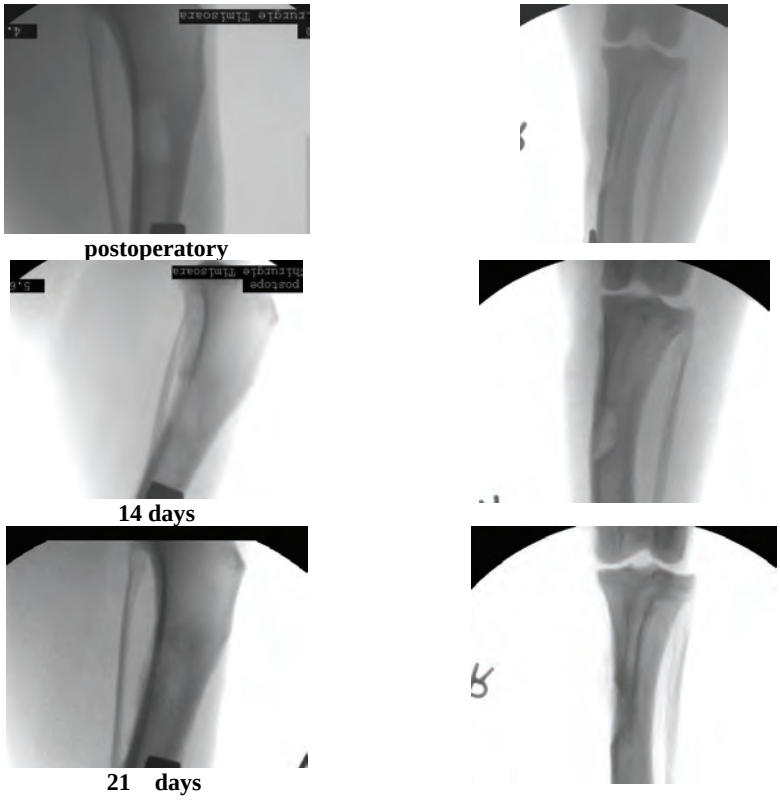
After the intervention, the analgesia was assured using Butorfanol 0.25 mg/kg b.w. administrated every 4 hours in the first 16 hours postsurgery.

The postoperatory observation of the dogs was made by clinical examination daily for 63 days. The X-ray evaluation was performed by digital radioscopy at 7 days intervals (0, 7, 14, 21, 28, 35, 42, 49, 56, 63 days) for nine weeks. It was followed the density and the growth of new tissue on the defect site compared with the healthy bone tissue.

2. RESULTS AND DISCUSSIONS

The postoperatory clinical observation of the all dogs, revealed dates which are similes with the dates reported after minor orthopaedic interventions. All the subjects presented discreet lameness for 1-3 days. All the subjects had unchanged behavior in the rest of the observation period. Healing of the skin wounds occurred in 7–10 days.

The radiographic evaluation of the Group A (fig.1), reveal the increase of the defect density and low periosteal reaction starting with 14 days. At 21 days the defect is filled with low density tissue up to the cortical level of the healthy bone, the periosteal reaction persist. At 35 days the new tissue exceeds the cortical level. At 49 days the density of new tissue is similar with the density of the healthy neighboring bone, keeping the level of new formed tissue. At the end of the monitoring period the changes of the defect are alike with the results obtained at 49 days.

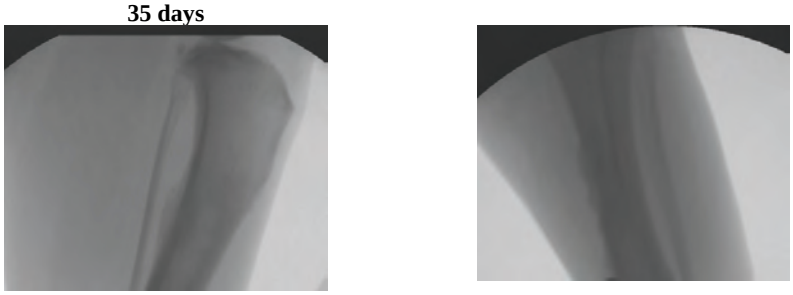


postoperatory

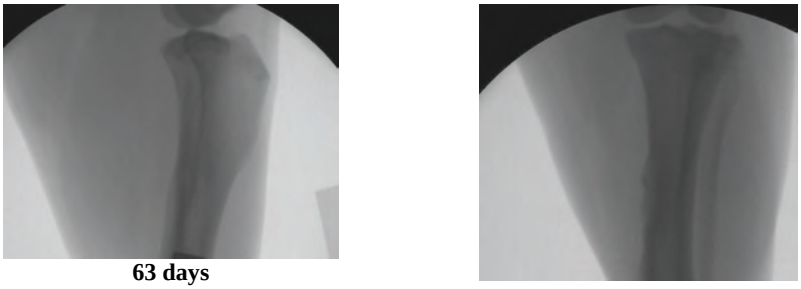
14 days

21 days

35 days



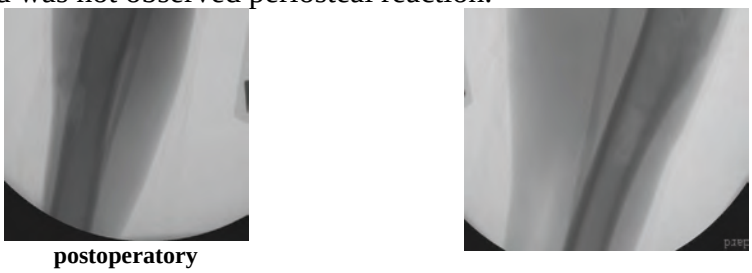
49 days



63 days

Fig. 1 Radiological evolution of Group A

The evaluation of Group B (fig. 2), show at 14 days after the surgical intervention, the upbringing of the defect site density. At 21 days the density is unchanged and the defect is lade 90%. At 42 days the defect is lade 100%, the density of new tissue is similar with the adjacent bone. Until at the end of the observation period 63 days the density of new tissue become identical with the adjacent bone, during this period was not observed periosteal reaction.



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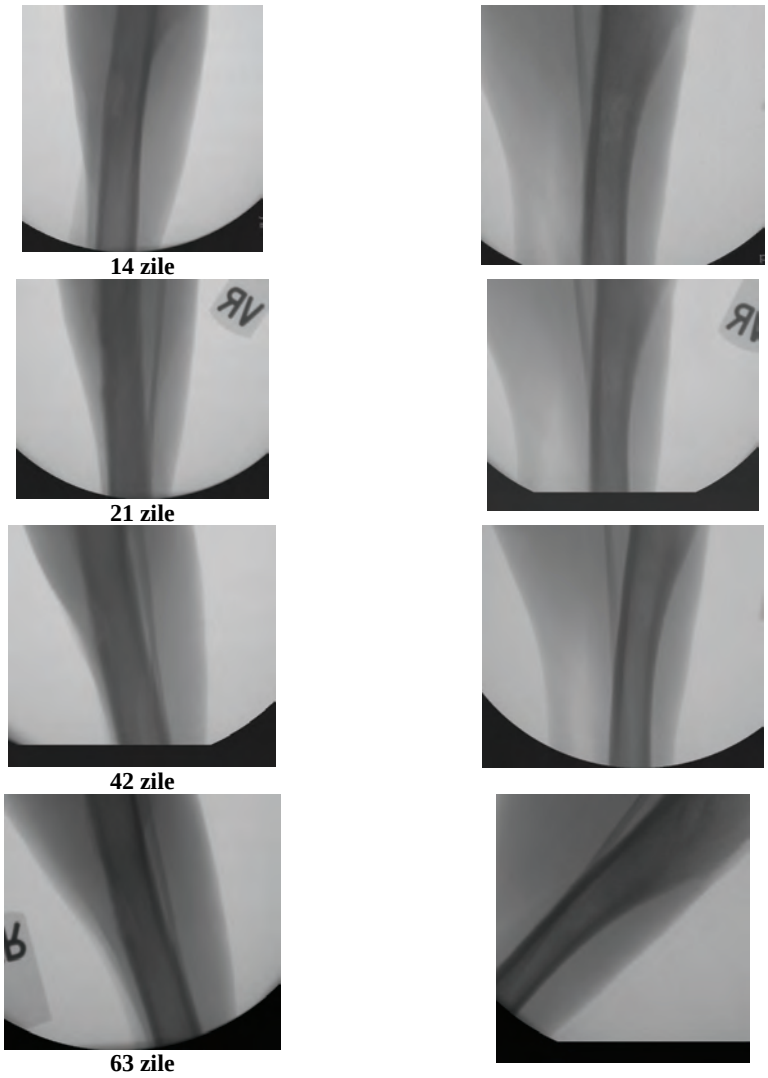

Fig. 2 Radiological evolution of Group B

The examination of Group C (fig. 3), remark no changes between the images performed immediately and at 14 days postoperatory, the defect density increase after 21 days. At 28 days the new tissue plump the defect but the density remain similar than at 21 days. At 35 days the new tissue surpass the cortical level of the adjacent bone, the density is unchanged. At 56 days the new tissue exceed the cortical level, his density is similar with the healthy bone adjacent. The results at the end of examination period are identical with the result from eight weeks.

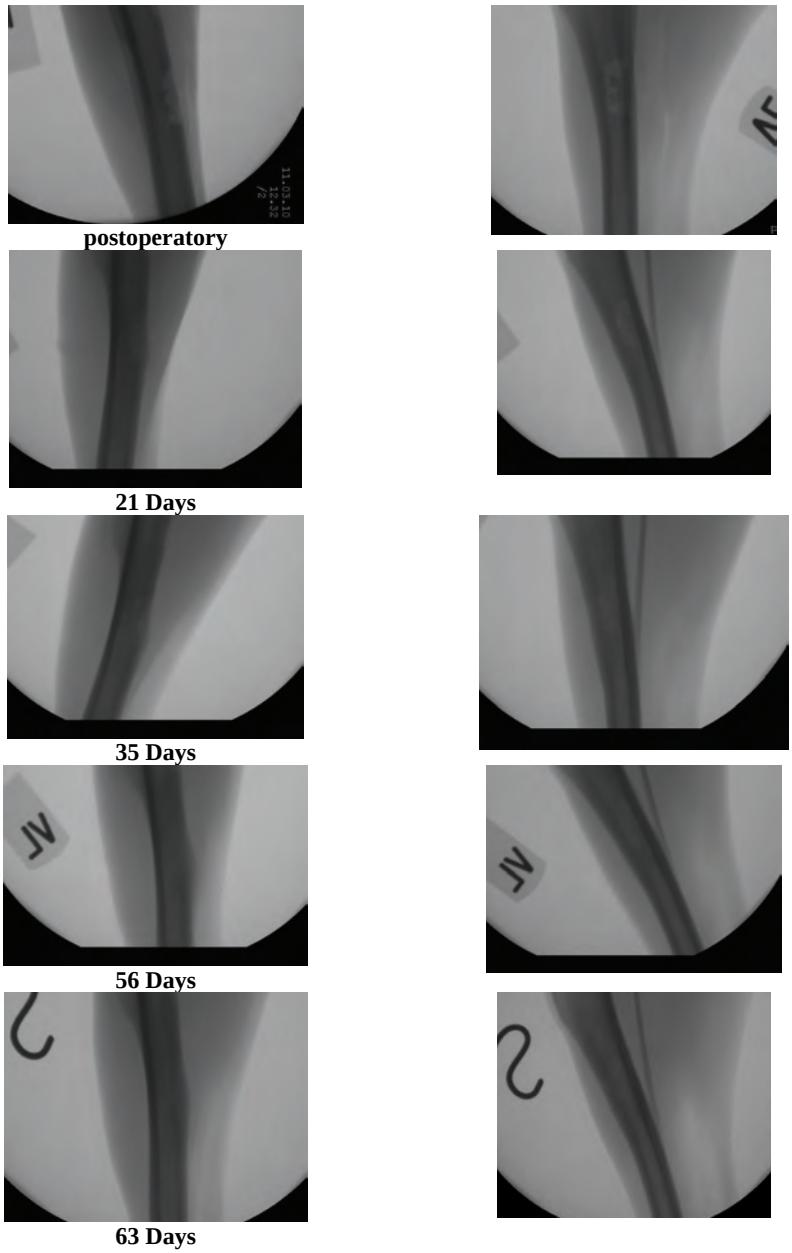

Fig. 2 Radiological evolution of Group C

The healing process of the bone defects in all subjects of the three groups is included in the physiological limits (Koivukangas, 2002).

The lag of changes observed in the Group C, in the first 14 days was indebted at the period necessary for cellular reaction and colonization of

β-TCP by osteoblasts from the cellular layer of the periosteum (Gao *et al.*, 1997, Nienhuijs *et al*., 2010).

The increased of defects density in all groups, between three and nine weeks are the consequence of calcium and hydroxyapatite presence at the mineralization site of the soft callus and make it radiologically visible (Greenbaum & Kanat 1993, Einhorn 1998) cited by Koivukangas (2002).

The periosteal reaction observed in groups A and C, is part of the physiological bone healing process (Koivukangas, 2002), the missing of periosteal reaction in group B maybe due to the periosteal cells loaded on the collagen matrix witch reduce the space of the defect, or to the collagen properties, influences cell differentiation, possesses sites for cell recognition and stimulates cell migration and infiltration, limiting the formation of soft callus in the first stage of healing and the next stages of the healing occur on the reduced soft callus (Koivukangas, 2002).

3. CONCLUSIONS

3.1. All the three methods used to filled the bone defects are viables.

3.2. The bone healing in presence of collagen matrix loaded with periosteal derived cells, occurs faster and with minimum of callus.

3.3. In presence of the β-TCP the healing attained with a tall callus.

ACKNOWLEDGMENTS

This work was supported by CNCSIS – UEFISCSU, project number PNII – IDEI 130/2008

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

HETEROTOPIC NEO-OSTEOGENESIS FROM VASCULARIZED PERIOSTEUM AND BONE GRAFTS

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Key words: neo-osteogenesis, corticoperiosteal, flap, free, vascularized, dogs

SUMMARY

In this study we have investigated the capacity of free vascularized corticoperiosteal flaps to generate heterotopic neo-osteogenesis. On eight common breed dogs, heterotopic transplantation of some tibial corticoperiosteal flaps was realized into the popliteus muscle. The postoperatory surveillance of the dogs was made by daily clinical examination for 63 days. The X-ray evaluation of the osteogenic potential of the corticoperiosteal flaps was performed by digital radioscopy at 7 day intervals for ten weeks. The dual-energy absorptiometry (DXA) was using to measure (at 1, and 2 months) bone mineral content (BMC) and bone mineral density (BMD) for compared tibial fracture healing at the donor site and the osteogenic potential of the corticoperiosteal flaps heterotopic transplantation. At the 63 days, biological samples were obtained for histological examination by classical microscopy and by fluorescent microscopy methods.

On all individuals the radioscopic exam revealed the presence of the corticoperiosteal flaps which maintained their position and radiographic density until 21 days. On radioscopic investigations performed after 21 days, a reduced radiodensity of the bone flaps was observed in all the subjects of the group, followed by accentuated mineral losing in the next 35-63 days.

The histological exam of corticoperiosteal flap tissue revealed a degenerative process of detached compact bone even in conditions of vascularisation and periosteal continuity maintaining. The presence of the fluorochromes indicates the location, time and amount of bone deposition. At the evaluation of the osseous tissue formed in the defect area (from where a corticoperiosteal fragment was detached) intense osseous growing activity inside the calcificated area was observed. The exam of the sample of corticoperiosteal fragment revealed a low bone growing activity The results reveal a significant correlation between BMD and BMC and the histomorphometric determination of bone mass.

Vascularized bone grafting seems to be a valuable reconstructive technique for the treatment of the skeletal defects. Periosteal grafts and flaps have been used for various purposes by numerous techniques. The osteogenic activity of the periosteal tissues has a great importance regarding the purposes of reconstruction. Up to now, experimental and clinical results regarding their osteogenic capacity have been a subject of debate. The role of vascularized periosteum flap as a graft and the factors stimulating it into osteogenic activity remain obscures (Chen *et al*., 2009).

In rats, the studies of ectopic bone formation in the groin and orthotopic bone formation in the femoral defect demonstrates that optimal bone formation requires four factors: bone morphogenetic protein (rh-BMP-2), a biodegradable matrix, osteoprogenitor cells, and blood supply (Kusumoto *et al.*, 1998, Kusumoto *et al.*, 1997, Vogelin *et al.*, 2000, Vogelin *et al.*, 2002). Few articles recognized its osteogenic process without the dependence of stress stimulation or bony contact (Chen *et al.*, 2009). Experimental study on rabbits reported that vascularized periosteal flaps presented strong osteogenic capacity in heterotopic conditions (Chen et al., 2009, Dailiana et al., 2002, Ortak et al., 2005, Rahn, 2003, Saridogan et al., 1993).

The information above was the basis of our investigations in this study, in which we aimed to evaluate the heterotopic osteogenic capacity of free vascularized corticoperiosteal flaps in the dogs.

1. MATERIAL AND METHOD

The study was made on eight dogs, commune breed, both sexes, aged between 2 and 5 years and body weight between 16 and 32 kg, on which, under general anaesthesia, heterotopic transplantation of some tibial corticoperiosteal flaps vascularized by tibial anterior artery into the popliteal muscle was realized. The surgical technique was presented by Igna *et al.*, 2010.

The subjects were monitorised intraoperatory by a standard protocol which included the measure at fixed time intervals (5 minutes), of the blood pressures (systolic, diastolic and mean), the oxygen saturation of hemoglobin, the heart rate, the pulse rate and the respiratory rate. After the intervention, the analgesia was assured using Butorfanol 0.25 mg/kg b.w. administrated every 4 hours in the first 12 hours postsurgery.

The postoperatory surveillance of the dogs was made by daily clinical examination for 63 days. The X-ray evaluation of the osteogenic potential of the corticoperiosteal flaps was performed by digital radioscopy at 7 day intervals (0, 7, 14, 21, 28, 35, 42, 49, 56, 63 days) for ten weeks. It was followed by the existence, formation, emplacement and radiodensity of the transplant.

The dual-energy absorptiometry (DXA) was using to measure (at 1, and 2 months) bone mineral content (BMC) and bone mineral density (BMD) for compared tibial fracture healing at the donor site and the osteogenic potential of the corticoperiosteal flaps heterotopic transplantation. A fan beam Xray bone densitometer (Hologic Delphy W, Elite, Hologic, USA) is then used to perform 2 repeat scans, with an

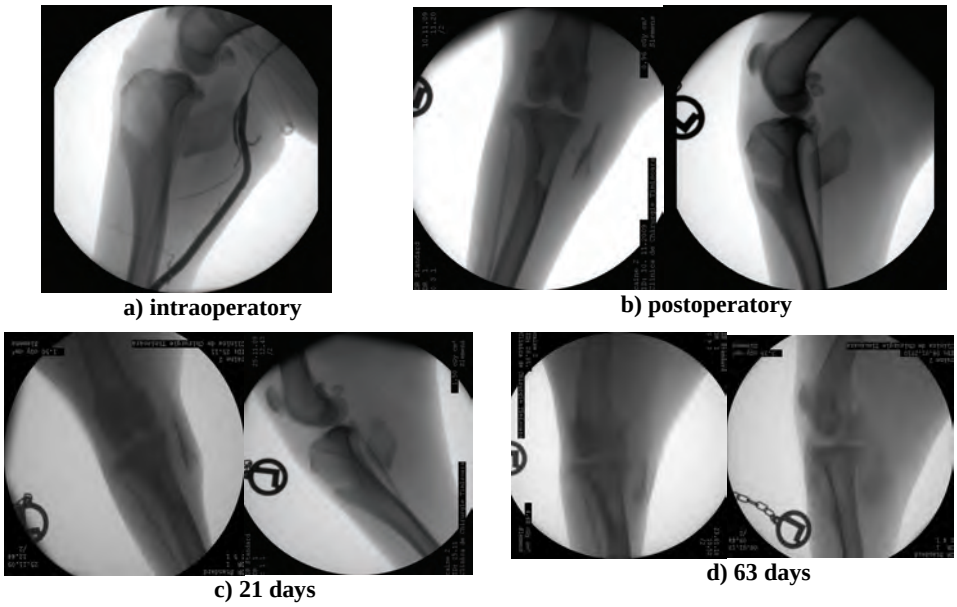
identical longitudinal, rotational and vertical alignment procedure, but with a 180^0 change in orientation. Projected images then undergo pixel analysis using human lumbar spine acquisition software for x-ray (photon) absorption. Estimated area (EA, cm^2), bone mineral content (BMC, g) and bone density (BMD, $g.cm^{-2}$) for each of the two tests are then recorded on Excel® spreadsheet software 11.1:7. As part of a quality control procedure repeat scans of samples were performed if there was a > 1 cm^2 difference in estimated area between the two scans.

For histological postoperative evaluation of the osteogenesis process three fluorochromes - tetracycline, red alizarin, and calceine - (De Yuehuei and Kylie, 2003) were injected following the protocol described by Igna *et al.*, 2010. At the end of this time interval, the subjects were submitted to a new surgical intervention meant for biological sample obtaining (normal bone fragment in the third middle tibial diafisis, bone fragment from the place of corticoperiosteal flap sample, and fragment from the corticoperiosteal transplanted flap) for histological examination by classical microscopy and by fluorescent microscopy methods. Sample processing (undecalcified bone) was prepared for histology, histomorphometry and fluorochrome analysis (of the multiple fluorochrome labels) after the protocol described by Igna *et al*., 2010.

2. RESULTS AND DISCUSSIONS

The postoperatory clinical observations in the all dogs revealed data which are similar to the ones reported after minor orthopaedic interventions. All the subjects presented discreet lameness for 1-3 days. All the subjects had unchanged behaviour in the rest of the observation period. Healing of the skin wounds occurred in 7–10 days.

Bone radiographic evaluations were consistent with viable bone in all the vascularized corticoperiosteal flaps. In the donor site, evidence of the healing bone was observed after 21 days. On all individuals the radioscopic exam revealed the presence of the corticoperiosteal flaps which maintained their position and radiographic density until 21 days. It was not concluded any radiodensity change of the surrounding tissues including the assumed projection area of the flap. On radioscopic investigations made after 21 days, a reduced radiodensity of the bone flaps was observed in all the subjects of the group, followed by accentuated mineral losing in the next 35-63 days – fig 1.



a) intraoperatory **b) postoperatory**

c) 21 days **d) 63 days**

Fig. 1. Cranio-caudal and medio-lateral radiographic views showing the proximomedial corticoperiosteal tibial flap and their evolution in the postoperatory intervals (1-63 days). There was evidence of radiographic accentuate radiodensity losing of the flap after 21 days.

Preliminary results for mean whole bone density (BMD) and bone mineral content (BMC) at the four selected sites from the dogs with heterotopic transplantation of some tibial corticoperiosteal flaps are shown in Table 1.

Table 1

Mean (±SEM) areal bone density (g.cm^2) and bone mineral content (g) in dog bone (n=8)

Site / Time	BMC			BMD		
	presurgery	30 days	60 days	presurgery	30 days	60 days
Tibia proximal opposed leg	2,33 ± 0.02	2,30 ± 0.02	2,34 ± 0.03	0.825 ± 0.028	0.828 ± 0.031	0.834 ± 0.033
Tibia proximal – donor site	2,29 ± 0.03	0,80 ± 0.08	1,98 ± 0.04	0.823 ± 0.031	0.411 ± 0.028	0.787 ± 0.021
Corticoperiosteal flap	-	0.23 ± 0.01	0.02 ± 0.01	-	0.163 ± 0.024	0.051 ± 0.028

The presence of the fluorochromes indicates the location, time and amount of bone deposition, and enhances the information contained in bone specimens (Penteado *et al*., 1990). The exam made with fluorescence microscope showed that the tetracycline was fixed on the growth areas of bone cells represented by green colour. Calcein is a fluorescent-imino-diacetic acid complex which fluorescens bright green

when combined with calcium. It is easily distinguished from tetracycline which fluoresces yellow. Alizarin Red indicates the calcium deposits except the oxalates, represented by red-orange colour. Both alizarin red and calcein were fixed in calcificated areas of bone tissue.

The exam of healthy bone tissue (fig. 2) reveal the calcification of the periosteal adjacent zone and the presence of growth bone activity adjacent to the calcification area, which correspond with width growing of the bone (Svindland *et al*., 1995, Takaaki *et al*., 2001). At the evaluation of the osseous tissue formed in the defect area (from where a corticoperiosteal fragment was detached) it was observed the presence of intense osseous growing activity inside the calcificated area (fig. 3), results which are similar with those reported by Takeuchi *et al*., 2010. The exam of the sample of corticoperiosteal fragment revealed a low growing bone activity and also a diffuse calcification of the examined tissue (fig. 4). Howship's lacunae are labeled with different colors. All these data confirm the imagistic observations and BMD and BMC determination, regarding the osteoresorbtion of the detached bone fragment.

The results obtained in this study contradict with the data from literature which mention that periosteal flaps that include a bone fragment and have vascular support can generate bone tissue at the implantation site in the human patients (Carlton, 1984, Kaminski *et al*., 2009, Sauerbier *et al*., 2007). In some experimental cases, the transplants were in contact with deficiency areas of bone substratum – maxilla, mandible, tibia, iliac spine. The contradiction is relative, our study investigating the heterotopic transplantation (muscular).

Other results that show osteogenic potential of ectopic transplanted periosteal flaps were reported by Vogelin *et al*., 2000 and 2002, in studies on rats that include the following conditions (factors) for optimal bone formation: osteoinductive bone morphogenetic protein (BMP), a biodegradable matrix, osteoprogenitor cells, and blood supply (Vogelin *et al*., 2000, Vogelin *et al*., 2002). The studies of Kusumoto *et al*., 1998, showed that ectopic osteoinduction occurred in the rat latissimus dorsi muscle flap and depended upon the dose of BMP, which suggests that osteogenesis is possible even in the absence of osteoprogenitor cells with the condition of BMP addition (Kusumoto *et al*., 1998).

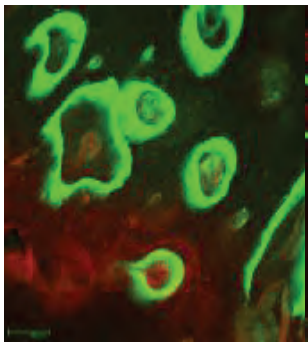


Fig.2. Fluorescence image bone tissue

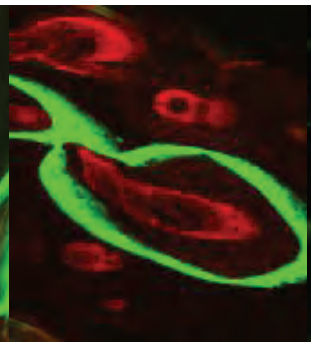


Fig.3. Fluorescence image osseous tissue from defect area

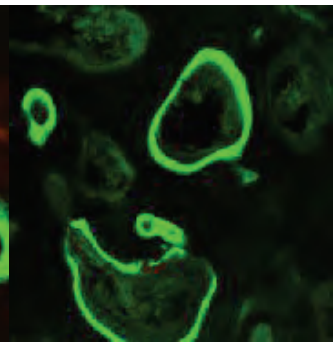


Fig.4. Fluorescence image healthy corticoperiosteal flap

Our study brings data to sustain the hypothesis that osteogenic capacity activation of the osteoprogenitor cells contained by corticoperiosteal flaps occurs only in the condition of direct informational signals existence from the contact with a structural bone defect or by addition of bone morphogenetic protein. This evolutional model represents the confirmation of osteogenic reaction incapacity of the free vascularized corticoperiosteal flaps in heterotopic transplantation in the dogs. It will be to explain away the contribution of flap to osteogenesis and the importance of mechanical stress of the transplanted flap into osseous defect.

3. CONCLUSIONS

3.1.In the dog, only the free vascularized corticoperiosteal flaps in heterotopic transplantation reveals osteogenic reaction incapacity.

3.2. The results reveal a significant correlation between BMD and BMC and the histomorphometric determination of bone mass.

3.3. Involvement of the periosteum in bone healing process in dogs shows distinct features from literature reviews in the rats and rabbits.

ACKNOWLEDGMENTS

This work was supported by CNCSIS – UEFISCSU, project number PNII – IDEI 130/2008

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

PRELIMINARY OBSERVATIONS ON THE DISTRIBUTION OF CANINE HAEMOPARASITES IN TIMIŞ COUNTY

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Key words: haemoparasites, distribution, dogs.

SUMMARY

In this study we have proposed ourselves to identify the haemoparasites of dogs from Timiş County. Venous blood was collected from 144 domestic animals of different sex, breed and age. In blood of dogs from Timiş County were identified four species of haemoparasites: *Dirofilaria immitis*, *D. repens*, *Babesia canis*, *B. gibsoni*. The prevalence of *Dirofilaria* spp. was 2.77% and *Babesia* spp. was identified in 9.02% from investigated samples. The sex, breed or age of dogs had no influence on distribution of haemoparasites. *Hepatozoon* spp., *Mycoplasma* spp. and *Ehrlichia* spp. were not identified.

Dirofilariosis is a disease caused by filarial worms of the genus *Dirofilaria* transmitted by mosquitoes. This genus consists of 27 valid species, and 15 species of questionable validity (Canestri Trotti *et al*., 1997). Definitive hosts are mammals, mainly primates and carnivores, and adult worms occur in subcutaneous tissues or in the heart.

Canine babesiosis is increasing in incidence and prevalence and is now a major problem in dogs. *Babesia* species are tick-transmitted apicomplexan parasites infesting a wide range of wild and domestic animal hosts (Cernăianu, 1958). Canine piroplasms belong to two distinct species, the large (4–5 μm) *Babesia canis* and the small (1–2.5 μm) *Babesia gibsoni* (Bashir *et al*., 2009).

Canine hepatozoonosis is a disease caused by protozoa of the genus *Hepatozoon*, transmitted by ticks. Since the first description, the occurrence of the parasite has been reported in several countries of the world. Two different species of *Hepatozoon* infect dogs, *H. canis* in Europe and South America, and *H. americanum* in the southern USA (Baneth *et al*., 2000).

The *Ehrlichiae* are a group of small, gram-negative, pleiomorphic, obligate intracellular cocci that infect different blood cells in various animal species and in humans. In dogs diseases are caused by bacteria in

the genus *Ehrlichia*, namely, Canine Monocytic Ehrlichiosis (caused mainly by *Ehrlichia canis*) and Canine Granulocytic Ehrlichiosis (caused by *Ehrlichia ewingii*).

Haemobartonellosis is a tick transmitted (and sometimes flea transmitted) disease that affects both dogs and cats, targets the red blood cells. Haemobartonellosis in dogs is caused by *Mycoplasma haemocanis*, formerly known as *Haemobartonella canis*.

The aim of the study was to investigate possible parasitism with *Dirofilaria* spp., *Babesia* spp., *Ehrlichia* spp., *Hepatozoon* spp., and *Mycoplasma haemocanis* in dogs from Timiş County for establishing of their prevalence.

1. MATHERIALS AND METHODS

The dogs were examined in the Parasitology and Parasitological diseases Department of the Faculty of Veterinary Medicine, Timisoara, Romania.

For identification of haemoparasites, venous blood was collected from 144 domestic dogs of different sexes (79 male and 65 female), breeds and age, from Timiş County. Blood samples were collected into 3 ml EDTA tubes from canine patients.

The blood was kept at room temperature and the samples were analyzed in 2 - 12 hours after prelevation.

The presence of microfilariae in the blood samples was assessed by modified Knott's technique (larvae were identified by morphological criteria) and *Fresh blood smear*, and adults by specific antigen test – Speed® DIRO / HEARTWORM (Bio Veto Test, France).

The identification of *Babesia* spp., *Hepatozoon* spp. and *Mycoplasma* (*Haemobartonella*) spp. was search by direct observation of the protozoa in blood smears stained with Diff-Quik® (Medion Diagnostics) method in accordance to the manufacturer instructions. The stained slides were examined with x100 objective.

H. canis gamonts can be diagnosed by microscopic detection of intracellular in stained blood smears. The gamonts are found in the cytoplasm of neutrophils or monocytes, have an ellipsoidal shape and are about 11 by 4 micrometers.

For detection of *Ehrlichia* Speed EHRLI test (BVT Virbac) – rapid detection of antibodies *Ehrlichia canis* in dog was used.

2. RESULTS AND DISCUSSIONS

All the dogs were examined for identification of a haemoparasotosis. Regarding dirofilariosis, circulating microfilariae were found in 4 of 144 dogs (Amstaf, German shepherd, Coker and mix - breaded) both by the Knott test and in direct examination blood drop. Prevalence values were 2.77% for *Dirofilariosis*. Patent infections were monospecific in four of four cases. Serological testing detected *D. immitis* antigen in three from four dogs (2.08%), one case was positive to *D. repens* (0.69%).

From 144 canine blood samples collected form Timiş County and analyzed using Diff-Quik method, 13 (9.02%) presented *Babesia* spp. parasites. At the microscopically examination of blood slides, in 11 dogs (7.63%) the infection with large *Babesia* species was observed. Large *Babesia* spp. appears blue colored, pear shaped, present in the centre of red cells. In colored slides from 5 dogs (3.47%) small *Babesia* spp. were observed as rod or coci shaped, small and blue colored.

Samples analyzed for detection of *Hepatozoon* spp., *Ehrlichia* spp. and *Mycoplasma haemocanis*., was negative, but this aspect not interrupted researches for identification etiological agents.

Evaluation of dirofilariosis prevalence by sex has yielded contradictory results. No significant differences between the sexes were reported in some researches (Song *et al*., 2003, Oge *et al*, 2003, Duran-Struuck and Hernandez, 2005). On the other hand some other researchers (Montoya *et al*., 1998, Selby *et al*, 1980) have reported significantly higher prevalence rates in males.

In our study, dirofilariosis prevalence was found equal in males and females. No breed, and age - related differences were observed, but *dirofilariosis* was identified in dogs of 2, 5 and 7 years old.

In Romania *D. immitis* was found in 35% of dogs tested (Olteanu, 1996), but we have seriously doubts concerning this high prevalence. Tudor *et al*. reported a prevalence value of 29.31% for *D. immitis* in Bucharest area. The infection prevalence was 55.82% in male dogs and 44.22% in female dogs (Tudor *et al*., 2008).

The presence of babesiosis in Romania was observed by other authors too (Amfim *et al*., 2008, Imre *et al*. 2009, Mircean and Mircean, 2006, Orăşanu *et al*., 2008).

Göthe and Wegerdt observed the presence of babesiosis in dogs that were in a hunting trip in our country (Göthe and Wegerdt, 1991).

Babesia canis infections were reported in Central and Eastern Europe countries. Canine babesiosis is increasing in incidence and

prevalence and is now a major problem in dogs. A study conducted in Lahore, Pakistan shown a prevalence of canine babesiosis of 2.62%. The male dogs were more prone to disease than female dogs (3.39 vs. 1.32%), whereas the incidence of disease was higher in younger dogs (6.9%) than older age groups. Crossbreds were more prone to the infection (10.9%) than purebreds (Bashir *et al*., 2009).

In Europe, most autochthonous infections with *B. canis* were detected in southern countries (Hornok *et al*., 2006, Fok Éva, 2005).

Hamel *et al*. have investigated 30 clinically healthy dogs from suburban areas of Tirana, Albania for *Babesia canis, Hepatozoon* spp., *Leishmania* spp., *Dirofilaria* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Rickettsia* spp. using direct and indirect methods. 63 % of dogs have shown antibody titers against *B. canis*, *E. canis* and/or *A. phagocytophilum.* On the other hand *Babesia c. canis*, *Babesia c. vogeli*, *Hepatozoon* spp., *D. immitis* and/or *E. canis* were identified in 43% dogs. There was no evidence for *Leishmania* spp., *Bartonella* spp. and *Rickettsia* spp. infections (Hamel *et al*., 2009).

3. CONCLUSIONS

1.1. In dogs from Timiş County were identified four species of haemoparasites: *Dirofilaria immitis*, *D. repens*, *Babesia canis*, *B. gibsoni.*

1.2. *Hepatozoon* spp., *Mycoplasma* spp. and *Ehrlichia* spp. were not identified.

1.3. The prevalence of *Dirofilaria* spp. was 2.77% and of *Babesia* spp. was 9.02% from samples investigated, respectively.

1.4. The sex, breed or age of dogs has not influenced the distribution of haemoparasites.

ACKNOWLEDGMENTS

This study was supported by the CNCSIS – UEFISCSU, ROMANIA, Grant TE_277 / 2010.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

PRION PROTEIN GENE POLYMORPHISMS IN SCRAPIE- AT TURCANA SHEEP

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Key words: scrapie ,genotype, resistance, selection intensity

SUMARY

The Turcana sheep is the first most important romaine breed. Reported here is the prion protein (PrP) haplotype frequency distribution for scrapie-related codons (136, 154 and 171) and a sequencing study of the complete PrP gene open reading frame for this breed. The most frequent PrP haplotype in both scrapie was ARQ, which was found at significantly higher frequency in scrapie-affected sheep. The susceptibility-associated VRQ haplotype was found at low frequencies in seven sheep. The resistance-associated ARR haplotype was found in all breeds.15 genotypes were defined, which are considered reliable in diagnosing the disease, genetic mutations affecting the sequence 136, 154, 171. It was identified ARR / ARR genotype, which determines resistance to scrapie. The research team collected samples from animals in the area to identify valuable genotypes in order to preserve the gene bank. The results of country analysis shows evidence Turdas 46 alanine (A) at codon 136 that confers resistance to scrapie prion structural changes. The presence of glutamine (Q) or histidine at codon 171 may send some characters of resistance to scrapie that was not detected in these samples.The analytical results from 56 samples the presence of glutamine (Q) in codon 171 of prion structural changes that confer resistance to scrapie prion. But classes G5-5 genotype 2 samples were detected with G4 genotype (ARR / VRQ) and 2 evidence-G5 (VRQ / ARQ), which are capable of prion disease. The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females. Any replacement females or breeding males should originate from flocks/herds not known to be affected with scrapie and under management practices precluding the introduction of scrapie or, in the case of sheep, should be of resistant PrP genotypes.This way it is posible to make the selection of individuals with the most valuable genotypes resistant to diseases such

Scrapie is a fatal, neurodegenerative disease that affects sheep and goats and belongs to the transmissible spongiform encephalopathies (TSEs) or prion diseases, which include bovine spongiform encephalopathy (BSE) in cattle and chronic wasting disease (CWD) in deer from the USA. Scrapie is a disorder characterized by the deposition of the prion protein (PrP^{sc};associated with TSE) in the central nervous system and lymphoreticular system. Characteristic clinical signs of the disease are behavioural disturbances, pruritus and increased difficulty in locomotion. Scrapie is the oldest known TSE. It was first recognized in sheep in Great Britain and other countries of Western Europe over 250

years ago. Scrapie has been reported in sheep worldwide, affecting many sheep-producing regions.Transmission of the scrapie agent is not completely understood, and apparently healthy sheep infected with the agent can transmit disease. Susceptibility to infection and incubation period in sheep has been shown to be affected by sheep genetics and breed. The long incubation period between exposure and clinical disease may allow animals to shed the agent for an extended period. The scrapie agent is thought to spread most commonly from the ewe to her offspring and other lambs through contact with the placenta and placental fluids, and sheep and goats are typically infected as young lambs or kids. Placental infectivity occurs in the incubation/preclinical stage of disease but is not constant with every pregnancy. Genetically susceptible lambs born to dams that develop clinical scrapie have a higher risk of developing the disease. Ram genetics will contribute to scrapie susceptibility in their offspring. Direct horizontal transmission likely accounts for scrapie cases in heavily infected flocks, where spread most likely occurs via an oral route. Infection also likely occurs via ocular exposure or contact with abraded skin or mucous membranes.Transmission to lambs through milk from infected ewes has been reported, as well as subsequent horizontal transmission among .Other infectious tissues have also been found, including: central nervous tissue, lymphoid tissue, peripheral nerves, blood, muscle, liver, nasal mucosa, and salivary glands.

For classical scrapie the codons at positions 136 and 171 in the gene that code for amino acids in the prion protein (PrP) have been associated with scrapie susceptibility. Codon 171 is thought to be the major determinant of susceptibility, with glutamine (Q) and histidine (H) conferring susceptibility and arginine (R) resistance. The effect of lysine (K) at codon 171 on scrapie susceptibility is unknown due to its infrequent occurrence. Codon 136 affects susceptibility to the less common valine-dependent classical scrapie, with alanine (A) and valine (V) conferring resistance and susceptibility, respectively.

All QQ sheep are susceptible to the more common valine-independent classical scrapie and can transmit the disease to susceptible flock mates. Conversely, ARR sheep are nearly completely resistant to this classical scrapie. These sheep are highly unlikely to carry or transmit scrapie. ARQ are rarely infected, and it is unknown whether infected ARQ sheep can transmit scrapie.

1. MATERIAL AND METHODS

In september 2010 were collected other samples, which revealed other valuable genotypes in the Orastie (Turdas) area. The original method Typi Fix - internationally patented from Prof.Brem - was first used by researchers in Romania CSCBA to discover DNA polymorphism and its applicability in the identification and traceability of meat species biodiversity. By this method one can follow the following aspects - determination of individual identity of animals in animal cells and finished products, control of paternal or maternal origin, detection of genes that influence the quality of raw material (meat and milk in our case), analysis determination of genetic variability, etc

The TypiFix™ ear tag system is a combination of a conventional ear tag with a simultaneous tissue sampling technology. By ear tagging the farm animals, the tissue samples are automatically collected and sealed in the TypiFix™ sample containers, where the tissue samples are preserved at ambient temperature and can be used for protein or DNA based assays. The easy handling of the TypiFix™ ear tag system allows economic sampling of whole populations and is therefore an effective tool for analysis of genetic markers for paternity control, traceability and breeding traits. The Typi-Fix®-System is a procedure for the collection of DNA containing tissue samples avoiding all these hurdles and problems.

DNA analyses of resistance/susceptibility genes for Scrapie in Turdas (HD) (partially results)

				Results				
				AA Codon1				Genotype-
No.	Animal ID	Breed	Owner	136	154	171	PrP Genotype[2]	class[3]
1	1090449204	sheep	Ferma Turdas	AA	RR	RQ	ARR/ARQ	G2
2	1090449202	sheep	Ferma Turdas	AA	RR	RQ	ARR/ARQ	G2
3	1101791366	sheep	Ferma Turdas	AA	RH	QQ	ARQ/AHQ	G3
4	1061894750	sheep	Ferma Turdas	AA	RR	QQ	ARQ/ARQ	G3
5	1090449205	sheep	Ferma Turdas	AA	RR	RQ	ARR/ARQ	G2
6	1101791385	sheep	Ferma Turdas	AA	RR	QQ	ARQ/ARQ	G3
7	1101791384	sheep	Ferma Turdas	VA	RR	QQ	VRQ/ARQ	G5

8	1046068062	Sheep	Ferma Turdas	AA	RR	QQ	ARQ/ARQ	G3
9	1075859507	Sheep	Ferma Turdas	AA	RR	RQ	ARR/ARQ	G2
10	1101791368	Sheep	Ferma Turdas	AA	RH	RQ	ARR/AHQ	G2
11	1101791381	Sheep	Ferma Turdas	AA	RR	RQ	ARR/ARQ	G2
12	1101791382	Sheep	Ferma Turdas	AA	RR	RH	ARR/ARH	G2
13	1101791383	Sheep	Ferma Turdas	AA	RR	RQ	ARR/ARQ	G2
14	1101791390	Sheep	Ferma Turdas	AA	RR	QQ	ARQ/ARQ	G3

2. RESULTS AND DISCUSION

Natural scrapie and bovine spongiform encephalopathy (BSE) infections have essentially not been diagnosed in sheep homozygous for the $A^{136}R^{154}R^{171}$ haplotype of the prion protein. This genotype was therefore assumed to confer resistance to BSE and classic scrapie under natural exposure conditions.

The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. All the probes in Turdas (Orastie) have in the 136 codon the alanine. In results of analysis in Turdas (Orastie) 82,14% the probes present the alanine (A) at codon 136 of the prion protein who confers resistance to the structural change of prion scrapie. The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep. In results of analysis in Turdas-(Orastie) 90 % the probes present the glutamine (Q) at codon 171 of the prion protein who confers resistance to the structural change of prion scrapie.

The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females. Any replacement females or breeding males should originate from flocks/herds not known to be affected with scrapie and under management practices precluding the introduction of scrapie or, in the case of sheep, should be of resistant PrP genotypes. Susceptible ewes of unknown or questionable disease status should be bred to RR rams or separated from the rest of the flock prior to and following lambing until there is no vaginal discharge to minimize spread to other animals. Another method used by some producers is selective breeding to reduce overall flock susceptibility based on PrP genotype. This method consists of breeding only with rams that are RR or QR.

3. CONCLUSION

3.1.It was analysis the prion protein for scrapie resistance genotyping *as codon*- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype. The TypiFix™ ear tag system is simple, one-step collection and preservation of tissue samples. The TypiFix™ ear tag system is fast, fully-automated and economical preparation of DNA. This method is to be performed much more quickly and economically than is currently possible with the traditional methods of sample preparation.

3.2. In results of analysis in Turdas 82 % the probes present the alanine (A) at codon 136 of the prion protein who confers resistance to the structural change of prion scrapie. The analytical results from 56 samples the presence of glutamine (Q) in codon 171 of prion structural changes that confer resistance to scrapie prion. But classes G5-5 genotype 2 samples were detected with G4 genotype (ARR / VRQ) and 2 evidence-G5 (VRQ / ARQ), which are capable of prion disease.

3.3. The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females It is important to breeding only with rams that are RR or QR.

Acknowledgments. This work was cofinanced from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 ”Postdoctoral school for zootechnical biodiversity and food biotehnology based on the eco-economy and the bio-economy required by eco-san-genesys”

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

IDENTIFICATION OF GENOTYPES VALUABLE RESISTANT IN SCRAPIE BY GENOTYPING METHODS

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Key words: scrapie, ,genotype, resistance, selection intensity

SUMARY

The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females. Any replacement females or breeding males should originate from flocks/herds not known to be affected with scrapie and under management practices precluding the introduction of scrapie or, in the case of sheep, should be of resistant PrP genotypes. Susceptible ewes of unknown or questionable disease status should be bred to RR rams or separated from the rest of the flock prior to and following lambing until there is no vaginal discharge to minimize spread to other animals. Another method used by some producers is selective breeding to reduce overall flock susceptibility based on PrP genotype. This method consists of breeding only with rams that are RR or QR..In our study and research, the best genotype class G1 (ARR/ARR) and G2 (ARR/ARH) it was display in the samples of Totesti in percentage of 80%.

Infection with the scrapie agent is determined by the detection of the abnormal prion protein accumulation in nervous tissue and/or lymphoreticular tissues and/or histopathologic lesions in central nervous tissue in susceptible species. The characteristic histopathologic change of nervous tissue is vacuolation of neurons, producing a distinctive appearance of spongiform change. The vacuolar changes may be accompanied by other microscopic features, such as neuronal degeneration, neuronal loss, gliosis, and cerebrovascular amyloidosis. Typically, the histopathologic lesions have bilaterally symmetrical distribution, although the distribution pattern and changes may vary between type of agent and host genetics.

The use of selective breeding and culling to increase genetic resistance to scrapie infection raises concern regarding the practices effect on the genetic diversity of the domestic sheep population and on production traits. A number of studies have been completed evaluating effect of PrP genotype selection and production traits with some studies providing limited evidence of associations between PrP genotype and traits.Overall, when observed, associations between PrP genotype and performance traits tended to be neither strong nor consistent across populations, and there was no tendency for associations between scrapie-

resistant PrP alleles and performance traits to be adverse (Dawson et al. 2008; Sweeney and Hanrahan 2008). A study did find that producer perception of animal quality was not influenced by animal susceptibility to scrapie (as determined by PrP genotype).

The rationale for conducting surveillance and scrapie eradication are as follows:

- economic impact: Scrapie is a non-febrile and insidious disease. Infected flocks with a high percentage of susceptible animals can experience significant production losses. Over several years, the number of infected animals in a flock increases and onset of clinical signs occurs in younger animals, making these flocks economically inviable. Female animals sold from infected flocks can spread scrapie to other flocks.

- potential public health : The apparent transmission of bovine spongiform encephalopathy (BSE), another TSE, to humans in the United Kingdom has resulted in a call for the eradication of all TSEs in food-producing animals. Recent research has demonstrated that BSE could be successfully transmitted to sheep and goats orally, and that sheep genotypes traditionally resistant to scrapie were susceptible to BSE. This has resulted in increased public concern. There is no scientific evidence to indicate that scrapie poses a risk to human health or that scrapie of sheep and goats is transmitted to humans. Because BSE in sheep is detected using the same tests as scrapie, however, early detection and eradication of scrapie would protect human health from the theoretical risk of BSE in sheep, should it occur, by eliminating the risk of BSE being masked by scrapie.

1. MATERIALS AND METHODS

For prelevation the samples we use the new method *TypiFix™ ear tag system* which is a combination of a conventional ear tag with a simultaneous tissue sampling technology. The easy handling of the TypiFix™ ear tag system allows economic sampling of whole populations and is therefore an effective tool for analysis of genetic markers for traceability and breeding traits. The Typi-Fix®-System is a procedure for the collection of DNA containing tissue samples avoiding all these hurdles and problems. *DNA purification with DNA FIX* columns an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix™ samples. The sorbents retain protein and other contaminants, while the DNA passes the column in the exclusion volume. DNA isolation and purification can be automated through the use of a pipetting robot and a

special one-step procedure (Nexttec technology). PCR reactions with these samples can also be prepared automatically. The results of the multiplex PCR analyses are linked with the scanned identification number and saved in the animal data bank. This aspect is very important for studying traceability and domestic animal biodiversity. *Gel electrophoresis of NCC™ purified DNA from 88 TypiFix™ eartag samples* : 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/ EtBr gel. The DNA concentration is about 10 ng/µl or greater = negative control. These higly-performant analyses are conducted at the well-known AGROBIOGEN laboratories in Germany, by Prof. Brem and his team, with their patented methods.

2. RESULTS AND DISCUSSION

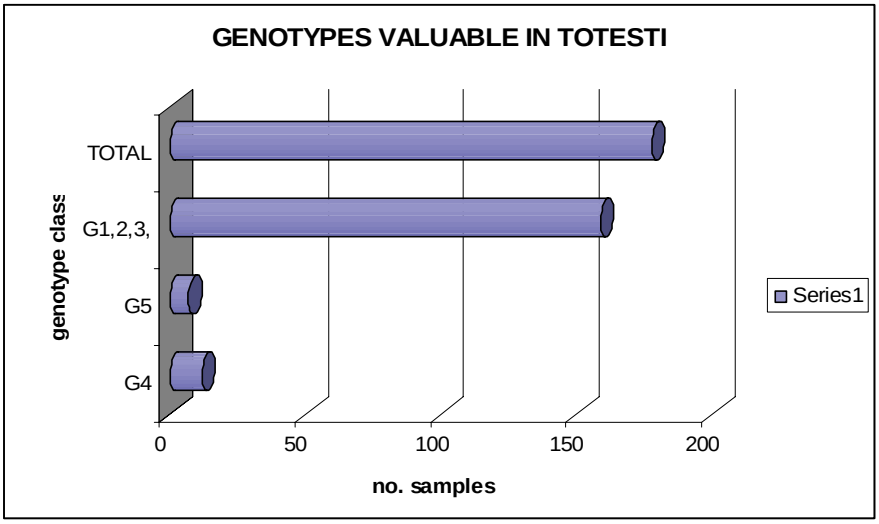
The analytical results of samples taken from the project area 41 samples had arginine (R) at codon 171 of prion protein, which confers resistance to structural changes of scrapie. Also present alanine (A) at codon 136 confers resistance to structural changes associated with scrapie. The results of country analysis shows evidence Hateg 102 alanine (A) at codon 136 that confers resistance to scrapie prion structural changes. The presence of glutamine (Q) or histidine at codon 171 may send some characters of resistance to scrapie that was not detected in these samples.The analytical results from 90 samples the presence of glutamine (Q) in codon 171 of prion structural changes that confer resistance to scrapie prion. But classes G5-5 genotype 4 samples were detected with G4 genotype (ARR / VRQ) and 5 evidence-G5 (VRQ / ARQ), which are capable of prion disease. Owners were notified and they took the decision to isolate those animals and not use them for breeding.This way it is posible to make the selection of individuals with the most valuable genotypes resistant to diseases such. In period 2009-2010 were collected other samples, which revealed other valuable genotypes in the Hateg county areea.

Tab 1

DNA analyses of resistance/susceptibility genes for Scrapie in Totesti (partially results)

			Results				
			AA Codon[1]				Genotype-
No.	Animal ID	Owner	136	154	171	PrP Genotype[2]	class[3]
1	1082661857/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
2	1082661701/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
3	1082661684/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
4	1082661622/F	Ferma Totesti,	AA	RR	RQ	ARR/ARQ	G2
5	1082661728/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
6	1082661726/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
7	1084191429/F	Ferma Totesti,	VA	RR	QQ	VRQ/ARQ	G5
8	1090445880/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
9	1082661742/F	Ferma Totesti,	VA	RR	RQ	ARR/VRQ	G4
10	1082661640/F	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3
11	1084191442/F	Ferma Totesti,	AA	RR	RQ	ARR/ARQ	G2
12	1084191401/M	Ferma Totesti,	AA	RR	RR	ARR/ARR	G1
13	1084191464/M	Ferma Totesti,	AA	RR	RQ	ARR/ARQ	G2
14	1084191417/F	Ferma Totesti,	AA	RR	RQ	ARR/ARQ	G2
15	1090445934/M	Ferma Totesti,	AA	RR	QQ	ARQ/ARQ	G3

The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. All the probes have in the 136 codon the alanine. The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep.The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep.But for genotype class (genotype classifications by the German Society of Animal breeding-DGfZ in Totesti we detected 12 probes with genotype G4 (ARR/VRQ) and 7 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease.The parmers it was notifie and the farmers took the decision to isolate those animals and not use them for breeding.



3. CONCLUSION

3.1.It was analysis the prion protein for scrapie resistance genotyping *as codon*- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype. The TypiFix™ ear tag system is simple, one-step collection and preservation of tissue samples.The TypiFix™ ear tag system is fast, fully-automated and economical preparation of DNA.

3.2. In Totesti we detected 12 probes with genotype G4 (ARR/VRQ) and 7 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease.The parmers it was notifie and the farmers took the decision to isolate those animals and not use them for breeding.

3.3. The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females. Any replacement females or breeding males should originate from flocks/herds not known to be affected with scrapie and under management practices precluding the introduction of scrapie or, in the case of sheep, should be of resistant PrP genotypes.

Acknowledgments. This work was cofinanced from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 ”Postdoctoral school for zootechnical

biodiversity and food biotehnology based on the eco-economy and the bio-economy required by eco-san-genesys”

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

MOLECULAR CHARACTERIZATION OF HUMAN *CRYPTOSPORIDIUM* ISOLATES IN BANAT REGION, ROMANIA

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Key words: *Cryptosporidium*, PCR-RFLP, human, Banat region.

SUMMARY

The aim of the present study was the genetic characterization of *Cryptosporidium* isolates, from humans with diarrhea attending to different hospitals located in Banat region. A total of 78 fecal samples were examined by modified Ziehl-Neelsen staining method. Five microscopically positive samples were investigated by PCR-RFLP of the SSU rRNA gene. The species and/or genotypes were determined using restriction endonuclease enzyme digestion with S*sp*I and V*sp*I. The results indicated the presence of *Cryptosporidium parvum* in three samples and *Cryptosporidium* cervine genotype in another two samples. These data suggest the animal origin of this zoonotic species and genotype.

This is the first study of molecular epidemiology in human cryptosporidiosis that has been made in Romania.

Members of the genus *Cryptosporidium* are parasites with large host specificity, infecting many vertebrate species (mammals, reptiles, birds and fishes), including humans.

Infections with *Cryptosporidium* species has been documented in both immunocompromised and immunocompetent patients, worldwide.

Epidemiological studies from humans, based on molecular genetics tools, have reported the identification of eight species (*C. parvum*, *C. hominis*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, *C. suis*, *C. andersoni*) and three genotypes (cervine genotype, chipmunk genotype, monkey genotipe) (Ong et *al*., 2002; Blackburn et *al*., 2006; Feltus et *al*., 2006; Leoni et *al*., 2006; Soba et *al*., 2006; Trotz-Williams et *al*., 2006; Xiao and Ryan, 2008; Imre, 2010; Dărăbuş and Imre, 2010). Usually, the first two enumerated species of *Cryptosporidium* are the most common species infecting humans.

Worldwide, the most frequently used gene, for *Cryptosporidium* species and genotype identification, has been the SSU-rRNA (18S) gene.

In Romania, the reports regarding the molecular analysis of *Cryptosporidium* isolated from humans are absent.

The primary objective of the present study was to determine the *Cryptosporidium* species/genotypes involved in human diarrhea cases and their epidemiological significance for humans in Banat region, Romania.

1. MATERIAL AND METHODS

Fecal samples from 78 patients (52 children's and 26 adults) with diarrhea were collected from different hospitals (Table 1), located in Banat region. The samples were stored in sterile plastic bottles at 4°C in 2.5% potassium dichromate until further processed.

All the stool samples were previously processed for the *Cryptosporidium* oocysts identification. The samples were microscopically examined with Ziehl-Neelsen modified (mZN) staining method. The microscopically positive samples were selected for PCR-RFLP analyses of the small subunit rRNA gene (18S) (Alves et al. 2001).

DNA was extracted from fecal samples using the QIAamp® DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

The nested polymerase chain reaction protocol was performed in two steps (Xiao et al. 2001) and the reactions were prepared in 25 µl volumes. For the primary PCR reaction (about ~1325 bp), the PCR master mix was prepared by mixing: 10X *Taq* Buffer $(NH_4)_2SO_4$, dNTP (2.5 mM), $MgCl_2$ (25 mM), *Taq* DNA polymerase (5U/µl), genomic DNA (7-95 ng/µl), forward and reverse primers, and nuclease free water. After initial complete denaturation at 94°C for 3 min the reaction was performed for 35 cycles (denaturation at 94°C for 45", annealing at 55°C for 45" and strand extension at 72°C for 1 min) followed by final extension at 72°C for 7 min (Xiao and Ryan, 2008). The conditions for the secondary/nested PCR reaction (expected amplicon size ~ 840 bp) were the same as for the primary PCR, except the use of a different pair of primers and the annealing at 58°C (Xiao and Ryan, 2008). The specific primers used were SSU-F2 forward and SSU-R2 reverse for primary PCR and SSU-F3 forward and SSU-R4 reverse for secondary PCR (Xiao and Ryan, 2008, Xiao et *al*. 1999).

Amplification products were separated using horizontal gel electrophoresis on a 1.5% agarose multi-porpose gel containing ethidium bromide and using the 100-bp ladder as the control. Gel images were visualized under UV light and were captured using a gel documentation system.

Restriction Fragment Length Polymorphism Analyses (RFLP) of SSU rRNA (18S) gene PCR products was performed according to Xiao et al. (1999).

For detection and differentiation of *Cryptosporidium* species and genotypes 5 µl of the secondary nested PCR product was subjected to restriction digestion with V*sp*I and S*sp*I enzymes. The reaction mixture contained: amplicon DNA, nuclease free water, VspI/SspI enzyme (12U/µl) and their respective buffers. All restriction digestions were carried out 37°C for 2 h in a humid chamber.

RFLP products were analyzed on 1.5% agarose gel and visualized after ethidium bromide staining.

2. RESULTS AND DISCUSSIONS

Of the 78 human origin fecal samples, five (6,41%) were positive for *Cryptosporidium* oocysts. With modified Ziehl-Neelsen staining (mZN), the oocysts are clearly marked ovoid or round shaped, colored in red on a green ground.

Based on analysis of the SSU rRNA gene the nested PCR products were observed at 850 bp region. This specific amplification confirms the presence of *Cryptosporidium* spp. infection (fig. 1).

The RFLP analysis with S*sp*I revealed bands at 454 and 274 bp for three samples, and 465 and 395 bp for two samples (fig. 2).

The RFLP analysis with V*sp*I, for specie diagnosis, revealed bands at 624 and 111 bp for three samples which clearly indicated that the isolates were *C. parvum* (*C. hominis* would have given bands at 561 and 115 bp). For two samples, processed RFLP with V*sp*I enzyme, the revealed band was at 460 and 172 bp. This fragment sizes confirm the presence of *Cryptosporidium* cervine genotype (fig. 3).

More human cases have been associated with the *Cryptosporidium* cervine genotype, which has been reported in one patient in Slovenia, three patients in the United States, ten patients in Canada, and one in England (Ong et *al*., 2002; Blackburn et *al*., 2006; Feltus et *al*., 2006; Leoni et *al*., 2006; Soba et *al*., 2006; Trotz-Williams et *al*., 2006; Xiao and Ryan, 2008).

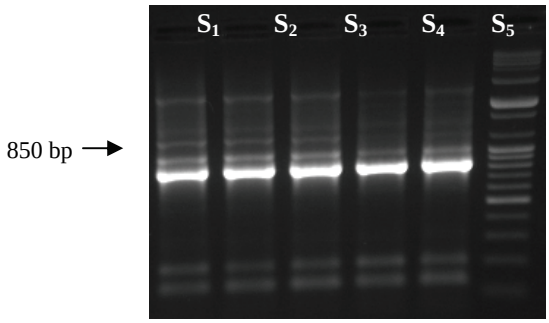

Fig. 1 Nested PCR diagnosis for genus *Cryptosporidium* (850 bp); S_{1-5}- processed samples; M –molecular marker

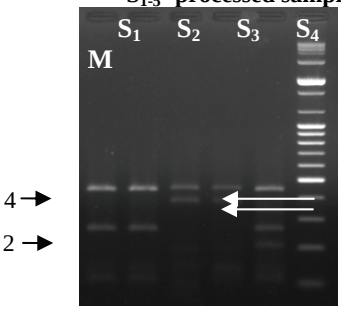

Fig. 2 Digestion fragments from SSU-rRNA (18S) gene with S*sp*I enzime; M - molecular marker; $S_{1, 2, 5}$ – characteristic migration bands for *C. parvum* S_3, $_4$ - characteristic migration bands for *C.* cervine genotype

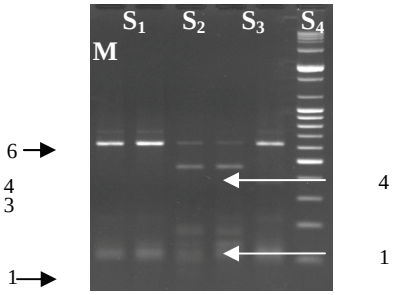

Fig. 3 Digestion fragments from SSU-rRNA (18S) gene with V*sp*I enzyme; $S_{1, 2, 5}$ – characteristic migration bands for *C. parvum*; S_3, $_4$ - characteristic migration bands for *C.* cervine genotype

Table 1

***Cryptosporidium* isolates and PCR-RFLP analysis at the SSU rRNA (18S) locus from humans in Banat region**

Sample origin from humans	Total no. of isolates studied	No. of isolates positive for:		Negatives
		Cryptosporidium parvum	*Cryptosporidium* cervine genotype	
"Louis Țurcanu" Children Hospital	19	1	2	16
"Victor Babeș" Hospital, from Timișoara	28	2	-	26
City Hospital from Reșița	25	-	-	25

Most species and genotypes of *Cryptosporidium* infect a limited range of animals, and when the host range or infectivity includes humans, the parasite acquires public health significance.

The predominance of *C. parvum* in humans may be due to high prevalence of bovine cryptosporidiosis in this zone (Imre, 2010; Dărăbuş and Imre, 2010).

C. parvum, the most spread specie, was reported in many countries worldwide (Xiao and Ryan, 2008; Xiao, 2010).

The increasing member of humans infected with the cervine genotype might be related to its wide range of mammalian hosts.

This is the first study of molecular epidemiology in human cryptosporidiosis that has been made in Romania.

3. CONCLUSIONS

3.1. Using molecular analysis of SSU-rRNA (18S) gene for *Cryptosporidium* oochist isolates from five human patients from Banat area, a parasitism with *Cryptosporidium parvum* was identified in three persons and *Cryptosporidium* cervine genotype was identified in two persons.

3.2. Parasitism with *C. parvum*, in humans, demonstrates the presence of a zoonotic specie, having as possible source of infection the ruminants.

3.3. The presence of *Cryptosporidium* cervine genotype was reported for the first time in Romania.

4. ACKNOWLEDGMENTS

The current research was based on grant 51-034/2007 PN II - Parteneriate) obtained by Prof. Dărăbuş from CNMP. Acknowledgments to Cristina Popescu, Molecular Genetics Laboratory CEEX II USAMVB Timişoara, for help in the interpretations of results.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

CHECK METHODS OF EXPIRY DATE OF MEAT PREPARATIONS AND IMPORTANCE THEREOF

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Keywords: expiry date, meat preparations, food-borne infections

SUMMARY:

Purpose of the paper – This paper fits the scope of current concerns in the food safety field and brings to the fore the compliance methods of meat preparations from a sensory and physicochemical point of view with expiry dates and the necessity to check expiry dates with a view to marketing quality products that are safe for consumption for the purpose of protecting the consumers.
Working methods – The paper presents the determination methods of expiry dates of meat preparations, for which organoleptic and physicochemical tests are carried out in order to assess the freshness of meat products by determining the level of easily hydrolysable nitrogen and the stage of fat oxidation (Kreis test). In event of occurrence of organoleptic and physicochemical changes of parameters, the assessment of the healthiness of the product follows, carried out by microbiological determinations of microorganisms incriminated for food-borne infections of interest for the meat industry and preparations.
Results – In order to observe the organoleptic features within and after the expiry term, analyses were carried out on categories of meat preparations for a certain period of time, while for the check of the expiry and assessment of freshness a number of samples were collected starting with January 2009 onwards and sent to the laboratory and subsequently conclusions were inferred.
Conclusions – food preparation production for consumption must mainly consider the protection of life and health of population, the meeting of the consumption necessities of all consumer categories and the compliance with the conditions imposed by legislation regarding labelling and supply of all necessary information with respect to the keeping, handling and consumption.

It is imperative that the food preparation production for consumption develops under the current law and takes mainly into consideration the protection of life and health of the population, the meeting of the consumption necessities of all consumer categories and the conformation to hygiene and environmental conditions with respect to the fabrication, handling, storage and transport of products.

The date of minimum durability represents the time frame set by the manufacturer during which the product should be consumed and during which it should maintain its quality and safety properties up to the expiry date if the criteria of transport, handling, storage, keeping, use and consumption had been properly complied with. With respect of the food products, this time frame refers to the deadline of consumption which means that the availability period of the product ends at a certain

date previously set by the manufacturing company and listed upon the technical specification and the label attached to the product. This type of guarantee expressed by the expiry date is specific to perishable foodstuffs or to those foodstuffs that could become a danger for consumer's health shortly after the expiry date.

The determination of expiry dates of meat preparations is carried out by each and one of the manufacturers on their own responsibility or through collaboration with research units and specialised laboratories according to the Government Order Number 42 from 1995 regarding the food preparation production for consumption republished 2008, on the basis of scientific laboratory' determinations, analyses and tests on the product and following a standard operating procedure established within national programmes, depending on the type of the product, the way in which each product mix is stored (by curing, smoking, chilling or heat treatment), depending on the input materials, but also on the type of the membrane (natural or artificial). Another important factor which contributes to the product durability is the way of packaging meaning that the bulk products have a shorter time limit than the ones which are vacuum packed or under controlled atmosphere.

The expiry date for each product, along with the temperature and the air humidity in which the product should be stored, delivered and marketed are listed upon the technical specification. The possible restrictions to one of the existent ingredients are also listed upon the technical specification.

On the label attached to the product or on the product packages is listed the date of minimum durability, the date up to which the products should maintain their specific characteristics under proper storage conditions and that would be preceded by the item "Best before…" followed by the day, month and year. The respective items are replenished with the indication of the keeping and storage conditions that should be followed throughout the whole availability period. (Government Resolution Number 106/2002 amended on 2009 regarding foodstuffs' labelling).

With respect of the foodstuffs that are highly perishable and are expected to become an immediate danger to consumer's health, the date of minimum durability is replaced with the deadline of consumption. The date of the manufacturer is therefore preceded by the mention "use before...", followed by the day, month and year, in this order, mention to which the indication of the storage conditions that should be kept is added. The information regarding the way in which the foodstuffs should be stored must be pointed out in an easy-to-see manner.

On the label attached to meat preparations, the batch code is also listed. It is useful in establishing the product's traceability as in the identification when needed of the technological layout of the respective product.

This paper fits the scope of current concerns in the food safety field and brings to the fore the compliance methods of meat preparations from a sensory and physicochemical point of view with expiry dates and the necessity to check expiry dates with a view to marketing quality products that are safe for consumption for the purpose of protecting the consumers.

The objectives of the research work were as they follow:

- the determination of expiry dates of meat preparations;
- methods of testing the expiry date by assessing the freshness and the healthiness of meat products;
- the observation of the organoleptic and physicochemical features within and after the expiry term of meat preparations within a meat processor.

1. MATERIAL AND METHOD

1. The method of testing the expiry date for new products.

For the new products – the expiry date is determined by laboratory studies for the assessment of freshness after the products have been kept under various conditions that simulate situations with which the product meets between the time the production is completed and the time it reaches the consumer. Both ideal, as well as unfavourable conditions are simulated – such as temperature and humidity fluctuations.

Laboratory tests are carried out on samples by determining organoleptic features (aspect, colour, taste, smell, consistency) and the physicochemical parameters (the interest ones) at the beginning and the end of tests of each type of product, in order to determine the degradation conditions stage in various stages and are compared to standards admitted by law. The expiry term begins upon completing the production process and it is established not according to the ideal conditions, bur rather according to the real conditions that the product meets until consumption, so that if the product is consumed by mistake immediately after the expiry date, there is no risk of food-borne infection.

Before setting the expiry date for a product that is going to be marketed, specialists analyse the factors that may influence the validity of the product (thermal treatment, raw material, packaging, additives,

etc). If the product concerned has appropriate results at the end of the expiry date, both from a sensorial point of view, as well as from a physicochemical point of view, the same is marketed as new product.

2. Check method of expiry date of already existing products

In order to check the expiry dates organoleptic analyses are carried out (aspect, taste, smell), as well as physicochemical analyses at the beginning and the end of the expiry period in order to assess the freshness of the meat preparations.

- Organoleptic examination

On a monthly basis The quality control department keeps counter samples until close to the expiry date or even after the expiry date; they are checked from an organoleptic point of view by a commission specially appointed by the company management.

The sensory features of meat preparations differ according to: production recipe, form of presentation and intended use of the products. The sensory analysis involves the investigation of the following features: exterior aspect and sectional aspect, form and size, colour on the surface and sectional colour, section aspect, smell and taste, consistency. Therefore the aim is that the products subject to sensory analysis do not show organoleptic changes compared to the ones established in the technical specifications of the product and to preserve their organoleptic quality over the entire expiry period.

Following the organoleptic check, a product assessment report is drawn up, where the products checked against the corresponding lots are registered, as well as the corresponding expiry date and the findings for each product. Any sensory change experienced by the product during the entire expiry period shall be clarified by physicochemical determinations and possibly microbiological determinations and involve a revaluation of the set expiry date by the specialists.

- Physicochemical examination

The assessment of meat preparation freshness aims at identifying and at determining the degradation products resulted. In order to perform the physicochemical analysis, a product of each group of products is sent to the laboratory close to the final expiry date with a view to checking the keeping of the quality features until expiry of the validity. Random samples are collected so that during one year all groups of products are checked. The analyses requested for expiry date check are the easily hydrolysable nitrogen and the Kreis Test.

The measured qualitative parameters must comply with the values in the technical specifications of the product drawn up according to the legislation in force.

DETERMINATION AND LEGISLATIVE FRAMEWORK

a. Determinations for freshness

1. Determination of easily hydrolysable nitrogen

The easily hydrolysable nitrogen and the ammonia in meat and meat preparations are determined with the help of the standard SR 9065-7/2007.

The determination of the easily hydrolysable nitrogen and of ammonia is carried out by:

- qualitative method with Nessler's reagent for ammonia identification
- quantitative method by direct titration with chlorhydric acid
- quantitative method by indirect titration with sodium hydroxide.

Principle of determination method of easily hydrolysable nitrogen by the qualitative method with Nessler's reagent. The nitrogen in the ammonia groups is released by weak base hydrolysis and together with the free ammonia is engaged by water vapour distillation in an acid solution, which is known from a quantitative and qualitative point of view. The excess of acid is determined by titration with an equivalent alkaline solution.

Interpretation:

The values for the easily hydrolysable nitrogen parameter expressed in mg/100 g are presented in the Order number 210/2006 on the approval of Rules regarding the marketing of meat products and must comply with the limit of 30 mg/100 g for fresh products (boiled meat products), 45 mg/100 g for smoked, boiled and smoked, boiled and double-smoked, 200 mg/100 g for durable (raw-dry) meat products.

2. Kreis test

Determination of fat oxidation stage is carried out with the help of the standard SR 9065-10/2007 and it allows for the assessment of the fat oxidation assessment by the intensity of colours that the treated fat indicates with a specific reagent.

Method principle. Fat is extracted from the analysed product and treated with cu fluoroglucine in the presence of chlorhydric acid.

Interpretation:

- The reaction is deemed to be negative when the fluid stays colourless.
- The reaction is deemed to be weakly positive in the case of appearance of pink coloration of various intensities
- The reaction is deemed to be in the case of appearance of red coloration with violet shades.

Assessment for the determination of the fat oxidation stage (Kreis test) is presented in the Order number 210/2006 for the approval of Rules regarding meat product marketing an it must be negative.

b. Determinations for healthiness

In event of occurrence of organoleptic and physicochemical changes of the parameters presented, the assessment of healthiness follows. Alteration is the set of changes that meat undergoes under the action of harmful microorganisms (bacteria, yeasts, moulds), especially under the action of aerobic and anaerobic putrefaction bacteria, which decompose proteins and consequently meat changes its sensory features (aspect, colour, consistency, smell) in a negative way. The first changes that mark the alteration of meat reside in the appearance of volatile chemical substances (NH3, H2S, mercaptans), simultaneously with the change of meat colour (it becomes bright red and then grey red, green red), consistency (it becomes very soft), smell (it becomes acid and then altered, putrid). The most advances stage of alteration is putrefaction, characterised by advanced decomposition of meat chemical compounds, forming indole, scatol, toxic nitrogenous bases, with fetid emanations.

In event the product indicate the beginning of alteration, changes in the normal organoleptic features begin being noticed. Thus, the consistency of products decreases, the mass becomes loosened due to gases that are formed by decomposition of protein substances, while taste and smell become unpleasant. In addition, the covering looses adherence to composition.

Incriminated microorganisms in food-borne infections are Campylobacter spp., Clostridium botulinum, Clostridium perfringens, Escherichia coli (E. coli 0157:H7 enterohemoragic), Listeria monocytogenes, Salmonella spp, Staphylococcus aureus, Yersinia enterocolitica, but of a specific interest for meat preparations is the test for Salmonella, Escherichia coli and Listeria monocytogenes pursuant to the Commission Regulation (CE) number 1441/2007 of 5 December 2007, amending the Regulation (CE) number 2073/2005 regarding the

microbiological criteria for food products, which establishes the microbiological criteria for food products and microbiological criteria for certain pathogenic microorganisms and metabolism products, and the application rules that the operators in the food sector must comply with when they apply general and special hygiene measures, as well as the obligations incumbent upon operators regarding the monitoring and actions necessary in consequence of the results.

The method for the determination of the presence of Salmonella-type bacteria is presented in the standard SR EN ISO 6579, the Identification of Escherichia coli bacteria with the help of the standard ISO 7251/2005, while the Listeria monocytogenes bacteria are detected by the method described in SR EN ISO 11290-1/2000.

2. RESULTS AND DISCUSSIONS

Food products are under the impact of environmental agents, and their features are constantly changing, until finally reaching the stage where they are inedible, when the extent of negative changes renders the products inappropriate for consumption and they must be excluded from the food circuit. Nowadays, when the variety of food products is so extended, quality guarantee is even more important as it represents a competitive factor worth of consideration. The producer directly guarantees the quality over the entire expiry term and during such term the product is going to be packaged, transported, handled and kept according to regulations, in order to preserve its original quality unaltered. Assumption of guarantee entails a high risk since any non-conformity not detected in time may compromise production, quality and reputation of the company.

The products analysed were produced within a meat processing establishment producing and marketing a varied range of meat products and having two flows, one for Specialities, Hams and Rolls and the second one for Salamis, Sausages, Hot-dogs, Bolognas and Hams in casing. Products are divided on groups of products according to the thermal treatment applied and they may be: boiled meat products, smoked meat products, smoked and boiled meat products, boiled and double smoked meat products. Technical specifications of products are organised from S.T. 1- S.T. 11 according to the product group they belong to and to the thermal treatment applied.

Table no.1

Products under study for organoleptic check

No.	Name of product	Product expiry period (days)	Lot	Date of production	Expiry	Date of analysis	Result of analysis	Number of days until/from expiry
	House salami	21	17	17.06.2009	08.07.2009	08.07.2009	conform	0
	Torpedo salami	21	16	16.06.2009	07.07.2009	08.07.2009	conform	+1
	Praga Extra ham	45	30	01.06.2009	16.07.2009	15.07.2009	conform	-1
	Polish sausages	30	16	16.06.2009	16.07.2009	15.07.2009	conform	-1
	Ready-to-cook minced meat rolls	5	9	09.07.2009	15.07.2009	15.07.2009	conform	0
	Spicy salami	21	30	30.06.2009	20.07.2009	20.07.2009	conform	0
	Summer salami	21	27	27.07.2009	17.08.2009	24.08.2009	conform	+7
	Transylvanian salami	21	30	30.07.2009	20.08.2009	24.08.2009	conform	+4
	Rustic salami	21	09	09.10.2009	30.10.2009	30.10.2009	conform	0
0	Praga Extra ham	30	05	05.02.2010	07.03.2010	03.03.2010	conform	-4
1	Transylvanian sausages	21	01	01.02.2010	22.02.2010	03.03.2010	conform	+10
2	Smoked pork pastrami	30	23	23.01.2010	22.02.2010	03.03.2010	conform	+4
3	Pork pate	-	-	-	10.03.2010	03.03.2010	conform	-7
4	Smoked pork sausages	21	09	09.02.2010	02.03.2010	03.03.2010	conform	+1
5	Peasant-style bacon	15	09	09.01.2010	24.02.2010	03.03.2010	conform	+7
6	Praga Extra ham	30	6	06.03.2010	06.04.2010	15.04.2010	Slightly sour smell	+9
7	"Outlawry" fillet	30	16	16.03.2010	15.04.2010	15.04.2010	conform	0
8	Victoria salami		8		02.04.2010	15.04.2010	Slightly sour	+13

							smell	
9	House sausages	30	15	15.03.2010	14.04.2010	15.04.2010	conform	+1
0	Trandafir sausages	21	25	25.03.2010	15.04.2010	15.04.2010	conform	0
1	House salami	21	20	20.03.2010	11.04.2010	15.04.2010	conform	+4
2	Transylvanian pork loin ham	20	24	24.03.2010	14.04.2010	15.04.2010	conform	+1
3	Chicken breast hot-dogs	21	24	24.04.2010	13.05.2010	13.05.2010	conform	0
4	Chicken Polish sausages	21	12	12.05.2010	02.06.2010	03.06.2010	conform	+1
5	Fox sausages	4	26	26.08.2010	31.08.2010	02.09.2010	conform	+2
6	Extra summer salami	21	13	13.08.2010	04.09.2010	02.09.2010	conform	-2

During the period 08.07.2009 – 02.09.2010 26 meat products from various groups of products were analysed with a view to checking organoleptic features within and after the expiry term. The products were kept until expiry, complying with the conditions stipulated in the technical specifications of the analysed products. As may be seen from table 1, 7 (six) products analysed were analysed in the last expiry day, 2 (two) had one more day before expiry, 1 (one) product had two more days before expiry, 1 (one) product had four more days before expiry, 1 (one) other product had seven days before expiry, 5 (five) products were analysed from a sensory point of view one day after the expiry date, 1 (one) product was analysed from a sensory point of view two days after the expiry date, 3 (three) products were analysed four days after the expiry date, 2 (two) products were analysed seven days after expiry, 1 (one) product after nine days, 1 (one) product after 10 days from expiry and 1 (one) product 13 days after expiry. Half of the analysed products were kept for several days after expiry in order to be able to observe possible organoleptic changes.

From the analyses carried out according to categories of products with a view to observing organoleptic features within and after the expiry date the following were noticed: none of the products showed changes in aspect, colour, smell and taste, although in some of the products the expiry date was exceeded by several days. Only in two cases the products showed changes in smell, but the check thereof from

an organoleptic standpoint was carried out many days after the expiry date, namely 9 (nine) or 13 (thirteen) days respectively. Following the sensory analysis, fact-finding reports were drawn up and the results of the sensory analysis were recorded.

Table no.2

Products under study for physicochemical analysis

No.	Name of product	Product expiry period (days)	Lot	Date of production	Expiry	Date of analysis	Easily hydrolysable	Kreis test	Number of days until/from expiry
	House sausages	21	6	06.02.2009	28.02.2009	26.02.2009	32.62	negative	-2
	Baneasa salami	15	23	23.02.2009	10.03.2009	06.03.2009	30.00	negative	-4
	Smoked deboned ham hock	21	28	28.02.2009	16.03.2009	13.03.2009	30.81	negative	-3
	Polish sausages	21	18	18.03.2009	08.04.2009	07/08.04.2009	23.32	negative	0
	Extra smoked bacon	20	25	25.04.2009	16.05.2009	18.05.2009	18.12	negative	+2
	Peasant-style chicken breast bologna	30	25	25.05.2009	25.06.2009	25.06.2009	23.01	negative	0
	Pork salami	21	26	26.06.2009	17.07.2009	16.07.2009	29.80	negative	-1
	Pork bologna	45	13	28.08.2009	28.08.2009	25.08.2009	26.33	negative	-3
	Vacuumed-packed portioned pork nape	20	27	27.07.2009	11.09.2009	10.09.2009	27.16	negative	-1
0	Victoria salami	30	7	07.09.2009	06.10.2009	09.10.2009	23.67	negative	+3
1	Paprika pork fat	30	-	14.10.2009	14.11.2009	12.11.2009	15.40	negative	-2
2	Extra Praga ham	30	6	06.11.2009	09.12.2009	09.12.2009	32.64	negative	0
3	Victoria salami	30	-	12.12.2009	11.01.2010	11.01.2010	21.78	negative	0

4	House salami	21	18	18.01.2010	09.02.2010	11.02.2010	35.41	negative	+2
5	Peasant-style ham salami	30	16	16.02.2010	19.03.2010	20.03.2010	39.89	negative	+1
6	Smoked deboned ham hock	21	22	22.03.2010	12.04.2010	13.04.2010	33.59	negative	+1
7	Chicken breast hot-dogs	21	-	24.04.2010	15.05.2010	14.05.2010	27.52	negative	-1
8	Peasant-style bologna	15	18	18.05.2010	03.06.2010	03.06.2010	27.70	negative	0
9	Smoked fillet	15	24	24.06.2010	10.07.2010	09.07.2010	38.98	negative	-1
0	Bologna	45	14	14.07.2010	28.08.2010	27.08.2010	21.91	negative	-1
1	Extra Praga ham	30	24	24.07.2010	25.08.2010	26.08.2010	29.52	negative	+1
2	Pork hot-dogs	21	06	06.08.2010	27.08.2010	29.08.2010	27.70	negative	+2
3	Peasant-style ham salami	30	26	26.07.2010	26.08.2010	27.08.2010	25.70	negative	+1

The products analysed were collected starting with January 2009 onwards and the last products was analysed on 14.07.2010; random samples were collected, selecting alternatively one product of different groups of products so that during one year all groups of products could be checked.

Collection is carried out on the basis of a self-control programme established at the beginning of each year. The purpose of the analysis thereof is to check the maintenance of quality features and freshness until expiry.

During the above-mentioned period 23 samples were sent to the laboratory to perform the physicochemical analysis and the analyses requested for expiry date check were the following: easily hydrolysable nitrogen and Kreis test. From among the products sent for expiry check purposes, as may be seen from the annexed table, 5 (five) sample were sent on the last expiry day, 5 (five) products had one more day before expiry, 2 (two) products had two more days before expiry, 2 (two) products had three more days before expiry, 1 (one) product was

analysed four days before expiry, 2 (two) products were analysed one day after expiry, 2 (two) products were analysed two days after expiry and 1 (one) product was analysed on the third day after expiry.

In all products analysed for expiry check purposes the easily hydrolysable nitrogen parameter complied with the permitted limit stipulated by the technical specification and the legislation in force, while the Kreis test was negative which means that the products were fit for consumption during the established expiry term.

CONCLUSIONS AND RECOMMENDATIONS

3.1. Currently the safety and hygiene aspects of meat are mainly based on a preventive approach, such as the enforcement of good hygiene practices and the application thereof based on the principles of hazard analysis and critical control points (HACCP) and impose the taking of all measures so that the risk of inflicting diseases on consumers be reduced to the minimum. They must comprise the application of specific measures based on the prevention and control of possible contamination during processing and until the product reaches the end consumer.

3.2. During the study, the lots controlled yielded results that conformed from an organoleptic and physicochemical point of view, as they complied with the parameters imposed by the producer and by the legislation in force.

3.3. The conform results are the result of impeccable hygiene of production process which is ensued by: personnel trained with respect to the hygiene rules and the good production practices, of observance of refrigeration cycle during all stages of technological flow, clean and disinfected closed circuit modern equipment; discard packaging for vaccum packaging or packaging in controlled environment; moreover, they are the result of monitoring of activities and responsibilities regarding the assurance of traceability of product, starting with the acceptance of raw material, ingredients and materials necessary for production, until obtaining the final product, of the specific processing method of raw materials and of ingredients.

3.4. It is essential that each packaged product is correctly labelled in order to conform with the legal requirements on labelling and to reduce risks related to allergens that may be present in food.

Incomplete or incorrect information on the product and/or little knowledge on general hygiene may entail the inappropriate handling of the product during subsequent stages of food chain. Inappropriate handling may cause diseases or the products may become unfit for consumption, even if previously along the processing chain adequate hygiene control measures were taken.

3.5. The consumer, before buying a product, must pay special attention to the exterior and sectional aspect of products if possible, must personally get information on quality and authenticity of products, on identification and recognition criteria of false or slightly altered products, must check for the integrity of packaging of food products, which represent one of the guarantees for appropriate keeping of products, must buy food products from licensed places that guarantee the compliance with the food safety requirements.

3.6. Products must be kept according to producer's instructions on the packaging of products (storage temperature, humidity, etc.) since only under these conditions the producer guarantees the harmlessness of the product and the preservation of its qualitative features.

3.7. Education programmes regarding health must cover general food hygiene. These programmes must help consumers understand the importance of information on the product, must follow instructions accompanying the product and to make an informed choice. Consumers must be informed on the time – temperature relation and on the effects on food (microbe multiplication – alteration – disease).

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

RESEARCH ON THE COMPARATIVE QUALITY OF MEAT PRODUCTS OBTAINED IN SPECIALIZED UNITS

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Key words: meat products, quality, physicochemical determinations, legislation.

SUMMARY:

The study of the level of physicochemical parameters of meat products constitutes a direct method of quality research. The methodology includes food sampling, physicochemical analysis and interpretation of the results as compared to the norms in force..
The following physicochemical determinations were performed: humidity, fat substances, protein substances, collagen, the connection protein/ collagen, the sodium chloride and nitrites, expressed in $NaNO_2$ during the period 2008 – 2010 in authorized laboratories, for the meat product Victoria Sausage from five, seven concurrent companies respectively, as compared to the same analyses performed on the studied product Victoria Sausage. (Unit 1). In parallel, the sales level for the Victoria Sausage was monitored, relating the internal figures to the national market and the impact of the measures adopted for permanent improvement of this product's quality and image in its performances was analyzed.

According to the European Union and the World Health Organization – we are all responsible for the food's safety, starting from its origin and up to the moment it reaches the final consumer. The quality of meat products constitutes a legal obligation and is ensured through the product standards, the technical specifications respectively, and represents the producer's responsibility, found in the declaration of conformity.

The study of the meat products' quality can be performed by the determination of direct quality criteria, such as the physicochemical distinctive features.

The range Victoria Sausage is traded on the meat products market from different specialized units. Each company has its own recipe, presentation mode and selling price. According to the legislation in force, all companies must comply with the same physicochemical parameters.

This work intends to study the physicochemical properties of the product Victoria Sausage, from several concurrent companies as related to the product manufactured in a meat processing unit (Unit 1, represented by **S.C. FOX COM SERV S.R.L.**) equipped with the latest technological facilities, where the Quality Management System is

implemented, certified and applied at the most rigorous standards and to analyze the way in which the measures adopted for the permanent improvement of a product's quality and image is reflected in its performances and sales on the market.

1. MATERIAL AND METHOD

In **2008** and in **2009** the product Victoria Sausage was purchased from five (5), four (4) concurrent companies respectively, and the following physicochemical determinations were performed: humidity, fat substances, protein substances, collagen, the relation protein/ collagen, the sodium chloride and nitrites expressed in $NaNO_2$, as compared to the same analyses performed on the studied product Victoria Sausage. In **2010** the product Victoria Sausage was purchased from seven concurrent companies and two samples per each company were performed as compared to the product Victoria Sausage manufactured in the above mentioned processing unit, with the following physicochemical determinations: humidity, fat substances, protein substances, the relation protein/ collagen and the sodium chloride.

Thus, in 2008 one sample per unit was analyzed from Units 1-6, a total number of 6 samples being analyzed from a physicochemical point of view. In 2009 a number of 5 samples were analyzed, from Units 1, 2, 4, 5, 6. In 2010 a number of 7 samples were analyzed from a physicochemical point of view, from Units 1, 2, 4-7. The interpretation of the results of the physicochemical quality analysis performed on meat products was made by relation with the Romanian norms on food.

The product, the Victoria Sausage respectively, is boiled and smoke- dried; its preparation is subject to the technological rules specified in the Order no. 210/2006 for the approval of the Norms regarding meat products' trading.

The product Victoria Sausage, manufactured in Unit 1 (S.C. FOX COM SERV S.R.L.) is a sausage with a structure made of bradt and grist. The grist is represented exclusively by gammon, without bones and lard, exceeding 50 %, of the product's structure and the bradt represents the fine paste providing the product's consistency, homogeneity, elasticity and succulence. This type is manufactured according to the technological instructions approved on homologation with the compliance of sanitary and sanitary- veterinary norms in force. Victoria Sausage is a product obtained by raw material of highest

quality. Its cross-section looks like a tessellated mass, composed of big chops of gammon (with dimensions between 40-60 mm) and bradt.

Material and method for the physicochemical determinations:

1**.** The determination of humidity is established through the method described in **SR ISO 1442:2010**.

2. The determination of the content of free fat in the meat and meat products is performed according to the standard **SR ISO 1444:2008**

3. The determination of the sodium chloride content is made through the Volhard' s method (**SR ISO 1841-1:2000**) and the Potentiometer method (**SR ISO 1841-2:2000).**

4. The determination of the nitrites content is made according to the standard **SR EN 12014-3:2005**.

5. The light hydrolysable nitrogen and the ammonia in the meat and meat products is determined according to the standard **SR 9065-7/2007**.

6. The determination of the nitrogen content in the meat and meat products is performed according to the international standard **SR ISO 937:2007**.

7. The determination of the **collagen content** is made by using the standard **SR ISO 349:1997**.

2. RESULTS AND DISCUSSIONS

The physicochemical properties for the meat products are established according to the **Order 210/2006** of 8.01.2007 for the approval of the Norms regarding the meat products trading, regulating the conditions which must be fulfilled by the meat products in order to be manufactured and traded on the territory of Romania. The physicochemical properties for the product Victoria Sausage, the admitted maximum and minimum limits respectively, are presented in table no. 1 and established according to the legislation in force, as follows:

Table no.1

The physicochemical properties

PROPERTIES	ELIGIBILITY CONDITIONS	Analysis method
Humidity ,% max	66	SR ISO 1442:2010
Fat substances,% max	30	SR ISO 1443:2008
Sodium chloride,% max	3	SR ISO 1841-2:2000
Nitrites, mg/100 gr. of product, expressed in $NaNO_2$	10	SR EN 12014-3:2005
Light hydrolysable nitrogen mgNH_3/100g % max	45	SR 9065-7/2007
Total protein substances, %min	11	SR ISO 937:2007

The content of collagen substances is determined according to the standard SR ISO 3496/1997, the collagen in relation with the protein content of the product must not exceed the limit of 20% max. The reaction sulphurated hydrogen and the Kreis reaction are negative.

1. Analyzing the humidity parameter, it ranges between 64,21% - 75,54%, considering the fact that in the Order 210/2006 regarding meat products trading, the humidity parameter for boiled and smoke- dried products must not exceed **66%.**

Diagram no. 1 presents the level of the humidity parameter in 2008, 2009, 2010 for the analyzed product from Units 1-6. If Unit no. 2 recorded a constant evolution, even if this parameter does not fall under the legislation in force with regards to the humidity parameter, it is not the same for Unit no. 5, which recorded a fluctuation in time in the evolution of this parameter, which is considered to occur due to the use of a raw material of a different quality.

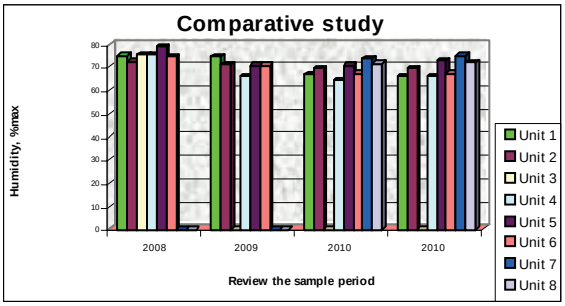
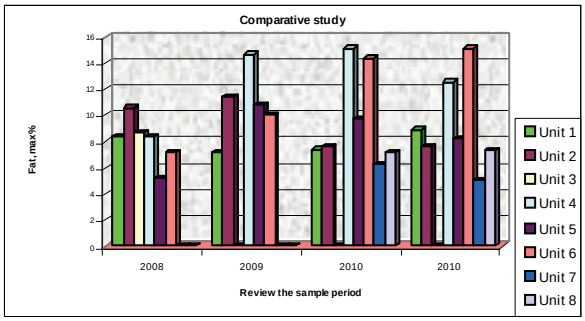


Diagram no.1 Comparative analysis of the Humidity parameter.

Analyzing the humidity for the product manufactured in Unit no.1 it is found that during the period 2008-2010 there was a decrease recorded from 75,04% (in 2008) to 66% (in 2010) falling at present under the legislation in force, following the implementation and the rigorous compliance with the legislation in force.

2. With regards to the Fat substances parameter, the result of the analyses for the products manufactured in the concurrent companies ranged between 5,23 – 15.13%, the maximum admitted value being of **30%**. As we easily notice in Diagram no. 2, the product Victoria Sausage, processed in Unit no. 6 records a double increase in 2010 as compared to 2008 for the Fat substances parameter, namely if in 2008 the Fat substances parameter recorded a percentage of 7,16, in 2010 the same parameter recorded a percentage of 15,11.



The situation evolves in reverse direction for the products manufactured in Unit no. 1, recording a decrease in the Fat substances percentage, from 8,89% in 2008 to 7,21% in 2010.

Diagram no.2 Comparative analysis of the fat substances parameter

3. For the total protein substances parameter, the boiled and smoke-dried products must fall under the minimum limit of **11%**, according to the legislation in force, the level of the total protein substances percentage being identified in Diagram no. 3 on the vertical (ranged).

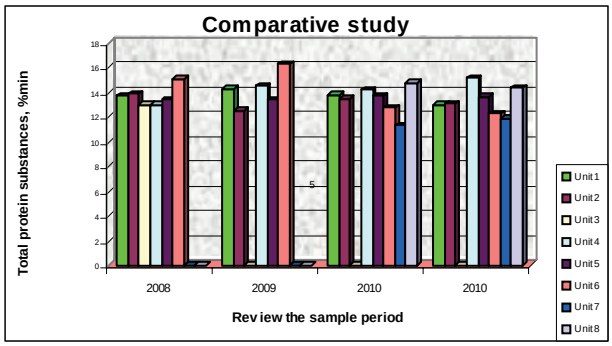


Diagram no.3 Comparative analysis of the total protein substances

For the analyzed products of the concurrent companies, the total protein substances parameter is comprised between 11,3%- 16,29%, which justifies the use of a qualitatively different raw material, from the point of view of the fat in the Units under study.

For the studied period, as revealed in Diagram no. 3, the product processed in Unit no. 6 presents the highest fluctuation for this parameter, ranging between 12,25 and 16,29. The analyzed samples

from Unit 1 resulted in a range from 12,97%- 14,3% and, as we can notice from Diagram no. 3, in the concurrent companies, the protein content is in many cases lower than in the analyzed products from Unit 1.

4. The maximum limit admitted for the salt parameter, is regulated as **3%** maximum, in the boiled and smoke- dried meat products. The analyses performed for the salt parameter (NaCl) on the concurrent products' products resulted in a range between 1,79% - 4%, the result of the samples analyzed in Unit 1 being 1,94% - 2,53%.

In Diagram no. 4 we can notice that the product manufactured in Unit 1 recorded a constancy with a slight decrease of the salt percentage in the products. The rest of the units recorded for the salt parameter variations or even exceeding of this parameter, such as the case of the product analyzed in 2009 in Unit 2, Unit 4 and Unit 5.

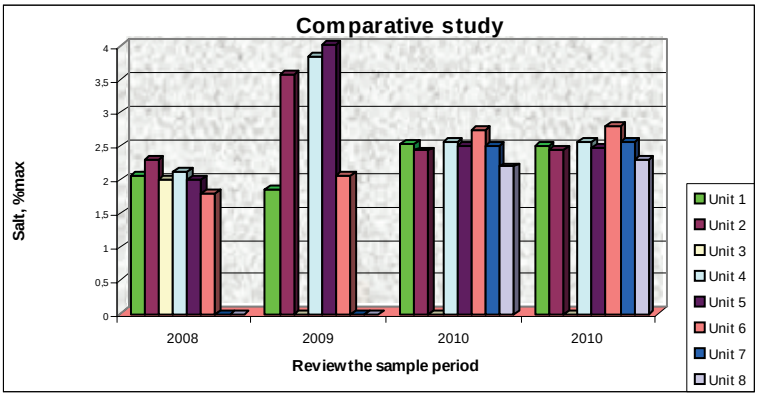


Diagram nr.4 Comparative analysis of the salt parameter

5. Nitrites

This parameter is regulated by the Order no. 711/2008 for the amendment of the Order no. 438/295/2002 regarding food additives, as having the limit of 15 mg/100 gr. of product.

Among the six units processing the Victoria Sausage, Unit no. 1 recorded in time a slight increase of this parameter, but the lowest content of nitrites as compared to all the units processing this product, as resulted from Diagram no.5. The lowest values are recorded in Unit no. 1 and the highest values are recorded in Unit no. 5, but all the analyzed samples comply with the legislation in force.

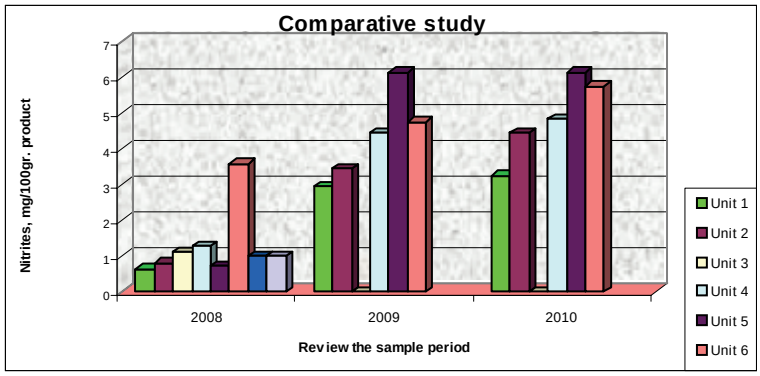


Diagram no.5 Comparative analysis of the nitrite parameter

6. From the Diagram it results variable collagen quantities, ranging between 0,68-2,72, these variations can be due to different quality of the raw material used in the technological process.

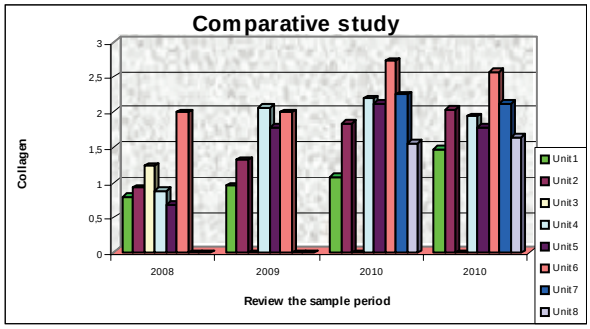


Diagram no.6 Comparative analysis of the collagen parameter

7. Additionally, the relation coallegn/ protein was also analyzed for all the samples of the concurrent companies as well as of Unit no. 1. All the analyzed samples comply with the legislation in force.

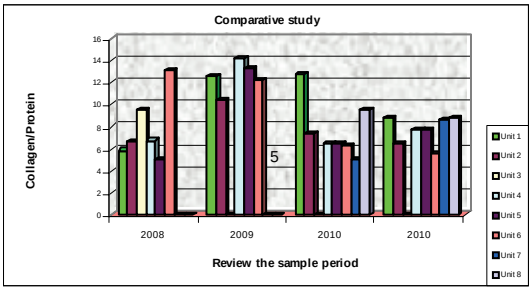


Diagram no.7 Comparative analysis for the relation: collagen/protein

As a conclusion, analyzing the results obtained, it was noticed that the humidity parameter was exceeded in most of the processing units and the salt parameter in Unit 2, Unit 4 and Unit 5, the rest of the analyzed parameters complying with the legislation in force.

Analyzing the physicochemical parameters for the product manufactured in Unit no. 1, we found the fact that the water percentage during the period 2008-2010 recorded a decrease from a higher value in 2008 to the value according to the legislation in force in 2010, the rest of the parameters recording values which comply with the legislation in force. The causes for which these limits were not exceeded are following the monitoring of the activities of production and processing of meat products, the implementation and compliance with the legislation in force and the permanent verification of the quality of the meat products obtained.

For the fat parameter all the products complied with the legislation in force, the maximum admitted value being **30%**, but most of the processing units recorded fluctuations for the total protein substances parameters as well as for the fat substances parameter in Unit no. 1, due to the fact that the technology of the processed products use raw material of constant quality.

The nitrites content had each year lower values for Unit 1 as compared to all the processing units of this product, the former maintaining its exigency in using the preserving agent in the meat products, even if the national and the European legislation provides its use in a higher quantity.

The collagen type proteins have a lower biological value due to their unstable content in amino-acids and the absence of certain essential amino-acids and the higher concentration of these proteins diminishes the biological value of the meat and meat products. It was noticed that the level of the collagen in the studied product was variable in all the processing units, but Unit 1 recorded the lowest values, the raw material used having a high biological value.

The analyzed unit (Unit no.1) wishes permanent satisfaction of the customers for the offered products ; in this purpose, it proposed several directions within its policy, which was communicated and understood within the company by periodical trainings, with the entire personnel involved in the supervision of the Quality Management System; these trainings are based on drawn- up minutes and clearly established debating themes.

The impact of all the measures taken over a product must be found in its performances.

We also proposed to monitor the sales of the Victoria Sausage, relating the internal figures to the national universe, taking into account all the existent ranges of Victoria Sausage, internally as well as

nationally. In general, the evolution of the sales depends on the following criteria, which are not ranged in the order of their importance (the hierarchy differs from one study to another):

- on- shelf availability (the product exists in the store)
- its placement into the main flow of buyers within the store
- a reasonable price
- the product's quality
- the product's taste
- the packaging' s attractiveness (its differentiation as compared to the concurrent products on the same shelf)
- advertising / publicity
- the product's support and signaling in the store

Starting with 2008, Unit 1 contributed in the qualitative improvement of the Victoria Sausage range, as we can notice in the following Diagram.

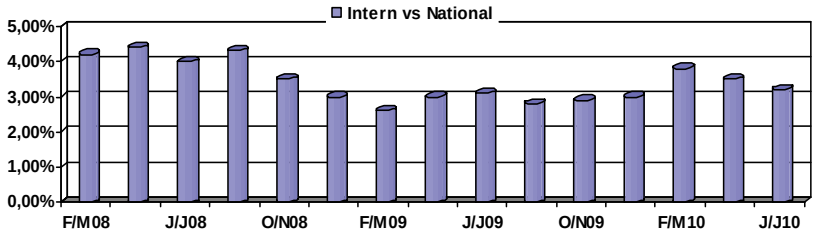


Diagram no.8 The evolution of the sales for the analyzed product manufactured by Unit 1, as compared to the national sales

We can easily distinguish two trends: a decreasing one until February / March 2009 and a slight but continuous one, until present. The decrease was mainly caused by the top management, by the adoption of an inadequate business strategy as well as by the world crisis, which is felt at the national level.

In order to return to the leading national sausages producers and to subsequently accede among the first three, the new top management team capitalized the entire company and took into consideration a set of ambitious and coherent measures, of which we mention:

- the adoption of the products portfolios upon the consumers' demand, in particular during this complex economic period
- the increase of the production capacities
- the increase of the sales power
- the extension of the fleet for an efficient distribution at the national level

- the adoption of the new design of the product's labels, which differentiated Unit 1 from its competitors by marking out the brand graphic elements
- the adoption of the new design of the company's price labels, placed in the traditional stores
- the constant increase of the product' s quality according to the attached analyses
- the increase of the on- shelf availability by the extension of the distribution at the national level
- the correct placement on the shelf (mercantization)
- the product's supporting on the delivery site (including by periodic degusting)
- the increase of Unit 1 brand's notoriety on the basis of its national supporting.

3. CONCLUSIONS

3.1 The objective of this work was the comparative study of the quality of the Victoria Sausage type meat product, obtained in several specialized units as compared to the product manufactured in a meat processing unit (Unit 1). In the latter, the Quality Management System is implemented, certified and applied at the most rigorous standards, process which is more and more reflected in time in the quality of the meat products in general and of the Victoria Sausage type product in particular.

3.2 Another objective was the analysis of the evolution of this product's sales on the sausages market following the permanent improvement of its *quality and image*.

3.3 As a consequence, 6 product samples from 6 specialized units were analyzed from a physicochemical point of view in 2008, a number of 5 samples from the same specialized units in 2009; the sixth unit could not be analyzed any longer as it is not present on the market anymore; in 2010 a number of 7 samples were analyzed, the same 5 units from 2009 and two more products from newly studied units.

3.4 The physicochemical determinations in 2008 and 2009 were the following: humidity, fat substances, protein substances, collagen, the relation protein/ collagen, sodium chloride and nitrites and in 2010 the following determinations were

performed: humidity, fat substances, protein substances, the relation protein/ collagen and the sodium chloride.

3.5 As a conclusion, during the entire period of the study, the product lots manufactured in Unit 1 had corresponding results, visibly improved from a physicochemical point of view, complying with the parameters imposed by the producer and the legislation in force.

3.6 The fact that the fat level is very low and the protein substances content is high makes from the product processed and traded by the manufacturing company a product which can be consumed by all the categories of consumers, being classified in the light products group. The product Victoria Sausage confers to the children as well as to the adults a high nutritive value due to the meat proteins.

3.7 Making a parallel between the Victoria Sausage type products from the concurrent companies and the studied product Victoria Sausage, we come to the conclusion that the product manufactured by Unit no. 1 is superior from the point of view of the constancy and the parameters' level, as compared to the similar product analyzed from the five, seven concurrent companies respectively.

3.8 All these measures, for the permanent improvement of the networks, the maintenance and the improvement of the Quality Management System, along with the marketing and sales strategies shall allow in the near future the FOX brand to become one of the significant players on the sausages market.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

GREEN GENERATION AND FARM ANIMAL BIODIVERSITY

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Key words: global heating, photosynthesis, , energy metabolism

SUMMARY

Rumors of the global heating of the Earth are increasing now days. Ecology as biological science concluded nature, without humans' implications, is in balance. The relations between living thing (biocenosis) and unanimated things where they live (biotope) are solved by the genetic variability of beings and through the natural selection controlled by environment. Biocenosis synthesizes organic matter from mineral compounds around: H_2O, CO_2, N, P and other chemical elements. Nature sustainability results from the primary and secondary trophic nets. When organic matter day it is mineralized. If part of it remains organic it is buried underground. Thus there are in nature "Telluric Carbon" underground, "Organic Carbon" inside the body of live or dead beings and "Atmospheric Carbon" as an external layer of Earth. Atmospheric carbon enters the composition of some gazes (CO2, CH_4, and CFC) able to retain heat out of sun rays. They express the so called "green house effect" together with the NO_2 (Nitrogen protoxide) and H_2O (water vapors). Global heating is due to the increase of green house gaze concentration which is saturated. All farm animals emit CO_2. Grazing animals emit CH_4 as well. Demographic explosion of humans increased production of "Organic Carbon" to feed them. It also increased the need for energy, most of it being obtained by burning "Telluric Carbon". To control the quantity of "atmospheric carbon" emitted to produce it is proper to use less "fossil fuel". "Biofuel" is of help. To decrease "Atmospheric Carbon" emission for feeding the same human population reducing the livestock of farm animals is possible if animal production performances are increased. Reducing the number of grazing farm animals in favor of grain consuming animals could help reducing CH_4 emission but will reduce resources of human food.

If seen from the cosmic space Earth, the third planet from the Sun, looks blue. If we look from down, from here where we are, we see it, or we would like to see it, green. Green is the color of chlorophyll, the pigment responsible for photosynthesis, for transforming atmospheric CO_2 into "Organic Carbon". Organic Carbon is the main element based on which chlorophyll uses energy of sun's light and some mineral matter, as H_2O, CO_2, N, P and few other elements from around to build up the bodies of green plants as genetic information commands.

Atmosphere is made up from 78% N, 20.94% O_2 and other minor gases. From the minor gases in atmosphere the CO_2, the CH_4, the CFC, the N_2O and the H_2O vapor are able to preserve heat from the sun raises to keep an constant average temperature of $+15^0$ at the solid surface of Earth and its variation amplitude as approximately $\pm 50^0$ C to 0^0 C.

Because of this effect that gases are called “green house gases”. An increased concentration of them is determining the global heating of the Earth and a higher mean temperature. Water vapors don’t participate in global heating being saturated.

As it can be seen three of the four green house gases contributing to the Earth global heating are compounds of the “Atmospheric Carbon” and are implicated in the live beings’ existence.

“Green generation” is a metaphoric paradigm concerning multiplication of green plants possessing chlorophyll as a mean to decrease concentration of atmospheric CO_2 by using it for organic matter synthesis of green plants. Thus the green color of the Earth will decline the concentration of “Atmospheric Carbon” and will act against the global heating of the Earth.

FARM ANIMALS’ PARTICIPATION TO THE GLOBAL HEATING

Animal life doesn’t use atmospheric Carbon in organic synthesis, on contrary living animals are emitting CO_2 into atmosphere as a result of their respiration. Organic synthesis in animals uses vegetal Organic Carbon from the fodder consisting of from lists of herbs up to seeds and fruits of other plants.

From the feeding point of view there are three kinds of farm animals: herbivorous, omnivorous and, less important, carnivorous. The herbivorous have a fermentation compartment in their digestive track populated with a micro flora able for anaerobic fermentation. Out of that high quantities of CH_4 result. The omnivorous have only the chemical stomach and are able to digest carbohydrates (starch and others) with small methane emission.

Carbon dioxide is produced by all animals in the so called metabolic process of energy. The emission of CO_2 is estimated to be 1 m^3 for each 5 Mcal of net energy including maintenance, deposited and excreted energy.

In a study of Maria Paraschivescu, CO_2 emission by swains has been estimated for 17 years between 1938 and 1997. She concluded that CO_2 emission reported to the fertility of sows and to the average daily growth gain is less when performances are higher. Same thing must happen concerning CO2 emission during the growing process of young animals in all animal farm animal species (Maria, Paraschivescu,1999).

In dairy cattle the cotta of maintenance energy within the total net energy used for maintenance and production will be smaller when milk yield will be bigger.

Animal production improvement of breeds of farm animals helps in decreasing the CO_2 emission per unit of human animal food.

Concerning CH_4 it must say no plant or animal is able to use it in any kind of organic synthesis. It can be consumed only by burning.

According to Cicerone and Ormland (1988), citied by D.E. Johnson and *alii*,28 % of the total methane emission is due to natural swamps and 20% to rice fields which commit anaerobic fermentation under water (Jonson, E.D., 1991),. Farm animals produce 14% of the total methane emission trough digestion process of fodder. That means farm animals produce methane to cover 2% of green house effect in global heating.

Same authors communicate the data proposed by Crutzen (1986) and Larmer (1998) as methane emission to be: 169 l in cattle, 192 l in buffaloes, 19 l in sheep, 19 l in goats, 223 l in camels, 5 l in swains, 76 l in horses and 0.2 l in humans, per head, per day (Jonson, E.D., 1991). The large herbivorous animals are producing the highest quantities of methane. Physiologically speaking small ruminants are producing more methane than cattle if the emission is reported to the quantity of dry matter ingested by animals. Since in ruminants methane is produced before digestion may be more CH_4 emission in small ruminants show a better availability for digestion of the fermented matter in sheep. Horses emit less CH_4 because in this genetic species fermentation takes place in caecum, a compartment which is placed after the chemical stomach.

It is known that digestibility of feed stuff or of mixed fodder is higher when the feeding matter has a higher energy concentration. Experiments of D.E. Johnson and *alii* with highly energy concentration diets (over 90% concentrate feed) have shown just a grater variance of energy losses as CH_4 emission. These losses varied between 2% - 12%, limits which weren't meet when fibrous fodder has been used.

HOW THE HUMANS COULD HELP GLOBAL HEAT PREVENTION

Molecules of green houses effect gases have a limited life in atmosphere. Thus CO_2 molecule can resist 100 years, the CH_4 molecule can resist 10 years, the N_2O molecule can resist 180 years and the CFC molecule can resist 400 years, if they were not involved in any chemical reaction (Tufescu, V.,1988). In nature there is a balance: green house

gases are produced in the quantities which are deleted by the physical phenomenon mentioned before. CO_2 enters in organic synthesis and is reproducing by organic matter mineralization. This process results also in CH_4 and N_2O, even these gases don't participate in organic synthesis. Mineralization of organic matter is delayed by the primary and secondary trophic nets. CFC are artificial gases, produced by humans, they are not produced in the nature. That means humans acting to produce their food and needed energy have to consume CO_2 in photosynthesis and to limit production of all green house gases.

It is possible to increase artificially the consumption of atmospheric CO2 cultivating forests and cultivating plants for "Biofuel" in addition to the culture for men food and for farm animals' fodder. Forestry culture is the most powerful tool to consuming CO_2 in organic synthesis but the resulted matter is few included in the primary trophic nets of the nature. Most biological production of woods died and enters the secondary trophic nets or goes directly underground and become "fossil fuel" (coal, oil or methane). The Organic Carbon of biological production of wood becomes "Telluric Carbon" which can't participate to the global heating of the Earth. Culture for "Biofuel" could be directed to obtain vegetal oil or carbohydrates. The first type of Biofuel has the advantage to firstly furnish energy and afterword CO_2. Obtaining the second type of Biofuel from Organic Carbon supposes firstly to produce CO_2 and only afterword energy is furnished. When this Biofuel is used the rest of CO_2 is emitted (Paraschivescu, M, 2009).

Products of agriculture cultivar directly consumed by humans have a better conversion of their contained energy in metabolic energy for the human body. That is good for food security. Out of these harvests vegetal residuals are resulting, as well. Some of the residues can be used as fodder for animals and that is good for delaying the formation of green house gases. The rest of them must suffer fermentation and mineralization. If fermentation is an aerobic one organic fertilizer and CO_2 will result, and that is good. If the fermentation is an anaerobic one CH_4 will result and this is less convenient.

Products of cultivar for fodder enter the trophic chain: soil, plant, farm animal, man. In this way the liberation of Organic Carbon atoms takes place step by step and is dallied. At the end the mineralization of the last dead biological production will be mineralized, as well.

Non digested organic matter of feed is eliminated as feces forming together with the urine the manure of farms. That is dead organic what must be evacuated to be mineralized. Urea from manure contains N and by fermentation can produce N_2O. N is present in every cell still

existing in the manure, too. If N is conserved as organic N it gives better value to the organic fertilizer.

WHAT COULD BE DONE TO KEEP GREEN HOUSE GASES UNDER CONTROL IN FARMING

There is not easy to control production of green house gases in animal farms. As long as animals are alive they will emit CO_2. The only one way to act is to increase the production genetic potential and to satisfy it as much as possible.

Concerning CH_4 emission E. D. Johnson and his team experiments have clearly shown that diets composition can't ensure a certain control of CH_4 emission as a digestion gas. When less CH_4 is emitted less ingested energy is lost, isn't it? Or on contrary, less rumen micro flora is developed?

Trials to reduce the CH_4 emission with ionophores were unsuccessful. Monensin, one of the ionophores, reduces the CH_4 emission but for 16 days only. Ionophores yet increased the daily body gain and decreased the CH_4 emission per kg of body weight.

The kind of grains included in diets showed some effect. List losses of energy as methane was registered when corn (losses 7, 1) were used instead of barley (losses 11.2). Greatest losses resulted when barley was substituted with dry beat pulp. But results remain uncertain.

The most difficult question to answer is if is better from the point of view of nature protection is to cultivate grain or forage to feed omnivorous or herbivorous animals. The answer has to pay attention to the problem of the food security which is not solved yet. What is sure is the fact that more animals mean more Organic Carbon and that is a protection against the Earth global heating.

Very important thing is the manure handling. If manure is evacuated hydraulically then it will be collected in lagoons and an anaerobic fermentation take place. So much quantities of CH_4 will be produced and N_2O will be produced too. Both gases have green house effect. It is much better to build floppy concrete ramps with kerbs, end stop and safety fences permitting to separate the watery part of the slurry, dilute it and pump and spread on the field (Tufescu, V., 1988). The solid part of the slurry, after aerobic fermentation, will become a valuable fertilizer.

CONCLUSIONS

1.1. A big mistake is to cut woods and reduce the forestry's surface. On contrary is a good action to plant trees and extend forestry, but biological production of woods doesn't feed humans nor farm animals. That doesn't help the food security.

1.2. More cultivars of green plants are useful against global heating of the Earth planet. The most intensive the agriculture is the better is for fixing carbon atoms as Organic Carbon, inactive in global heating. Intensive agriculture must be done without using chemical fertilizers since they contribute to N_2O formation. Using organic fertilizers has very good effect upon soil fertility.

5.3 More livestock of farm animals means more Organic Carbon deposited which is retarded from Atmospheric Carbon production, component of green house gases. That helps also the food security of humans.

5.4 Both herbivorous and omnivorous farm animals must be kept. Herbivorous are consuming biological part of the green plants production that can't be consumed by humans, even they are emitting methane in their nourishing process. The fodder they consume must be mineralized in any case and its anaerobic fermentation can't be excluded.

5.5 Important measure protecting against green house gases formation is to handle the slurry of the farms on floppy concrete ramps able to permit separation of watery parts and use them, after dilution as a liquid fertilizer.

5.6 Green generation understood as increasing of forests' surface, cultivating green plants food or fodder production, breeding improved farm animals to eat the fodder and produce food for humans is the best way to act against the global heating of the Earth planet.

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Scientific works, C series LVI(1), 2010
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RESEARCH ON THE LUNG, ESOPHAGUS, INTESTIN AND KIDNEY HISTOSTRUCTURE OF SWINE FETUS AGED 50 AND 60 DAYS OLD

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Key words: embryo development, lung, esophagus, intestine, kidney

SUMMARY

The authors have studied the histological structure of organs of the thoracic and abdominal cavity in swine embryos aged of 50 and 60 days after having used a fixing solution of saline formol a paraffin inclusion and after the serial division into section of the organs.
The sections have been coloured with HE methods, Mallory trichromical method, silver impregnation Gömöri method.
Lung at age 50 days shows a weak differentiation of bronchial tree is found to differ according to histological structure of the organization lung. Lobes lung are bounded by connective tissue which shows perilobular loose network of mesenchymal tissue. At the age of 60 days extralob bronchi shows advanced stage of differentiation, both in the bronchial epithelium and cartilage in the muscular tunic.
Esophagus in 50 days is going structural organization. Esophagus shows three tunics (mucosa, muscular and adventice), not differentiated submucosa. Muscle lacking mucosa. At 60 days, esophageal mucosa is under differentiation, being present both stratified pavement epithelium and corion soft type.
Small intestine from fetuses of 50 days shows villous extensive corion intestinal mucosa is poorly developed and lacking Lieberkuhnn glands. At 60 days, the intestinal epithelium shows no caliciform mucous cells, the apical pole of enterocytes is differentially present microvilii as forming "striated shelf.
Kidney at 50 days shows nefron differentiated morphologic structure. Malpighi corpusculuii are highlighted and appear differentiated podocitele of Bowman membrane structure.At 60 days tubules keep urine shows nefroread functional and appear at the apical pole poorly differentiated micro Vilia willingi form of ,,edge in the brush". Reticulin fibers are evident, not present structural elements of the juxtaglomerular apparatus.

The research on the ontogenetical development of swine embryo often concentrates on the embrionary period up to 45 days old, an extremely important period in terms of creating new reproduction biotechnologies (transfer of embrios).

Most embryology studies deal with swine embryonary development from 6-10 mm up to the age of 45 days (Hill M., 2003; Schoenwolf C.G., 1973).

The fetal development of swine embryo and fetus is not presently an usual subject of research in the specific literature, a limited number of articles exist on this topic, while most studies effectuated are in fact

electronomicroscopic researches on the microstructure of the organs in course of differentiation.

The studies effectuated on 45 days old swine embryo have proved the presence of developed villous, differentiated morphologic structure. Malpighi corpuscles and differentiation of bronchial tree (Petruț T. et al., 2006, 2009).

Also, at 45 days-old, the stomach microstructure has an epithelium in course of differentiation with PAS positive granules on its surface and in the structure of gastric epithelium cells (Georgieva R.K., K. Gerov, 1975).

1. MATERIAL AND METHOD

The swine fetus were picked up from the uteri of the females sacrified by necessity and were classified by length, with special focus on the 7 and 11 cm length embryos. This length corresponds to the age of 50 and 60 days of intrauterin development, at the limit of embrionary and fetal development.

The histological pieces collected were selected by dissection and fixed in saline neutral formol, being processed later for paraffin inclusion. The paraffin blocks were cut to 6 microns and coloured by the HE methods, Mallory trichromical and silver impregnation Gömöri.

2. RESULTS AND DISCUSSION

The lung at age 50 days shows a weak bronchial tree diferenciation and distinct histological structures are present according to the organizational level of the lung. It is constituted of lung lobes that present epithelial condensations made by the bronchial buds that branch dichotomous. Around the bronchial buds, the mesenchyme condenses and induces the bronchial epithelium differentiation (fig. 1).

The lung lobes are bounded by perilobular connective tissue showing loose networks of mesenchymal tissue. Trophic and functional blood vessels are present in peribronhial lobar mesenchymal tissue.

Bronchial epithelium during this period of embryonic development appears as a simple prismatic epithelium, the bronchial muscle (Reissessen) being present as well.

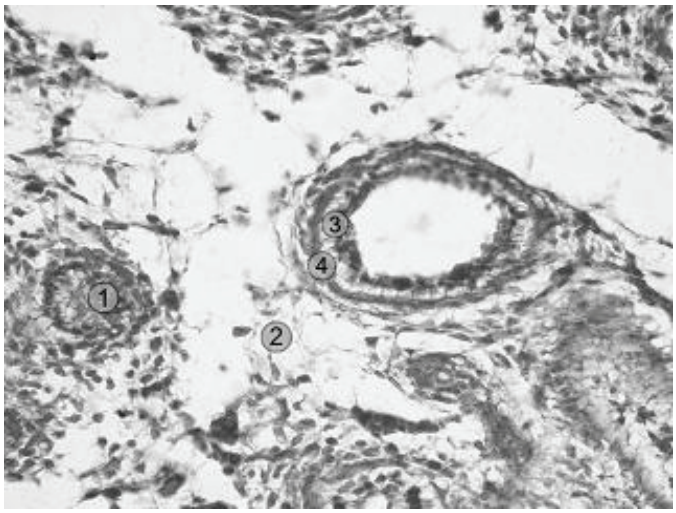

Fig. 1 – Swine fetus - 50 days, lung section, Mallory trichromic stain , Ob 40x
1. bronchial buds; 2. mesenchymal tissue; 3. bronchial epithelium; 4. bronchial muscle.

At 60 days, around the bronchial buds, mesenchymal cells are differentiated and with basal membranes, fibroblasts and smooth muscle fibers form the reticulate and connective network of intralobular bronchi. In the intralobular mesenchyma appear branches of pulmonary vessels and capillary vessels to accompany intralobular bronchi (fig. 2).

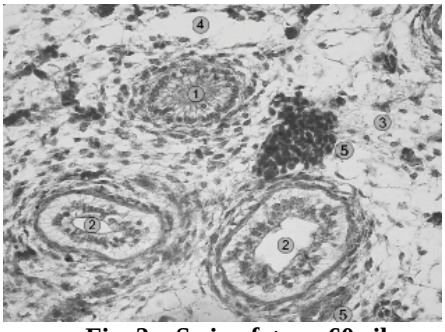

Fig. 2 – Swine fetus - 60 zile; secțiune prin lobul pulmon; Col. tricromic Mallory; Ob. 40x
1. bronchial buds; 2. intralobular bronchi;
3. mesenchymal tissue; 4. capillary limphatic;
5. capillary vessels.

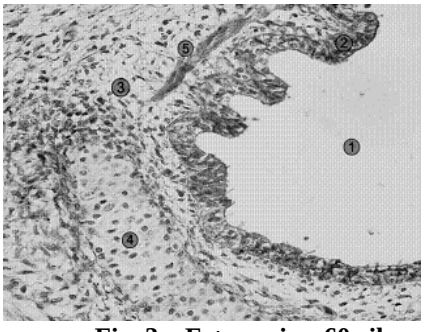

Fig. 3 – Fetus suin - 60 zile; secțiune prin pulmon; Col. HE; Ob. 40x
1. extralobular bronchi; 2. bronchial epithelium; 3. fiber- muscular-cartilaginous tunic; 4. cartilaginous nuclei; 5. bronchial muscle.

The extralobular bronchi present an advanced differentiation state, both in the bronchial epithelium and in the muscular-cartilaginous tunic (fig. 3). Extralobular bronchi present an ongoing organization epithelium of simple prismatic passing stage to pseudostratified prismatic stage. Pseudostratified bronchial epithelial cells have cilia on the apical pole and between ciliate cells mucous goblet cells appear in goblet form but without mucin cumulation at the apical pole. The basal membrane where the epithelium is set is obvious. In the bronchial chorion, collagen fibres and fibroblasts, mast cells and lymphocytes are present. In the fiber- muscular-cartilaginous tunic are differentiated the cartilaginous nuclei and smooth muscle fibres that form the Reissessen muscle. The fiber-muscular-cartilaginous tunic is continued with mesenchymal perilobular tissue.

Esophagus at 50 days is being organized structurally. The esophagus has three tunics (mucosa, muscular and adventitia), the submucosa not being differentiated yet. Mucosa`s muscular layer is missing Esophageal mucosa is being differentiated, the stratified epithelium and the corion are both present (fig. 4), without the mucosa`s muscular layer being formed which separates mucosa from submucosa.

At 60 days, esophageal mucosa is being differentiated, smooth stratified pavement epithelium and also the chorion are present. (fig. 5).

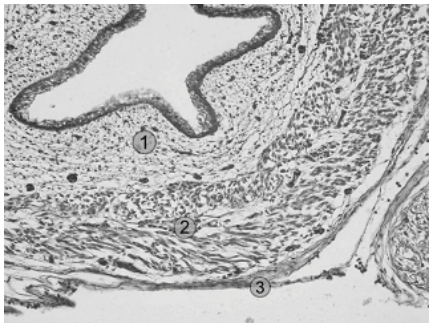

Fig. 4 – Swine fetus - 50 days, section through the esophagus; Mallory trichromic stain; Ob 40x
1. esophageal mucosa; 2. esophageal muscular layer; 3. adventitia.

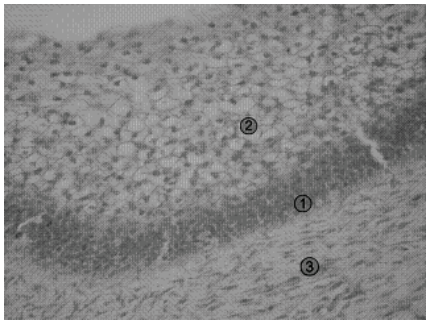

Fig. 5 – Swine fetus - 60 days, section through the esophagus; Col. HE; Ob. 40x
1. stratum basal; 2. stratum spinosum; 3. chorion.

Epithelium from the basal layer appears to consist of several layers of cells and has an intense mitotic activity, which generates cells of stratum spinosum. Stratum spinosum cells have undifferentiated tonofibrils, leading to an unorganized aspect of the epithelium. Cell

cytoplasm appears to be vacuolar, and in the superficial level the pavement cell leyer is missing.

Epithelium is separated from the chorion by a clear basal membrane. In the chorion are present both fibroblasts and fibrocytes spread among mesenchymal cells.

Small intestine from fetuses of 50 days has well developed villous and enterocytes have a prismatic shape with centrally disposed spherical core or 1/3 higher. Apical pole is different. Muscularis mucosa is under differentiation.

Tunics intestine differentiated mainly occur in the submucosa, muscular wall are being organized. Corion intestinal mucosa is weak developed and devoid of glands Lieberkuhnn. Submucosa is presented as a thin layer of collagen (fig.6).

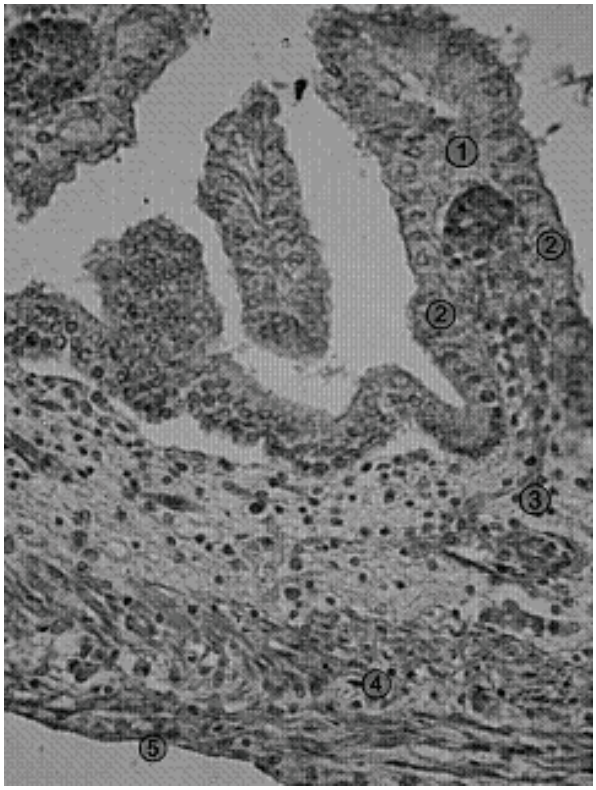

Fig. 6 - Swine fetus - 50 days; section through the intestine; Col. HE; Ob. 20x
1. intestinal villous; 2. intestinal mucosal epithelium; 3. chorion;
4. muscular layer; 5. serous.

At 60 days, the axis villus are obvious blood vessels and lymphatic tissue is accompanied by mesenchymal populated by mesenchymal cells, fibroblasts, and lymphocytes fibrocytes.

Villus intestinal small intestine have extensive at this age. Intestinal mucosal epithelium cells do not cripple forms, the apical pole of enterocytes is different, being present microvill forming, shelf striated (fig. 7).

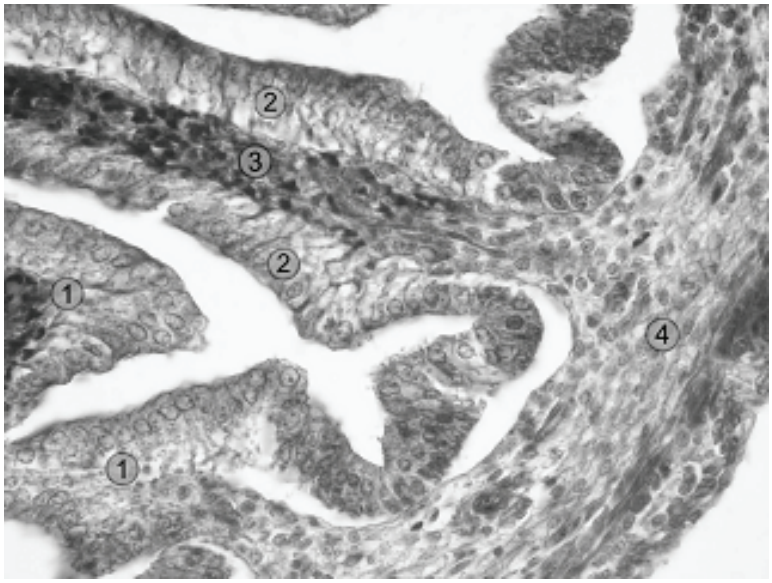

Fig. 7 - Swine fetus - 60 days; section through the intestine;
Col. Mallory trichromic stain; Ob. 40x 1. intestinal villous; 2. intestinal mucosal epithelium; 3. axis villous; 4. submucosa;

Kidney at 50 days have differentiated nephrons morphological structures. Malpighi corpuscles are clearly composed of fenestrated capillaries of the vascular huddle embedded in connective tissue of the kidney and mesangial Bowmann capsule (fig. 8). Podocytus of membrane structure occur Bowmann differentiated between two sheets of space capsule Bowmann is currently filtering.

At 60 days have nefroread uriniferi tubules arranged on the basement membrane form of a simple cubical epithelium.

Nephrocytes are present in tubules twisted being functional at the apical pole appear willing micro vill weak differentiated form of the brush border. Structural elements are not present in the juxtaglomerular apparatus.

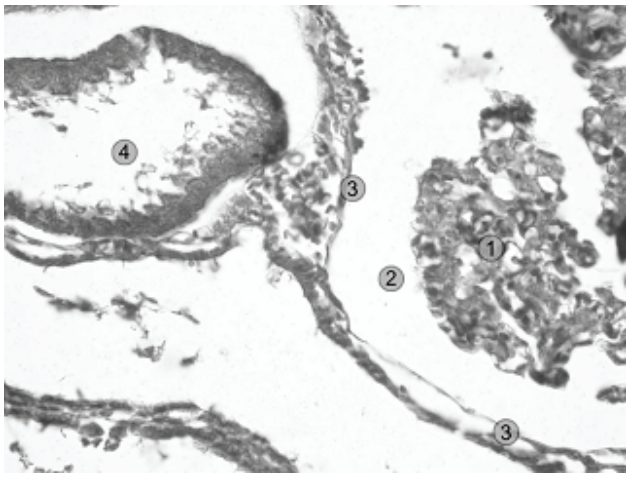

Fig. 8 - Swine fetus - 50 days, section through the kidneys; H.E. Col., Ob 20x
1. vascular glomerule; 2. space filtering; 3. Bowmann capsule;
3. vascular glomerule 4. uriniferi tubules.

Located at interstitial tissue among the tubules and uriniferi glomerulata vascular reticulin fibers are evident (fig. 9). Located at interstitial tissue among tubules uriniferi interstitial vascular network is present.

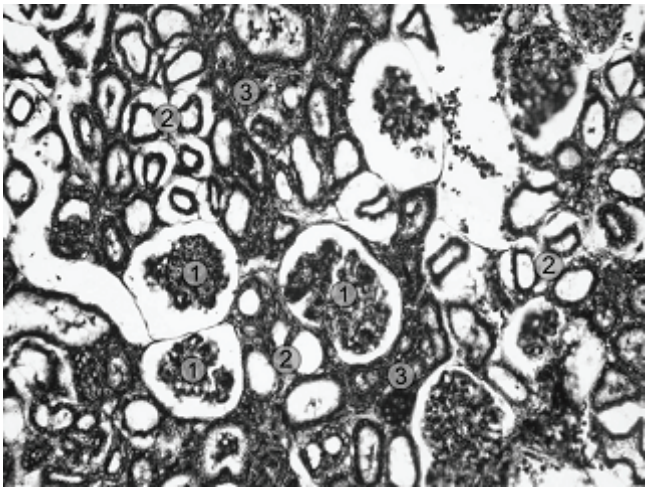

Fig. 9 - Swine fetus - 60 days, section through the kidneys;
Silver impregnation Gömöri method, Ob 20x
1. Malpighi corpuscles; 2. uriniferi tubules; 3. reticulin fibers.

3. CONCLUSIONS

3.1. Lung at age 50 days shows a weak differentiation of bronchial tree is found to differ according to histological structure of the organization lung. Lobs lung are bounded by connective tissue perilobular showing loose network of mesenchymal tissue. At age 60 days bronchi extralobs present advanced stage of differentiation, both in the bronchial epithelium and cartilage in the muscular tunic.

3.2. Esophagus in 50 days is being structural organization. The esophagus has three tunics (mucosa, muscular and adventicea), not differentiated submucosa. Muscle lacking mucosa. At 60 days, esophageal mucosa is under differentiation, being present both stratified pavement epithelium and corion soft type.

3.3. Intestine from fetuses of 50 days has well developed villous, corion intestinal mucosa is weak developed and devoid of glands Lieberkühnn. At 60 days, the intestinal epithelium has not caliciforme mucous cells, the apical pole of enterocytes is different, being present microvill forming, shelf striated".

3.4. Kidney at 50 days shows nefronului differentiated morphologic structure. Malpighi corpuscles are clearly differentiated and appear Bowmann podocitele of membrane structure. At 60 days uriniferi tubules present nefrocitele functional and at the apical pole appear willing microvill poorly differentiated form of 'edge in the brush. Reticulin fibers are evident, not present structural elements of the juxtaglomerular apparatus.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

HYGIENIC QUALITY OF RAW COW MILK FROM TRANSILVANIAN FARMS WITH TIE STALL HOUSING

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Keywords: total bacterial count (TBC); microbial contamination, season.

SUMMARY

The aim of this study was to assess the quality of raw cow milk based on the total bacterial count (TBC), in farms with tie-stall housing from Transylvania. The collection and analysis of the 408 milk samples from 17 farms (24 samples/farm) was made between September 2009 and August 2010. The total bacterial counts varied greatly in the milk from the investigated farms, the mean values ranging from 182 to 304,583 x 10^3 CFU/ml. Significant differences were demonstrated among the majority of the farms (Dunn's Multiple Comparisons Test, $p<0,05$). The mean values of TBC were significantly higher in summer than in winter ($p< 0,05$). In 29,4% of the studied farms the raw milk's bacterial counts exceeded the limits admitted by European standards. The obtained results indicate high microbial contamination of the raw milk in the majority of the investigated farms caused by neglecting the hygienic requirements.

The total bacterial count (TBC) is a way of measuring the overall hygienic quality of the milk in the farm (Marshall, 1985). Milk is synthesized by cells within the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, bacterial contamination can generally occur from three main sources: within the udder, outside the udder, and from the surface of the equipment used for milk handling and storage. Cow health, environment, milking procedures and equipment sanitation can influence the level of microbial contamination of raw milk (Bramley and McKinnon, 1990). Equally important are the milk holding temperature and length of time in which the milk is stored before testing and processing, factors that allow bacterial contaminants to multiply (Wiking *et al.,* 2002). All these factors will influence the total bacterial count. Regulation No. 853/2004 of the European Parliament and of the Council (EC) sets down the hygienic limit at $\leq$1,00 x 10^5cfu/ml milk for the total bacterial count in raw cow milk. Aseptically collected milk from clean, healthy cows generally has a TBC less than 1,00 x 10^3cfu/ml. Higher values suggest that bacteria have entered the milk

from a variety of possible sources. Though it is impossible to eliminate all sources of contamination, counts of less than 5,00 x 10^3cfu/ml are possible, while counts of 1,00 x 10^4 cfu/ml should be achievable by most farms (Marshall, 1985). The most frequent cause of a high TBC is poor cleaning of the milking system. Milk residues on equipment surfaces provide nutrients for growth and multiplication of bacteria that contaminate the milk at subsequent milking times. Other procedures that can elevate bulk-tank TBC are milking dirty udders, maintaining an unclean milking and housing environment, and failing to rapidly cool milk to less than 4°C. On rare occasions, cows with mastitis can shed infectious bacteria into the milk and can also cause a high TBC. In these circumstances, a concurrent elevation in SCC (Somatic Cell Count) should be evident.

The aim of this work was to assess the hygienic quality of the raw cow milk based on the total bacterial count (TBC) in farms from Transylvania.

1. MATERIAL AND METHODS

The material for the study comprised 408 samples of bulk-tank milk from 17 Transylvanian dairy farms (32-113 cows/barn). The cows were housed in all of the farms in tie stall barns, usually in short stalls. Manure cleaning was made by manual handling or mechanically. The cows were either tethered in the barns during the cold season (pasturing during daytime in the rest of the year) or permanently (without pasturing). The milking was done mechanically at stall. The milk samples were collected between September 2009 and August 2010, two samples per month from each of the farms. Sampling was done directly from the tank with raw milk in sterile test tubes. After collection, the samples were transported to the laboratory using ice boxes. For the total bacterial count (TBC) determination successive dilutions were performed from the milk samples: 1 ml of milk was taken and homogenized with 9 ml sterile normal saline solution (0.09%), obtaining the basic dilution 10^{-1}, from which successive dilutions were done afterwards: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. From each dilution 0,1 ml was transferred using a sterile pipette and spread on PC agar using a sterile glass spreader for each sample. The plates were then incubated at 30ºC for 24 to 48 hours (SR EN ISO 4833/2003). Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to

obtain the TBC. The TBC was expressed as the number of organisms of colony forming units per ml (cfu/ml).

The obtained data were statistically processed using the SPSS version 17 software. The descriptive statistical indicators (mean, standard deviation, median, minimum and maximum) were calculated for the TBC in the investigated dairy cattle shelters. The comparison of the values obtained was made with the Dunn's Multiple Comparisons Test.

2. RESULTS AND DISCUSSION

The descriptive statistical indicators (mean, standard deviation, median, minimum and maximum) for the TBC are presented in table 1. The bacterial counts varied greatly in the milk of the investigated shelters, the main values ranging from 182 (farm 9) and 304,583 x 10^3 cfu/ml (farm 10). The minimal values ranged between 100 and 270 x 10^3cfu/ml and the maximal values between 299 and 351 x 10^3 cfu/ml. It can be observed that in 29,4% of the farms the milk contains bacterial counts beyond the admitted limits stated in the European standards ($\leq$1,00 x 10^5cfu/ml milk). Our values are higher than those obtained by Cempirkova and Franciosi *et al*. (Cempirkova, 2007; Franciosi *et al.*, 2009) and lower than those obtained in other studies (Grega *et al.*, 2005; Samia *et al.*, 2009; Sraïri *et al.*, 2006). The values of microbial contamination of raw cow milk are influenced by the dairy cows' health and hygiene, by the hygiene of the environment where dairy cows are housed and milked, by methods of udder preparation and milking technique, methods of cleaning and sanitation of milking machines and milk cisterns, tenders' hygiene, speed of milk cooling to a required temperature and milk storage time (Bramley and McKinnon, 1990).

The higher values of microbial contamination of milk on farms 5, 10, 11, 13 and 17 were connected with the severely soiled environment of the barns and dairy cow udders with faeces and with the insufficient drying of udders with a synthetic cloth after their pre-milking washing, i.e. with deficiencies in the hygiene of the barn and milking that were consequently reflected in TBC values (McKinnon *et al.*, 1990; Holm *et al.*, 2004; Pavičić *et al.*, 2008). By the analysis of the dominant microflora in downgraded bulk milk (TBC > 3,0 × 10^4 /ml) Holm *et al.* found out that 64% of samples contained micro organisms connected with poor hygiene; 28% of samples were contaminated by the microflora also connected with poor hygiene, and with growing at low

temperatures (psychrotrophic bacteria) and in 8% of samples bacteria connected with mastitis were predominant (Holm *et al.*, 2004).

Table 1

Total bacterial counts (TBC × 10^3 × ml^{-1}) in bulk milk samples on the examined farms

Farm	n	Mean	SD	Median	Minimum	Maximum
1	24	12,640	6,969	10,575	4,325	27,700
2	24	25,384	10,338	25,150	11,250	48,000
3	24	52,444	18,379	54,550	24,650	83,000
4	24	82,234	19,258	82,050	51,000	111,650
5	24	149,417	70,215	113,000	98,000	287,000
6	24	16,887	1,728	17,000	13,700	19,400
7	24	0,173	0,057	0,170	0,100	0,299
8	24	0,628	0,176	0,606	40,000	95,000
9	24	0,182	0,056	0,190	9,700	26,300
10	24	304,583	22,727	300,000	270,000	351,000
11	24	192,000	10,640	192,500	172,000	207,000
12	24	41,504	16,727	35,750	22,800	65,100
13	24	160,371	12,366	159,500	140,000	179,000
14	24	10,810	5,750	9,500	3,550	21,100
15	24	42,195	13,318	37,050	28,000	71,000
16	24	0,498	0,238	0,520	0,170	0,807
17	24	174,954	12,264	174,000	156,000	193,000

Those farms in which the hygienic conditions were adequate (7,8,9,16) are those with the lowest TBC values. As it is shown in table 2 the differences we recorded between the studied farms were significant ($p>0,05$), very significant ($p<0,01$) and extremely significant ($p< 0,001$).

In table 3 we listed the TBC values in different seasons: in the winter, spring, summer and autumn. The highest mean value was recorded in summer and the lowest in winter, the difference being statistically significant (Dunn's Multiple Comparisons Test, $p<0,01$). The values obtained in spring and in autumn were close to each other. Similar observation was confirmed in the literature (Grega *et al.*, 2005; Kuczaj, 2001). Some authors demonstrated that commercial milk collected in spring and in summer contained nearly three times as many bacteria (868,9 × 10^3 cfu × cm^{-3}) as that produced in autumn and winter. However, the differences were statistically insignificant (Kuczaj, 2001).

Table 2

Statistical differences in TBC in relation to the farm

Farm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1		ns	ns	b	c	ns	ns	ns	ns	c	c	ns	c	ns	ns	ns	c
2	ns		ns	ns	b	ns	c	ns	b	c	c	ns	b	ns	ns	ns	c
3	ns	ns		ns	ns	ns	c	c	c	c	a	ns	ns	ns	ns	c	ns
4	b	ns	ns		ns	ns	c	c	c	a	ns	ns	ns	b	ns	c	ns
5	c	b	ns	ns		c	c	c	c	ns	ns	ns	ns	c	ns	c	ns
6	ns	ns	ns	ns	c		c	ns	ns	c	c	ns	c	ns	ns	ns	c
7	ns	c	c	c	c	c		ns	ns	c	c	c	c	ns	c	ns	c
8	ns	ns	c	c	c	ns	ns		ns	c	c	b	c	ns	b	ns	c
9	ns	b	c	c	c	c	ns	ns		c	c	c	c	ns	c	ns	c
10	c	c	c	a	ns	c	c	c	c		ns	c	ns	c	c	c	ns
11	c	c	a	ns	ns	c	c	c	c	ns		b	ns	c	b	c	ns
12	ns	ns	ns	ns	ns	ns	c	b	c	c	b		ns	ns	ns	c	a
13	c	b	ns	ns	ns	c	c	c	c	ns	ns	ns		c	ns	c	ns
14	ns	ns	ns	b	c	ns	ns	ns	ns	c	c	ns	c		ns	ns	c
15	ns	ns		ns	ns	ns	c	b	c	c	b	ns	ns	ns		c	ns
16	ns	ns	c	c	c	ns	ns	ns	ns	c	c	c	c	ns	c		c
17	c	c	ns	ns	ns	c	c	c	c	ns	ns	a	ns	c	ns	c	

a = p<0,05 considered significant; b = p<0,01 considered very significant; c = p<0,001 considered extremely significant; ns = p>0,05 considered not significant

Table 3

Total bacterial counts (TBC × 10^3 × ml^{-1}) considering the season

	Winter	Spring	Summer	Autumn
n	102	102	102	102
Mean	61,327**	70,276	93,700**	72,793
SD	80,920	85,032	102,244	85,336
Median	23,025	30,290	63,100	39,000
Minimum	97,000	0,100	0,210	0,153
Maximum	298,000	307,000	351,000	306,000

** P<0,01 considered very significant

3. CONLUSIONS

3.1. The obtained results indicate a high degree of microbial contamination of the raw milk in the majority of the investigated farms. This fact denotes the poor cleaning of the milking systems, milking dirty udders, maintaining an unclean milking and housing environment.

ACKNOWLEDGMENT

This work was supported by CNCSIS – UEFISCSU, project number 1095 PNII – IDEI 1492/2009.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

ASSESSMENT OF DAIRY COW LOCOMOTION IN FARMS FROM BRASOV COUNTY

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Keywords: locomotion scoring, lameness, tie-stall.

SUMMARY

This study aimed at assessing the locomotion of dairy cattle in farms from Brasov county, in order to identify the animals with locomotion problems. We evaluated 372 cows in five farms with tie stalls. The locomotion scoring was made using the system developed by Sprecher *et al*. Finally we calculated the percentage of the cows with normal locomotion (locomotion score LS = 1) and of those with different degrees of lameness (LS = 2, LS = 3, LS = 4, LS = 5) per farm and per total number of assessed cows. In the investigated farms the percentage of cows with score one (normal) varied between 21,87% and 40,47%. Score two (mildly lame) was found between 43% and 62,5% of the cows, score three (moderately lame) between 7,14% and 22%, score four (lame) between 2% and 4% and score five (severely lame) between 0% and 3,12%. Within the assessed 372 cows 118 (31,72%) cows had normal gait (LS = 1), 176 (47,31%) were mildly lame (LS = 2), 63 (16,93%) were moderately lame (LS = 3), 9 (2,42%) were lame (LS = 4) and 6 were (1,61%) severely lame (LS = 5). In the investigated farms the prevalence of lameness was as follows: 18,75% in farm A, 16% in farm B, 11,4% in farm C, 15,62% in farm D and 23,5% in farm E. The mean prevalence of lameness was 20,97%. The obtained results showed that more than half of the assessed cows presented locomotion problems, the severity of lameness degree varying among the investigated farms.

Locomotion scoring has proven to be a useful tool for not only determining the prevalence of lameness on a farm, but also for making farmers more aware of cows with lameness problems. This helps the earlier identification of the lame cows, preventing some more life threatening severe lesions that may be viewed as major cow welfare problems (Whay, 2002). Lameness in dairy cows is a painful condition and causes economic losses (Esslemont and Kossaibati, 1997) through early culling (Booth *et al.*, 2004) and reduced milk yield (Amory *et al.*, 2008). A variety of locomotion scoring systems have been developed for dairy cattle (Manson and Leaver, 1988; Tranter and Morris, 1991; Whay *et al.*, 1997; Sprecher *et al.*, 1997; Whay, 2002). There are important practical differences between the systems when they are applied in the field.

Locomotion scoring is a relatively quick and simple qualitative assessment of the cow's ability to display normal gait. If locomotion scoring is done monthly, it can be used to identify the individual cows at risk of becoming clinically lame. The score should be examined in order to find out the causes of their lameness. Group-profile lameness scores can be used to estimate the future losses in milk production at dairy level or at the level of a specific group of cows within the dairy. The estimated losses can help to determine whether general interventions are more beneficial or managerial or nutritional measures are more profitable. Finally, the locomotion score profiles kept in the records can provide a mobile indicator of the extent of lameness and can monitor the impact of the interventions meant to ameliorate lameness (Nordlund *et al.*, 2004).

The aim of this study was the assessment of cattle locomotion in farms from Brasov county to identify the cows with different degrees of lameness.

1. MATERIAL AND METHODS

The study was performed in five dairy cattle farms (noted as A, B, C, D and E) with tie stalls, in Brasov county, in January 2010. All the barns were closed, with solid flooring. In four of the investigated barns the manure removal was done manually (once or twice a day). All of the barns were naturally ventilated. The cows were kept on short beds. In four barns bedding was used (straw, sawdust) on the cows' bed. The cows were tied in the barns in the cold season (pasturing in the rest of the year). In four of the investigated farms the cows had access to paddocks in the cold season as well. Each of the farms was visited once. With the occasion of that visit, the locomotion scores of all of the lactating cows (48 cows in farm A, 50 in farm B, 42 in farm C, 32 in farm D and 200 in farm E) were recorded. A total number of 372 dairy cows were assessed. The locomotion score was determined by two observers working together, as the cows were moved to the paddocks. Each animal was observed standing and walking (on a concrete surface whenever possible) using the Sprecher *et al*. (1997) scoring system. According to this system one point is conferred to the cows with normal gait (lameness score LS = 1), two points to the mildly lame cows (LS = 2), three points to those moderately lame (LS = 3), four points to the lame cows (LS = 4) and five points to the severely lame cows (LS = 5). A total number of 372 dairy cows were assessed. The percentage of the cows with normal locomotion (LS = 1) was calculated and of those with

different degree of lameness (LS = 2, LS = 3, LS = 4, LS = 5) per farm and per total number of assessed cows.

2. RESULTS AND DISCUSSION

The results of the locomotion assessment of the cows in the five investigated farms are shown in table 1. It can be observed that the percentage of the cows with the lameness score of one (LS = 1 meaning normal) varied between 21,87% and 40,47%, the lameness score of two (mildly lame) between 43% and 62,5%, the score of three (moderately lame) between 7,14% and 22%, the score of four (lame) between 2% and 4% and the score of five (severely lame) was between 0% and 3,12% of the cows.

Table 1

Locomotion score distribution for investigated farms

Farm	Locomotion score				
	1	2	3	4	5
A	31,25	50	16,67	2,08	0,00
B	32	52	10	4	2
C	40,47	47,62	7,14	2,38	2,38
D	21,87	62,5	9,37	3,12	3,12
E	31,5	43	22	2	1,5

Figure 1 presents the distribution of the locomotion scores in the 372 assessed dairy cows. Among all of the evaluated cows 118 (31,72%) had normal gait (LS = 1), 176 (47,31%) were mildly lame (LS = 2), 63 (16,93%) were moderately lame (LS = 3), 9 (2,42%) were lame (LS = 4) and 6 (1,61%) were severely lame (LS = 5).

The locomotion scoring system suggested by Sprecher *et al.* (1997) was used because of the clear objective descriptions that differentiate each score. The percentage of cows without any gait abnormalities (LS = 1) was slightly higher than the percentage obtained by Espejo *et al.* (2006) in their assessment of cows housed in 50 free-stall barns in Minnesota (approximately 20%) and more reduced than the percentage reported by Cook (2003) in 30 dairy farms in Wisconsin (54,9%). Almost half of the cows were classified as having locomotion abnormalities (LS = 2), but not clinically lame. This result is congruent with those obtained by Espejo *et al.* (2006). Many reasons could explain this imperfect locomotion such as the presence of mild or chronic lesions that are not painful enough to cause clinical lameness, abnormal

conformation of legs or claws, pain in other body areas or problems in the evaluation because of slippery or irregular floors.

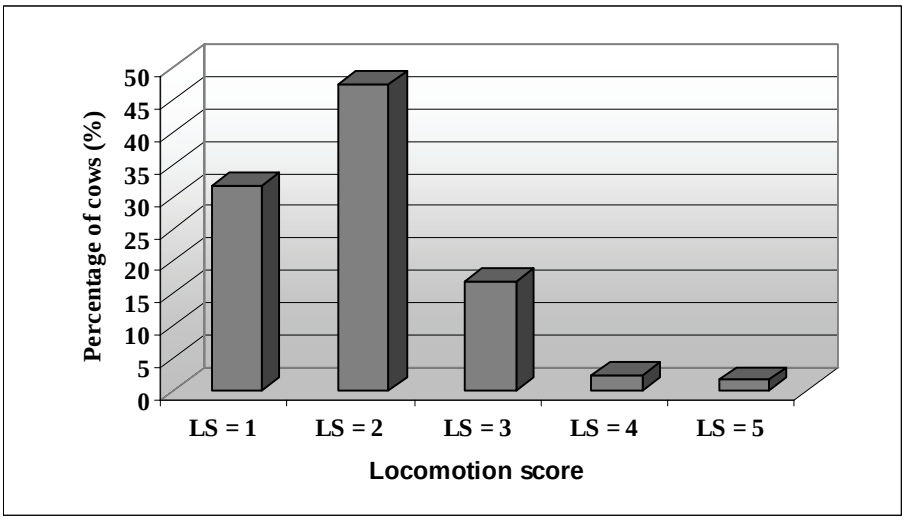

Fig. 1. Locomotion score distribution in the 372 assessed dairy cows

The cows with the locomotion score of three represented 16,93% of the total, less than half of the percentage obtained by Tadich *et al.* (2010) in their study. Approximately 4% of the cows had LS ≥ 4. This percentage is smaller than that observed by Espejo (2006), in which the proportion of cows with LS ≥ 4 was 6%. In our study the percentage of cows with lameness score five was higher than that reported by Tadich *et al.* (2010).

In all of the farms most of the cows had a lameness score of two, being considered mildly lame. Among the evaluated farms the highest percentage of cows with different degrees of lameness was recorded in farm E. By putting in the "lame" category all the cows which obtained a lameness score higher than two (Amory *et al.*, 2006) the lameness prevalence can be established at farm level (for each of the farms) and also the mean prevalence. In the investigated farms the lameness prevalence was: 18,75% in farm A, 16% in farm B, 11,4% in farm C, 15,62% in farm D and 23,5% in farm E (Fig. 2).

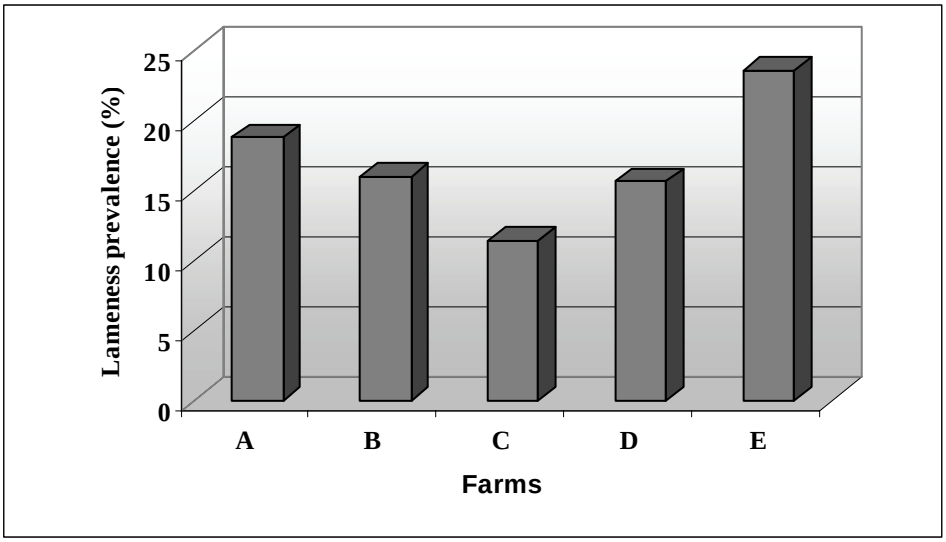

Fig. 2. The lameness prevalence in the investigated farms

The mean prevalence of lameness in the five farms was 20,97%. One of the causes for the higher percentage of lame cows in farm E could be the lack of exercise in the paddock in the cold season, as it is mentioned in the scientific literature (Regula *et al.*, 2004). The mean prevalence of lameness found in the farms we investigated is comparable with the results of the study done by Cook (2003) in tie-stall farms in Wisconsin (21,7%). Regula *et al.,* (2004) found a lameness prevalence of 21% (in 1999) and 17% (in 2000) in Swiss dairy cows kept in tie-stalls with minimal outdoor access during winter, whereas in tie-stalls with regular outdoor exercise the prevalence of lameness was lower throughout the year. If lameness prevalence exceeds 15% of the herd, it is important to differentiate the cause (Cook, 2003; Nordlund, 2004).

3. CONLUSIONS

3.1. More than half of the assessed cows showed locomotion disorders.

3.2. The severity of lameness varied among the investigated farms.

3.3. The obtained results indicate that specific measures need to be implemented immediately to decrease lameness in 60% of the evaluated farms.

ACKNOWLEDGMENT

This work was supported by CNCSIS – UEFISCSU, project number 1095 PNII – IDEI 1492/2009.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

CONSEQUENCES OF SIX MONTHS POTASSIUM DICHROMATE INTAKE ON SERIC TESTOSTERONE AND LH LEVEL IN MALE RATS

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Key words: chromium VI, male rats, sex hormones

SUMMARY

The aim of the study was the evaluation of potassium dichromate impact on biochemical biomarkers for testicular function (testosterone and LH level) in male rats after six months intake. The study was carried out on 28 white Wistar male rats equally divided in three experimental and one control group. Experimental group were exposed via drinking water as followed: E_1: 25 ppm Cr VI (LOAEL); E_2: 50 ppm Cr VI; E_3: 75 ppm Cr VI. The experiment was performed in compliance with the national law and international regulations: 143/400/2002; 471/2002; 205/2004; 206/2004; 9/2008; 86/609/CEE.

The study pointed out: significant decrease of seric testosterone level comparative to control group (E_1/C: -51.93%; E_2/C: -70.02%; E_3/C: -85.17%) and in inverse, significant correlation to exposure level (E_2/E_1: -37.65%; E_3/E_2: -50.50%; E_3/E_1: -69.13%); significant increase of seric LH level comparative to control group (E_1/C: +21.6%; E_2/C: +39.6%; E_3/C: +49.2%) and in direct, significant correlation to exposure level (E_2/E_1: +14.8%; E_3/E_2: +6.87%; E_3/E_1: +22.69%).

In experimental groups testosterone levels were under physiological limits (E_1/Ph: -35.2%; E_2/Ph: -59.6%; E_3/Ph: -80.0%), and LH levels were over physiological limits (C/Ph: +900%; E_1/Ph: +1116%; E_2/Ph: +1296%; E_3/Ph: +1392%).

In the world persists major concern about the increase of reproductive system disorders in human males. It is believed that the reason is the increased stress, lifestyle factors, and presence in the environment of endocrine altering chemicals. Occupational activities may involve constant exposure to toxic agents and may have detrimental effect on human reproduction (Giwercman *et al*., 1993).

Hexavalent chromium compounds, used in different industrial processes like stainless steel production, chromite ore processing, welding, chrome plating and tanning are widespread environmental contaminants and considered carcinogenic for animals and humans (Gambelunghe *et al*., 2003).

The aim of this study was the evaluation of potassium dichromate impact on biochemical biomarkers for testicular function (seric testosterone and LH level) in male rats after six months of exposure.

1. MATERIALS AND METHODS

The study was carried out on 28 white Wistar male rats divided in three experimental and one control groups. Experimental groups received potassium dichromate via drinking water for six months as followed: E_1: 25 ppm Cr VI (LOAEL) (EPA); E_2: 50 ppm Cr VI (2 x LOAEL); E_3: 75 ppm Cr VI (3 x LOAEL); control group received tap water without chromium content.

Blood samples for hormone assay were collected by cardiac puncture under anesthesia (Ketamine 50 mg/kg + Xylazine 5 mg/kg, intraperitoneal administration) following protocols and ethical procedures. Plasma samples were separated by centrifugation, frozen and stored at -20^o C until assayed. Seric testosterone and LH levels were determined by Tody Laboratories Bucharest (ISO 17025) using chemiluminiscence method. The amount of testosterone and LH was expressed as ng/ml.

Animals had free access to food and water.

The experiment was performed in compliance with the national law and international regulations: 143/400/2002; 471/2002; 205/2004; 206/2004; 9/2008; 86/609/CEE.

The results were statistically analyzed by Anova method and Student test.

2. RESULTS AND DISCUSSIONS

The results are presented in table 1 and figure 1.

Table 1

Seric testosterone and LH (ng/ml) level in control and experimental groups

Groups	Testosterone			LH		
	x±Sx	**SD**	**CI 95%**	**x±Sx**	**SD**	**CI 95%**
C	3.37±0.01	0.03	0.01	5.00±0,01	0.01	0.01
E_1	1.62±0.01*	0.01	0.01	6.08±0,01*	0.01	0.01
E_2	1.01±0.01*	0.01	0.01	6.98±0,01*	0.01	0.01
E_3	0.50±0.01*	0.01	0.01	7.46±0,01*	0.01	0.01

E/C *: p<0.01

In exposed individuals testosterone level decreased significantly (p 0.01) comparative to control group (E_1/C: -51.93%; E_2/C: -70.02%; E_3/C: -85.17%) being inversely, significantly (p 0.01) correlated to exposure level (E_2/E_1: -37.65%; E_3/E_2: -50.50%; E_3/E_1: -69.13%).

Testosterone level was higher than physiological limits (Ph) (2-3 ng/ml – Krinke, 2000) in C group (C/Ph: +34.8%) but visibly lower in E groups (E_1/Ph: -35.2%; E_2/Ph: -59.6%; E_3/Ph: -80.0%).

After six months of exposure in all E groups significant increase (p 0.01) of seric LH level comparative to C group (E_1/C: +21.6%; E_2/C: +39.6%; E_3/C: +49.2%), and in direct, significant (p 0.01), correlation to exposure level (E_2/E_1: +14.8%; E_3/E_2: +6.87%; E_3/E_1: +22.69%) was recorded.

Comparative to physiological limit (Ph) (0.5 ng/ml – Krinke, 2000) seric LH level was higher in all groups, more obvious in E groups (C/Ph: +900%; E_1/Ph: +1116%; E_2/Ph: +1296%; E_3/Ph: +1392%).

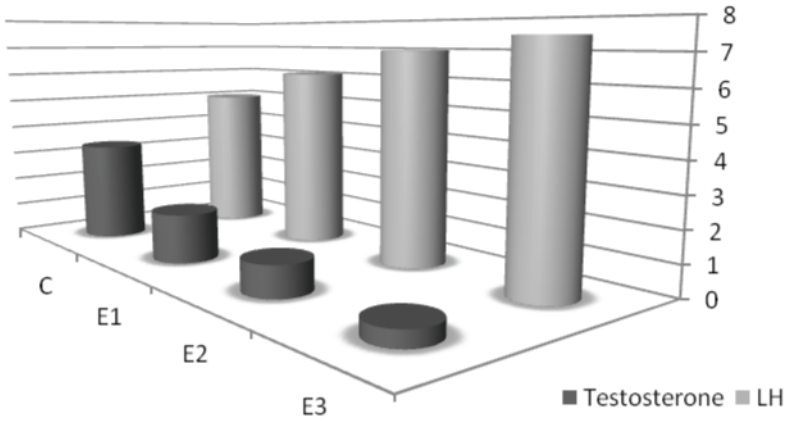

Fig.1 Seric testosterone and LH level dynamics after potassium dichromate exposure

Excessive generation of reactive oxygen species (ROS) and free radicals constitutes the oxidative stress in different organs of body, including testes. The oxidative stress is the major fact that has to be accepted for understanding male infertility (Shrama and Agarwal, 1996). ROS are produced during steroidogenesis and they are responsible for damages steroidogenic pathways (Zirkin and Chen, 2000).

Cao *et al*. indicated that overproduction of ROS reduced levels of enzymatic and non-enzymatic antioxidants in Leydig cells, thus resulting in testosterone secretion decline (Cao *et al*., 2004). Bekpinar and Tugrul described damage of testicular tissue and decrease of testosterone levels, suggesting that oxidative stress has effect at the level of gonadal steroid biosynthesis (Bekpinar and Tugrul, 1995).

Results obtained by Ernst and Bonde and Li *et al*., Muselin *et al*., Trif *et al*., Rankov *et al*. regarding LH seric level are similar with presented ones, but are in contradiction with Chandra *et al*. results (Ernst and Bonde, 1992; Li *et al*., 1999; Muselin *et al*., 2007; Trif *et al*., 2009; Rankov *et al*., 2009; Chandra *et al*., 2007)

Results related to seric testosterone level decrease are similar to those obtained by Ernst and Bonde, Yousef *et al*. and Chandra *et al*. (Ernst and Bonde, 1992; Yousef *et al*., 2006; Chandra *et al*., 2007).

Our results sustain affirmation that hexavalent chromium is an important reproductive toxicant as Office of Environmental Health Hazard Assessment (OEHHA) and the Developmental and Reproductive Toxicant Identification Committee (DART IC) mentioned in 2007.

3. CONCLUSIONS

3.1 The exposure of adult male rats for six months to potassium dichromate (Cr VI) in drinking water (25, 50 and 75 ppm Cr) determined:

3.2 Significant decrease, under physiological limit, of seric testosterone level comparative to control group and in inverse significant correlation to exposure level;

3.3 Significant increase, over physiological limit, of seric LH level comparative to control group and in direct, significant correlation to exposure level.

ACKNOWLEDGEMENTS

Acknowledgements to CNCSIS, grant no. 90/2008 obtained by Jelena Rankov.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

ANAESTHETHIC PROTOCOL FOR CLOSED REDUCTION OF HIP DISLOCATION IN THE DOG

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Key words: ketofol, dog, orthopedic procedure

SUMMARY

Fifteen dogs were anesthetized with ketofol prepared as a 1:1 mixture of ketamine 10 mg/mL and propofol 10 mg/mL mixed in same syringe, for fluoroscopic diagnostic and for closed reduction of hip dislocation. Median ketofol dose was 2.5 mg/kg of each agent. Twelve dogs tolerated the procedure well; the quality of analgesia was satisfactory in almost every case. Thirteen dogs recovered from anesthesia without complications. Dogs were able to walk unassisted in 18 minutes after last ketofol administration (range 15 to 37 minutes). Ketofol is effective for painful diagnostic examination and therapeutic orthopedic procedure.

Propofol, a sedative-hypnotic drug, had a rapid onset with very short duration of action, smooth induction and recovery, although frequently causes hypotension and apnea when administered in bolus form and provides little analgesia (Lee, 2006). Ketamine, a dissociative anesthetic, is one of the few anesthetic with cardiovascular stimulant properties, reported to provide potent somatic analgesia (Webster, 2001). The use of ketamine as a single anesthetic agent in dog has been limited by the occurrence convulsions when used alone and by the increase of muscle rigidity (Lee, 2006). Ketamine and propofol administered in combination from separate syringes has been used successfully in dog in a variety of settings (Intelisano *et al.*, 2008, Lerche *et al.*, 2000), however they are not used together by the veterinarians. Administering ketamine and propofol mixed in the same syringe, so-called ketofol, has been shown to be efficacious in the operating room and in ambulatory settings in human beings (Andolfatto *et al.*, 2010, Arora, 2008, Arora *et al.*, 2007, Camu and Vanlersberghe, 2002, Daabiss *et al.*, 2009, Mortero *et al.*, 2001). The ketofol anesthetic protocol use for dogs was selected on the basis of studies performed in humans, and the purpose of the study was to evaluate clinical efficacy for short painful diagnostic and orthopedic procedures.

1. MATERIAL AND METHOD

Fifteen dogs (12 males and 3 females, aged between 3 to 7 years, weights ranged from 5.5 kg to 21 kg) with presumptive diagnosis of recent hip dislocation were enrolled in the study. Dogs were anesthetized for fluoroscopic diagnostic and for closed reduction as deemed suitable.

Ketofol was prepared as a 1:1 mixture of ketamine 10 mg/mL and propofol 10 mg/mL mixed in a 10-mL syringe, and was administered intravenously, through an indwelling catheter placed in cephalic vein. The initial bolus was 1 mg/kg of each agent with repeat boluses of 0.5 mg/kg of each agent. Adjustment of ketofol doses, at the discretion of the treating veterinarian, was performed to maintain the level of profound sedation that permitted dogs to lie in lateral recumbency with minimal manual restraint.

Vital signs were recorded every two minutes during the procedure and every five minutes after the procedure until the dog was lying in strenal recumbency with head up.

Outcomes of interest were: the quality of analgesia, induction and recovery based on clinical experience, occurrence of significant hypotension, respiratory depression, the appearance of other adverse event, and times to standing.

Data are presented as percentage of frequency of occurrence or as median with ranges.

2. RESULTS AND DISCUSSIONS

All dogs were adequately sedated in less of two minutes. Induction was uneventful and smooth. Apnea and cyanosis is a characteristic of induction of anesthesia with propofol but not for ketofol. Twelve dogs tolerated the procedure well with ketofol anesthesia. The quality of analgesia was satisfactory in almost every case, no gross purposeful movements through 8 to 13 minutes after the last ketofol bolus administration. Three dogs (20% of patients) received an additional propofol bolus of 2 mg/kg to increase the depth of anesthesia and to counteract suboptimal muscle relaxation.

The median dose of ketofol administered was 2.5 mg/kg each of propofol and ketamine (range 1 to 3.5 mg/kg). The relative large ketofol dosage interval reflects individual variability in sedative response. Slightly lower propofol doses (2.5 mg/kg) were used than may be

typical in other reports (6-8 mg/kg after no premedication) represents an advantage reflected in the decrease of adverse effects or recovery time.

Cardiovascular effects were minimal, with slight increase in pulse rate and blood pressure. No dog had significant hypotension; changes in mean arterial pressure compared with the baseline (median 116 mmHg) had ranges of 110 to 128 mmHg (median 121 mmHg). No patient had a respiratory depression or required airway support.

Thirteen dogs (86.6%) recovered from anesthesia without complications. During the recovery period, one dog (6.6%) exhibit dysphoria and one (6.6%) had excessive salivation and vomiting. No other adverse reactions or excitatory responses occurred after ketofol. Dogs were able to walk unassisted in 18 minutes after last ketofol administration (range 15 to 37 minutes).

The use of ketamine in conjunction with propofol has been shown in procedural sedation for human beings to reduce the dose of propofol required to achieve sedation (Mourad *et al.*, 2004) and this combination is believed to result in less toxicity than either drug alone because their complementary effects enable the use of lower doses of each drug (Camu and Vanlersberghe, 2002). In theory, the opposing hemodynamic and respiratory effects of each drug might be complementary and minimize overall adverse effects (Willman and Andolfatto, 2007).

The median recovery time of 18 minutes in our study is comparable to that of other anesthetic protocols noted for their rapid recovery times. Only 20.66% of dogs showed a recovery time longer than 20 minutes. Studies on propofol, as injectable anesthetic agent, report that in unpremedicated dogs given one dose (mean dose 5.95 mg/kg) recovery was complete in a mean time of 18 minutes and after maintenance of anaesthesia by intermittent injection the mean recovery time was 22 minutes from administration of the last dose (Watkins *et al.*, 1987). Other report indicates a short duration of surgical anesthesia, 2-10 minutes, and a complete recovery within 20-30 minutes (Webster, 2001). When intravenous ketamine is used in a dose of ½ mg/kg IV somatic analgesia without catalepsies is up to 30 minutes (Slatter, 2003).

The reports in the literature showed that propofol induced a variable period of apnea in 85% of dogs (Smith *et al.*, 1993). In our study apnea and cyanosis/hypoxia didn't appear.

Propofol and ketamine are effective for painful diagnostic and therapeutic orthopedic procedures when used together in a single syringe. This combination showed a positive synergistic effect when compared to both drugs alone. The relatively small size of our study warrants further investigation regarding safety and the incidence of

adverse effects. Additional studies are needed to help delineate the optimal dosages and the most appropriate performed procedure with this anesthesia.

3. CONCLUSIONS

3.1. Ketofol is suitable alternative for dog deep sedation and analgesia, and appeared to be adequate when is performed a painful therapeutic orthopedic procedure.

ACKNOWLEDGMENTS

This work was supported by CNCSIS-UEFISCSU project number PN II-IDEI 130/2008.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

PREVALENCE OF *GIARDIA* SPP. INFECTION, ASSOCIATED OR NONASSOCIATED WITH *CRYPTOSPORIDIUM* SPP. AND OTHER PARASITES IN DOGS IN TIMIŞ COUNTY

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Key words: *Giardia* spp., dogs, parasitic fauna, prevalence.

SUMMARY

This study was conducted to determine the prevalence of the *Giardia* spp. infection, in dogs from Timiş County and to analyze the potential risk factors that support this infection (gender, age). The examinination of the samples was accomplished using flotation method (Willis), direct examination using Lugol solution and by *Cryptosporidium* and *Giardia* Rapid Test (Vegal Farmaceutica, Spain). Parasitic fauna of dogs from Timiş County was represented by *Giardia* spp., (48%) *Cryptosporidium* spp. (6 %), *Isospora* spp. (11%), *Toxocara* spp. (29%), *Ancylostoma* spp. (14%) and *Trichocephalus* spp. (9%). Age up to six months is an important risk factor. The breed and gender did not represent any considerable risk factors.

Giardiosis or lambliosis is a parasitical disease of the small intestine, found in a wide range of mammal and bird species, caused by a single-celled organism of the Genus *Giardia*. It can develop into a zoonotic disease. *Giardia* spp. is a cosmopolitan protozoan which leads to a syndrome of malabsorption found in animals and humans.

Giardiosis in dogs is considered an intriguing disease for the clinician and also for the specialists, because the prevalence of the parasite varies according to the diagnostic technique, area of study, and also according to the susceptibility of the individual host (Capelli, 2003, Carlin *et al*., 2006).

A fundamental aspect in understanding this disease is that the prevalence of giardiosis in dogs may be underestimated due to the presence of a subclinical infection, the intermittent evacuation of the cysts, and also due to low sensitivity of the diagnostic methods (Monis *et al.*, 2003).

The epidemiological surveillance regarding the prevalence of giardiosis and data regarding the main risk factors that contribute to the presence of the disease in dogs in Timiş County are unknown or incomplete (Mcglade *et al.*, 2003).

The aim of the study was to determine the prevalence of the *Giardia* spp. infection, in dogs from Timiş County and to analyze the potential risk factors that support this infection (gender, age).

Giardia spp. infection can evolve as unique pathogen agent or associated with other enteropathogen agents, including *Cryptosporidium* spp. (Hamnes *et al.*, 2007).

1. MATERIAL AND METHOD

The study period started in December 2009 and was finished in May 2010. This study included 35 dogs with different age and gender.

For an accurate epidemiological evaluation of the cases studied, records were kept in order to help in data interpretation. The dog breeds were: Beagle, German Shepherd, Bichon Frise, Bichon Maltese, Collie, Poodle, Cross Breed, Bull Mastiff, West Highland White Terrier, English Bulldog, Pinscher, Golden Retriever, Shar Pei.

The age of the dogs attending the study varied, ranging from two months old to 12 years. The dogs were divided into three study groups for statistical analysis of correlations among age and parasitic state of each dog. In study group one, dogs up to three months of age were included, study group two included dogs aged between three and six months old and study group three was formed of dogs over six months of age.

The dogs introduced in this study were from Timiş County and were examined in the Clinic of Parasitology and Parasitological Diseases of the Faculty of Veterinary Medicine Timişoara.

The samples were prelevated by vets or brought in by the owners of the dogs.

The examinination of the samples was accomplished using flotation method (Willis), direct examination using Lugol solution and by *Cryptosporidium* and *Giardia* Rapid Test (Vegal Farmaceutica, Spain). The results were submerged in the computer program Microsoft Excel in a statistical manner, using the *Anova Test* and the *Ttest*. The variable p in the results is significant when p is less or equal with 0.05.

2. RESULTS AND DISCUSSIONS

According to the data obtained from the field cases, the results regarding the parasite status of the three groups divided by age is:

Group one, consisting of 11 dogs, had 10 positive samples and one negative sample representing 9%. Among the nine positive samples

eight were found positive for *Giardia* spp., and among these, six were positive only for *Giardia* spp. (18%), and the other two were also positive for *Toxocara* spp. and *Isospora* spp. (18%).

Two samples were positive for *Toxocara* spp., *Isospora* spp. and *Ancylostoma caninum* (18%).

In the **second group** which had six dogs involved, five samples were found positive (84%) for parasites and one was negative (16%). From the positive ones, two samples were positive only for *Giardia* spp. (33%) and two had associated parasitic infection with *Ancylostoma* spp. and *Toxocara* spp. (33%). *Cryptosporidium* spp. was identified in one sample (16%).

Group three consists of 18 dogs had 11 positive samples (61%) for parasites and seven negative (39%). Among the positive samples five were infected with *Giardia* spp. In three samples was identified *Giardia* spp. as single parasite (17%). One sample was positive for *Giardia* associated with *Ancylostoma caninum* and *Toxocara* spp. (6%) and one associated with *Cryptosporidium* spp. (6%). In six samples *Toxocara* spp., *Isospora* spp., *Trichocephalus* spp., and *Ancylostoma caninum* (33%) were identified.

Measuring the results found in the three groups divided by age, the prevalence of the *Giardia* spp. infection was 73% in the first group 67% in the second group and 28% in the third group.

Regarding the gender factor, among the 35 samples examined, 18 belonged to males and 17 belonged to females. From the samples derived from **males**, 13 were identified positive for parasites. From these, five were positive only for *Giardia* spp. (29%), three were positive for *Ancylostoma* spp., *Giardia* spp. and *Toxocara* spp. (18%), and five samples were positive for other parasites than *Giardia* spp. (35%).

Among the samples derived from the **females**, 12 were positive for parasites. Six of these were positive only for *Giardia* spp. (35%), three were positive for *Giardia* spp., *Isospora* spp., *Ancylostoma* spp. and *Toxocara* spp. (18%), and in three samples other parasites than *Giardia* spp. were identified (18%).

In the final analysis of the results obtained in this study, 31% of the samples were found positive only for *Giardia* spp., and 17% of the cases *Giardia* spp. was associated with other parasites. Other parasites were found in 26% of the samples and 26% of them were negative for the parasites included in the study.

The samples from the dogs involved in the study had a parasitic fauna consisting of *Giardia* spp. (48%), *Cryptosporidium* spp. (6 %),

Isospora spp. (11%), *Toxocara* spp. (29%), *Ancylostoma* spp. (14%) and *Trichocephalus* spp. (9%).

In Romania, Jarca Adriana in 2008, identified a extensivity of giardiosis in dogs from seven localities from Satu - Mare County, of 51,08 % (Jarca Adriana *et al*, 2008).

A study conducted by Meireles Paola in 2008, regarding the dogs in the metropolitan area of the city of Curitiba, Parana´ South of Brazil, demonstrated a prevalence of the *Giardia* spp. infection with values from 16 to 24 %(Meireles Paola *et al.,* 2008).

Regarding the age of the dogs involved in the study, the highest prevalence was identified in dogs from three to six months of age, meaning 33 to 38%.

Age groups until three months and four to six months had high prevalence (73 and 67%) and prevalence in age group over six months was 28%.

The same study shows that by gender the prevalence was 23.73% for males and 24.39% for females. The study carried out in our clinic revealed a prevalence of 45% in males and 53% in females (Meireles Paola *et al.,* 2008).

In the study conducted in our clinic, the prevalence according to breed was not tacked into account because of the small number of medical cases.

Another study conducted in Sao Paulo SP, Brazil, showed a prevalence of 12.2% (Oliveira Sequeira *et al.,* 2002), in Rio Grande do Sol, the prevalence was 34% in a case study, in Floriano 32%, and 34% in Belo Horizonte-MG (Beck *et al.,* 2005, Gennari *et al.,* 2002). In Uberlandia-MG, Brazil, 29% of the dogs in the study were positive for the *Giardia* spp. infestation (Mundim *et al.,* 2007).

A study conducted in Pennsylvania, U.S.A., demonstrated a prevalence of 4.7% (O'Handley *et al*., 2000). In the Center and Northern Italy the prevalence had a value of 21.3% (Capelli, 2003) and in Japan the prevalence was 14% (Itoh *et al*., 2005).

Regarding to the groups divided by age, dogs up to six months were more receptive to the infection with *Giardia* spp. At the age of three months, cubs are separated from their mothers. Due to this fact, stress installs allowing different factors such as immunological estate, environment and water source to intervene, all three making the cubs susceptible to infections.

According to Mundim in 2007, the disease manifests clinically more frequent in young animals (Mundim *et al*., 2007). Lallo in 2003, say that the clinical expression of the disease is dependent upon the factors

related to host, immunity response and factors related to the parasite (Lallo *et al*., 2003).

In Italy, Paoletti Barbara, in 2006, suggested a prevalence of 26.6 % for *Giardia* spp. infection in dogs tested (Paoletti Barbara, 2006).

Barutzki and Schaper in 2003, in Germany, between 1999 and 2002, conducted a study about endoparasites in dogs and observed 16.6% positive samples for *Giardia* spp (Barutzki and Schaper, 2003).

The prevalence of *Giardia* spp infestation in dogs from Norway was determined by Hamnes in 2007 with the value interval of 6.9 to 11.4, in addition to the age group taking in consideration (Hamnes *et al*., 2007).

The prevalence of *Giardia* spp. infection is rated between 5.4 and 55.2 by different authors (Barutzki and Schaper, 2003, Papini *et al*., 2005).

Giardia spp. infections were reported in dogs in different parts of the world and by different authors in: Germany (Barutzki and Schaper, 2003, Cirak, 2004, Epe *et al*., 2004), Italy (Berrilli *et al*., 2004, Papini *et al*., 2005), Czech Republic (Dubna´ *et al*., 2007), Poland (Zygner *et al*., 2006), Finland (Rimhanen-Finne *et al*., 2007), Australia (Caccio, 2005, Monis *et al*., 2003, Thompson, 2004), Canada (Lefebvre *et al*., 2006, Shukla *et al*., 2006), USA (Carlin *et al*., 2006, Monis *et al*., 2003, Thompson and Robertson, 2003), Brazil (Mundim *et al*., 2007), Japan (Abe *et al*., 2003, Itoh *et al*., 2005), Korea (Lee *et al*., 2006), India (Traub *et al*., 2004), and Thailand (Inpankaew *et al*., 2007).

The prevalence found in the ones mentioned above is 10% in domestic mature dogs, between 30 and 50% in cubs, with the possibility of overtopping these values in dog shelters (Dubna´ *et al*., 2007, Hoar *et al.*, 2001, Rosa *et al*., 2006).

3. CONCLUSIONS

3.1. The prevalence of *Giardia* spp. in dogs from Timiş County was 48%.

3.2. The *Giardia* spp. infection developed as a single parasitic infection or as a multiple parasitic infection being associated with other protozoa and nematodes. During the study cestodes were not identified.

3.3. It is a proven fact that the age up to six months is an important risk factor.

3.4. The breed and gender did not represent any considerable risk factors.

3.5. Parasitic fauna, less *Giardia* spp., of dogs from Timiş County was represented by *Cryptosporidium* spp. (6 %), *Isospora* spp. (11%),

Toxocara spp. (29%), *Ancylostoma* spp. (14%) and *Trichocephalus* spp. (9%).

ACKNOWLEDGMENTS

This study was supported by National Centre for Programs Management Romania, Grant PNCDI 2 - PC 51 - 034/2007.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

USING THE DOPPLER TECHNIQUE TO EMPHASIZE THE EMERGENCE AND DEVELOPEMENT STAGES OF VASCULARIZATION IN THE BONE CALLUS DURING FRACTURE HEALING IN DOGS AND CATS

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Key words: doppler, callus, fracture healing, dog, cat

SUMMARY

The purpose of this study is to highlight the fitness and usefulness of normal echography techniqie (B-mode) and Doppler′s, in terms of their use for determining and monotoring the dynamics of bone healing, as a result of osteosynthesis at dogs and cats.

The neovascularization of bone callus can be visualized usig Doppler ecography, and we can establish four periods during the fracture healing, when the Dopplers signals are present until these are nule.

The purpose of this study is to highlight the fitness and usefulness of normal echography techniqie (B-mode) and Doppler′s, in terms of their use for determining and monotoring the dynamics of bone healing, as a result of osteosynthesis at dogs and cats. The neovascularization of bone callus can be visualized usig Doppler ecography, and we can establish four periods during the fracture healing, when the Dopplers signals are present until these are nule (Martinoli *et al., 1998).*

1. MATERIALS AND METHODS

We have been studied a number of 21 cases, 11 dogs and 10 cats. The animals under study were between 6 months and 10 years. The fractures were located in the limbs, healed by conventional surgical methods (plate with screws, centromedular with nails or rods). The ultrasound is an veterinary power Doppler for small animals, and the transducer is a linear one, with 7,5, 10,0 and 12,5 Mhz frequency. The radiographer is an Philips, Practix 33 plus.

2. RESULTS AND DISCUSSION

The methods employed studied for all 21 cases were the normal echography (B-mode), Doppler echography and radiology. These tests were repeated from 10 to 14 days, until the fractures was healed, and the zero day was the first day after surgery. Thus, there were setted four major periods in witch the Doppler signals had considerable variations. Time postoperatively was divided into periods of 12 days.

The first period, from zero day up to day 12 (a week and a half), was esteblished as a period of minimum activity even no activity of the neovascular sistem at the level of the fracture′s focus and adiacent tissues. We are able to see this proces in the image fig.1. Fig. 2 ilustrated a detail of Doppler activity at the level of fracture in the first ten days after surgery. We have also an X-ray image of the proces, at the same time, in fig.3.

The second period, from day 13 to day 24 (three weeks almost), was a period of maximum activity of the vascular processes in the fracture′s focus. This intens process is visible in fig. 4 and fig.5, an detail of Doppler window.

During the 3rd period, from day 25 to day 36 (3 weeks to 5 weeks), Doppler signals decrease in intensity and number, until the last period, the period of total healing of bone. So that in the 4th period that we established, namely from day 37 to end of healing process, to day 72-84, the signals become very weak and finally void zero. We see in fig.6 a decrese of vascular signals in the day 41 after surgery and a detail also of the process in fig.7. Fig. 8 ilustrated a image witt callus formation, at 6 weeks after reconsrtuction of the bone.

In the image fig.9 we can see a every poor image of the vascularity at the end of healing process, in the day 67 after surgery, and in the last picture, fig. 10 a detail of this image. The X-ray is the corespondent of the echographic image, at 2 and half months after surgery in fig. 11.

3. CONCLUSIONS

3.1. The normal process of bone healing at the level of the fracture, can be traced and stadialised using Doppler technique. One can esteblish four periods durind the normal process, when the variation of blood at the fracture healing place are major and obvious.

3.2. The neovascularization in the callus is a normal process during the period of healing, and variation of number and intensity of vessels

from the process, can be visualized and monitorized using Dopper technique.

3.3. Using ecografic and radiologic methods, we can monitoring the process of callus formation and development.

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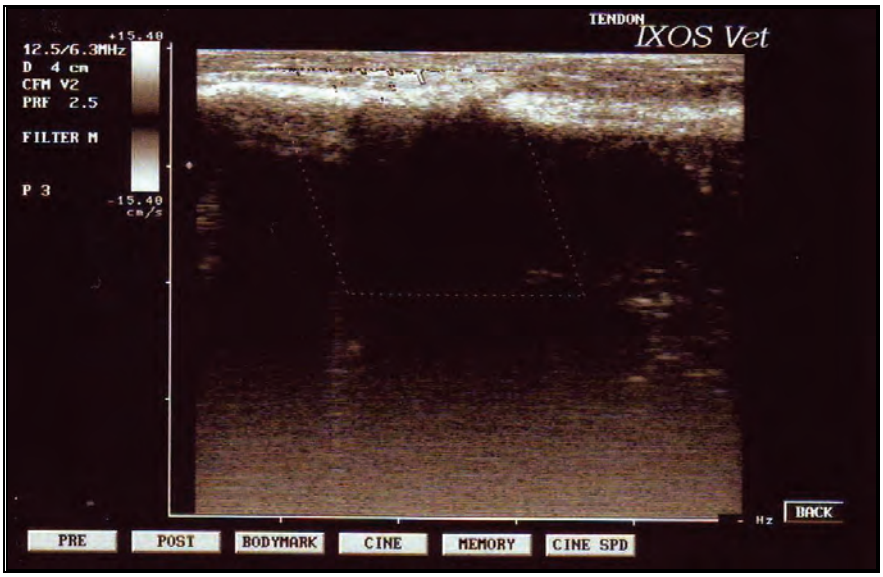

Fig. 1. Day 10 after surgery

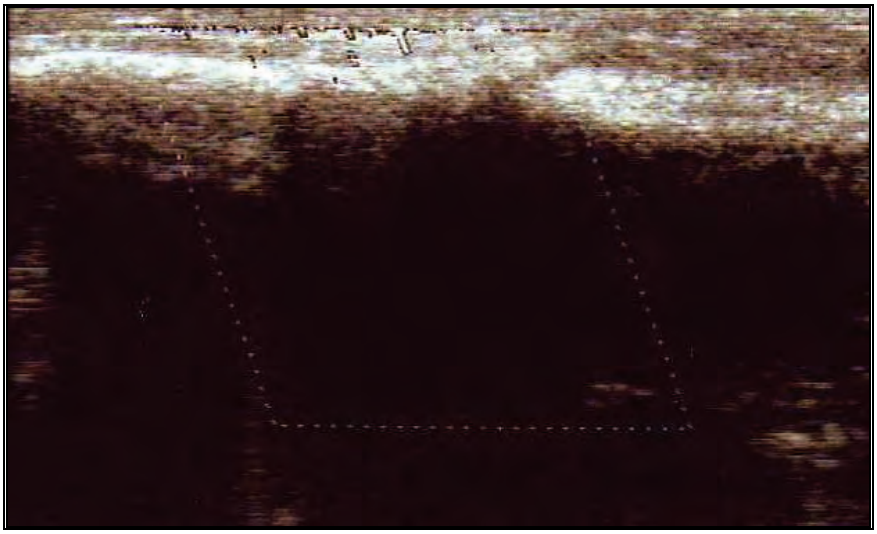

Fig.2. Detail of fig.1

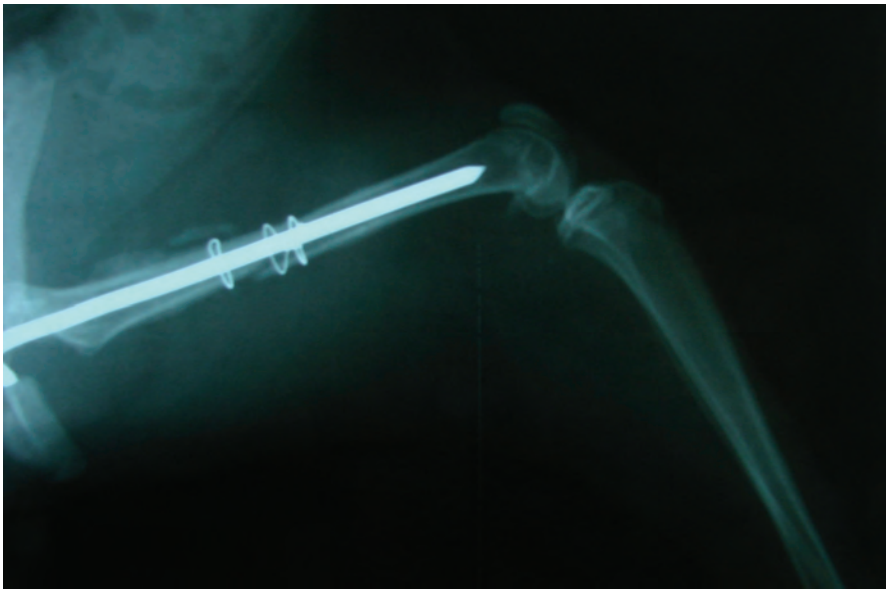

Fig.3. Radiological image after surgery, first 10 days

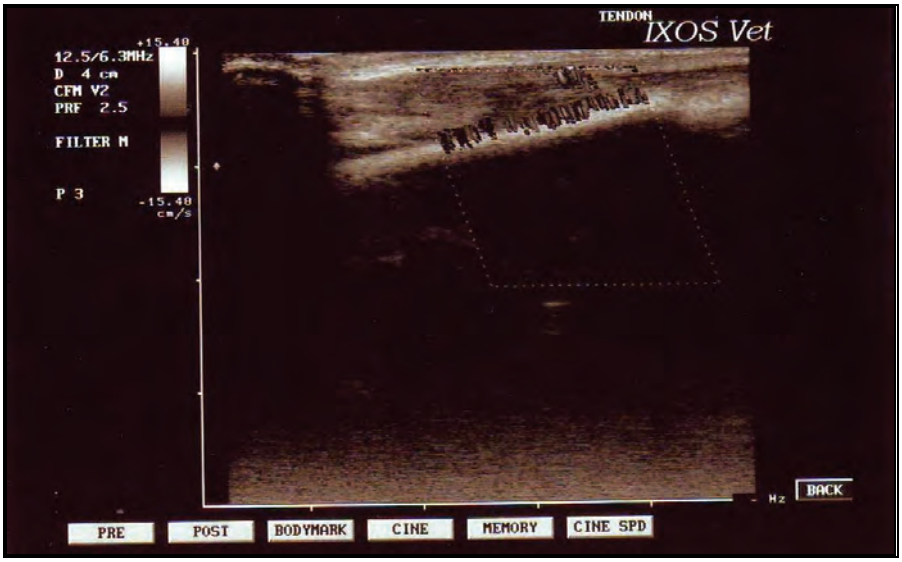

Fig. 4 Doppler image in the day 16 after surgery

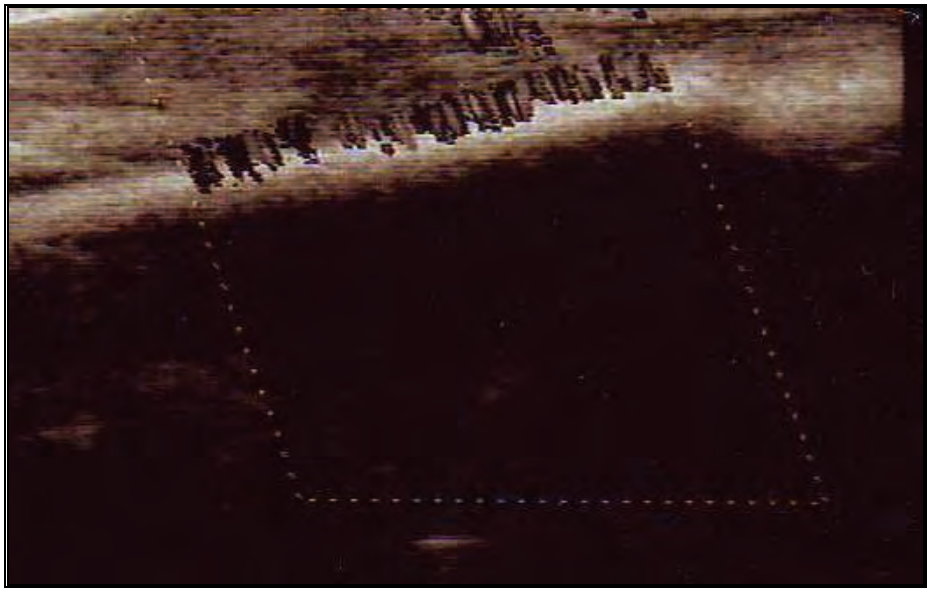

Fig. 5 Detail of fig. 4

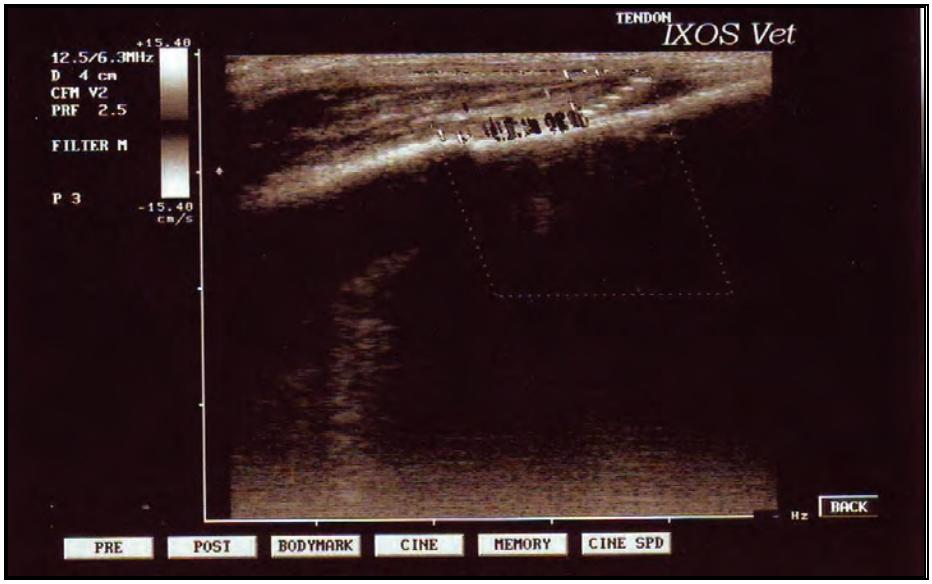

Fig. 6 Day 41 of healing process

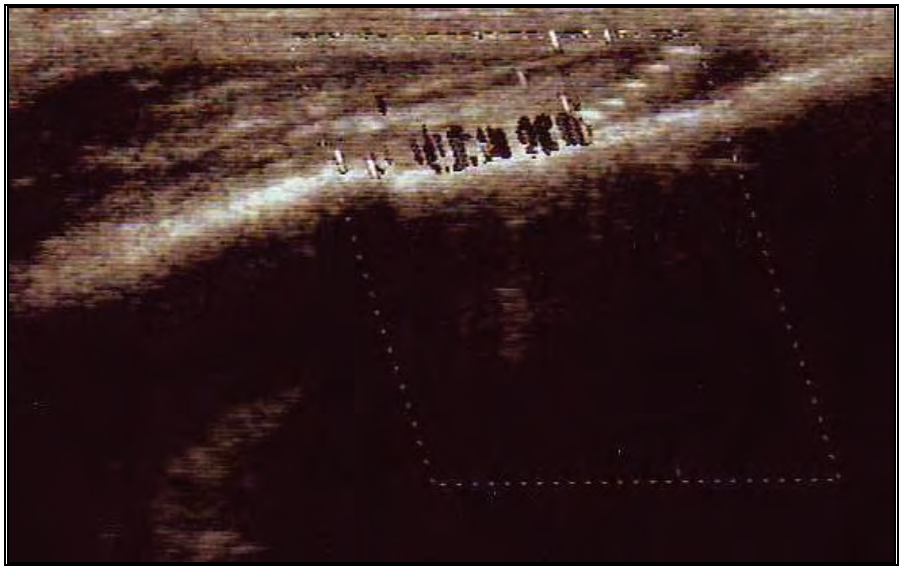

Fig 7. Detail of fig. 6

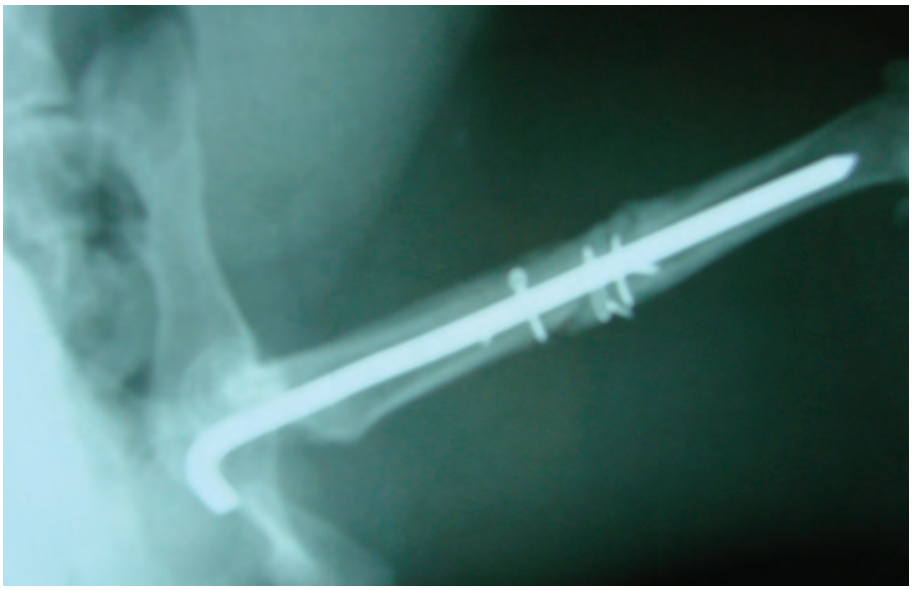

Fig. 8 Radiogfafic image of the healing process, at 6 weeks after surgery

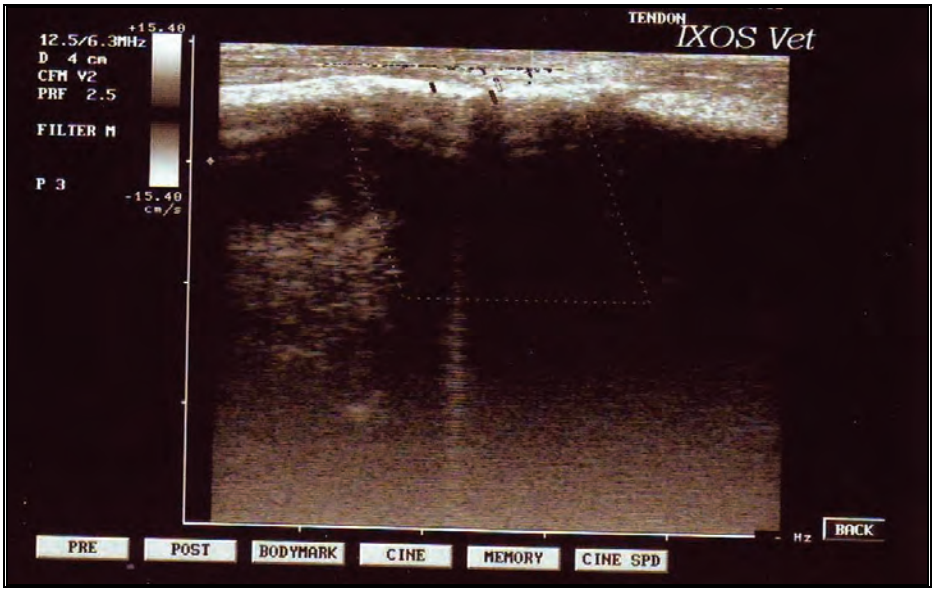

Fig. 9 Doppler image at 67 days of callus developing process

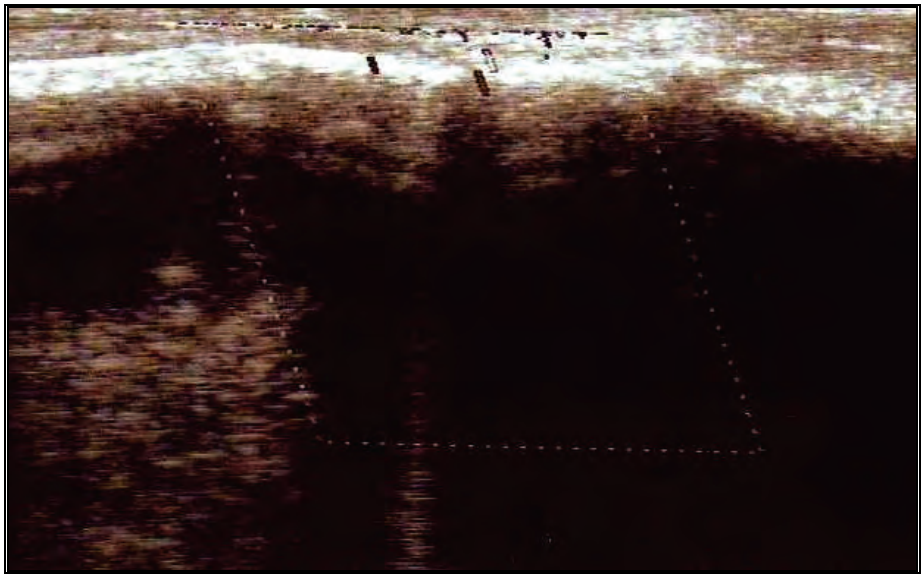

Fig. 10 Detail image of fig. 9

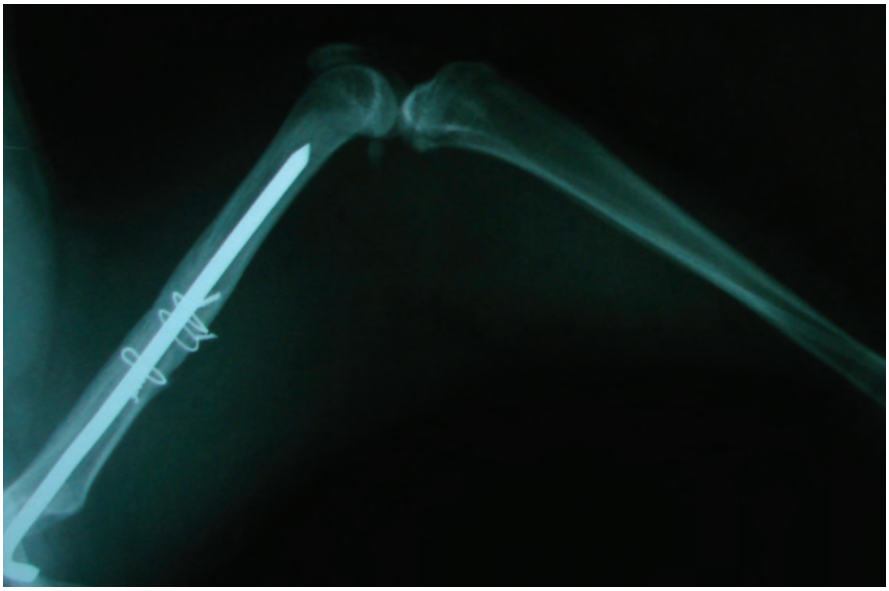

Fig.11 Radiograph image at 65 days after surgery

Scientific works, C series LVI(1), 2010
ISSN 1222-5304

STUDIES CONCERNING THE HUMORAL IMMUNE RESPONSE IN SHEEP INOCULATED AGAINST CONTAGIOUS AGALACTIA

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Key words: electrophoresis, serum protein fractions, lysosyme, G immunoglobulin

SUMMARY

The research was done on two batches of sheep> batch A (n=10) that did not get the vaccine, used as control batch, and batch B (n=10), experimental, that got the vaccine against contagious agalactia. The experiment lasted 65 days, during which time 4 blood samples were taken: when the first inoculation was performed (T_0), 21 days after the second inoculation (T_1), 45 days after the first inoculation (T_2) and 65 days after the first inoculation (T_3). The inoculation effect was evaluated by following in dynamics some humoral immunologic parametria: serum protein fractions, G immunoglobulin (IgG), total protein and lysosyme. The data was statistically analyzed using the Student-Fisher method. Using the computer analysis of electrophoresis an increase of the globulin values was seen, especially of gamma globulin in batch B in comparison with batch A, after the second inoculation (T_1). The average values of IgG and of the total protein registered a highly significant increase at T_2 and T_3 ($p< 0.001$) in the experimental batch in comparison with the control batch. The lysosyme showed high values at 45 days (T_2) and at 65 days (T_3) from the first inoculation in batch B in comparison with batch A ($p< 0.001$). The quantification of the anti-Mycoplasma agalactiae specific antibodies pointed out high values of DO after the second inoculation.

Contagious agalactia is a severe disease in sheep and goats produced especially by *Mycoplasma agalactiae* and characterized by mastitis, arthritis, keratoconjuctivitis, pneumonia and from time to time miscarriage (OIE Manual, 2008). The disease diagnosis is set after the isolation of the germ which can be identified using serological and biochemical tests and seldom by tests of molecular biology like polimerase chain reaction (PCR) (OIE, Terrestrial Manual, 2008). The antibodies detection using the complement clamping test (RFC) and the immunoenzymatic test (ELISA) give a fast diagnosis for the disease but not very sensitive for the animals in which the disease is chronic. The indirect ELISA test was used in the routine programs for the disease control. Recently the immunoblot test is proven to be the most sensitive and specific test for the *Mycoplasma agalactiae* detection (Manual of Diagnostic Tests and Vaccines for terrestrial Animals, 5th Edition, 2004). The research based on the P_{40} gene test show a new diagnosis

marker for the contagious agalactia in sheep using the PCR technique (Oracova, 2009; Rosati, 1999). The Mycoplasma induces both a humoral immune response and a cellular response in the host body with the participation of the complement, properdin, lysosyme, polymorphonuclear leukocytes, including citokines synthesis (Tîrziu, 2007; Tudose, 2010). Mycoplasma has an unspecific effect over the cells of the immune system, inducing suppression, polyclonal stimulation on B and T lymfocites, increase the macrofages cytotoxicity, NK cells, T lymfocites by inducement of the cellular receivers and by actuation of the complement system (Madanat, 2001). The specific prophilaxy of the disease is based on the usage of inactivated or live attenuated vaccines. The inactivated vaccine with formalin or with saponins, and adjuvants with aluminum hydroxide or with mineral oils gave good results in the current prophilaxy, and in the necessity one (Buonavoglia, 2007). The recent studies showed that using the adjuvant vaccines with mineral oils like Montanide are high immunogenic, and the antibodies last a long period of time, but they produce granulomatous reactions at the inoculation area (Buonavoglia, 2007). In this study we want to investigate the specific and unspecific humoral immune response for in need vaccinated sheep against contagious agalactia.

1. MATERIALS AND METHODS

Animals. The study was done on two batches of sheep: A batch unvaccinated (n=10) used as a control batch and B batch (n=10) vaccinated with vaccine against contagious agalactia. The second inoculation was done after 21 days from the first one. Blood samples were collected from these animals in the inoculation day (T0), 21 days before the second inoculation (T1), 47 days after the first inoculation (T2) and 65 days after the first inoculation (T3).

Electrophoresis in 1% agarose gel was done in Tris-barbital buffer, pH=8.6, using the horizontal electrophoresis Line 1.1 machine. The migration time was 30 minutes at 100V and 37mA. After they got dry the slides were coloured with 1‰ Amidoblack 10B solution. The electrophoregrams were obtained by computer integration, when relative and absolute values of the proteic fractions from the serum were revealed.

Simple radial immunodiffusion test (IDSR-Mancini) for the G immunoglobulin dosing (IgG) was done using 5 cm diameter slides. For this test we used a reagents set, prepared in the Spiru Haret Veterinary

Medicine Faculty, comprising of: rabbit serum sheep anti-IgG and sheep reference serum with a known IgG concentration. The IgG concentration (g/dl) values from the samples were read on a standard curve according to the diameters of the precipitation rings (mm).

Lysosyme determination was done by determining the plate lysis in 9 cm diameter plates in which we put 2% agar gel, prepared in phosphate buffer pH=6.2, in which the *Micrococcus lysodeicticus* culture was put into afterwards. Sera were distributed in wells with gel. The reaction reading was performed in 24 hours by measuring the lysis diameters (mm), and the lysosyme concentration values (µg/ml) were read on a standard curve. As a reaction witness we used 100 µg/ml solution of purified lysosyme (Merck).

The immunoenzymatic technique. The antigen: to catch and to quantize the antibodies anti-Mycoplasma agalactiae sera, we used as antigen *Mycoplasma agalactiae* diluted at 10µg/mL in a 0,1N NaOH solution. In the coated stage we added 100µL antigen in every hole. After the plates were put in the incubator for two hours at 37ºC, they were washed using PBS/Tween in a Mindray MV-12A plate cleaner. The sheep anti-Mycoplasma agalactiae sera was diluted in a ratio of 1/100 with a PBS/Tween buffer with an addition of 0,5% bovine serum albumin. The plates incubation was done at 37ºC, for 60 minutes. The conjugate: we used a sheep anti-IgG conjugate marked with peroxidase. It was diluted in a ratio of 1/100 in PBS/Tween buffer to which we added 1% bovine serum albumin and then we put 100µL in each well. The substratum: it contained 0,005 hydrogen peroxide and 0,6mg/mL ABTS in citrate buffer of 4 pH. We used 100µL substratum in each well and after one hour the reaction was stopped with 50µL 1,5% sodium fluoride. The reading: the optical densities (DO) were read at 405nm with a multichannel plates ELISA Apollo LB 911 **s**pectrophotometer (Berthold Technologies).

The biuret method for measuring the protein concentration was done colometrically using the Spekol machine at a wave length of 545nm.

The statistical analysis was performed by calculating the average (x), the standard deviation (ds) and the coefficient of variability (CV%). The significance tests were calculated using the Student-Fischer (T test) method.

2. RESULTS AND DISCUSSION

Dynamics analysis of serum proteic fractions in control batch A and the experimental batch B is presented in table 1 and 2. The albumin

concentration at T_0 in sheep batches A and B showed similar values (batch A=4,50±0,02g/dl and batch B= 4,49±0,12g/dl). At T_1 the values for batch A=4,48±0,02 were and for batch B=4,48±0,31; the relative average values of this fraction were at T_2 of 4,52±0,47g/dl for batch A and of 4,47 ± 0,47 for batch B, and at T_3 of 4,20 ± 0,74g/dl for the control batch and of 4,47±0,56g/dl for the experimental batch.

The average values (x±ds) and the relative ones (%) for the α_1-globulin in all four harvesting were ranged between 3,10±0,41% and 3,41±0,42% representing concentrations of de 0,30±0,06g/dl and 0,46±0,05g/dl for batch A and of 0,48±0,07% and 0,52±0,19, respectively of 3,56±0,53g/dl and 4,28±0,17g/dl for batch B. We noticed a slight increase in the level of α_1-globulin at T_2 and T_3 in the inoculated batch B (T_2=0,52±0,19g/dl; T_3=0,51±0,09g/dl) in comparison with unvaccinated batch A (T_2=0,30±0,06g/dl; T_3=0,33±0,08g/dl).

Table 1.

The average values (x±ds), the relative values (%), the absolute values (g/dl) and the coefficient of variation (%) of the serum proteic fractions in sheep from batch A

Fractions		x±ds			
		Period			
		T_0	T_1	T_2	T_3
Albumin	%	57,45±0,33	57,26±0,62	57,25±0,39	57,24±0,42
	g/dl	4,50±0,27	4,48±0,02	4,52±0,47	4,20±0,74
	CV%	5,27	4,93	5,27	6,46
α_1-globulin	%	3,41±0,42	3,26±0,35	3,10±0,41	3,26±0,82
	g/dl	0,46±0,05	0,34±0,07	0,30±0,06	0,33±0,08
	CV%	11,21	12,13	15,68	14,54
α_2-globulin	%	4,26±0,17	4,36±0,28	4,92±0,95	5,07±0,82
	g/dl	0,32±0,02	0,46±0,03	0,48±0,09	0,52±0,03
	CV%	7,26	8,32	8,56	9,34
β-globulin	%	8,25±0,35	8,24±0,35	8,19±0,27	8,20±0,42
	g/dl	0,64±0,04	0,63±0,03	0,61±0,02	0,60±0,08
	CV%	22,72	23,52	24,76	24,53
γ-globulin	%	26,47±1,80	26,20±1,72	26,17±1,16	26,09±1,07
	g/dl	1,92±0,04	1,91±0,03	1,90±0,03	1,89±0,04
	CV%	2,74	3,25	4,36	3,26

Table 2.

The average values (x ± ds), the relative values (%), the absolute values (g/dl) and the coefficient of variation (%) of the serum proteic fractions in sheep from batch B

Fractions		x±ds			
		Period			
		T_0	T_1	T_2	T_3
Albumin	%	56,73±4,25	53,49±3,76	53,79±2,26	52,73±2,46
	g/dl	4,49±0,12	4,48±0,31	4,47±0,47	4,47±0,56
	CV%	7,28	6,56	4,32	5,76
α_1-globulin	%	3,56±0,42	3,72±0,46	4,28±0,17	4,25±0,12
	g/dl	0,48±0,07	0,49±0,05	0,52±0,19	0,51±0,09
	CV%	10,27	14,35	12,73	11,26
α_2-globulin	%	4,25±0,19	4,47±0,29	5,73±0,28	5,56±0,13
	g/dl	0,33±0,14	0,56±0,13	0,79±0,32	0,78±0,15
	CV%	5,26	4,35	5,98	6,32
β-globulin	%	8,20±0,42	8,29±0,17	8,32±0,27	8,30±0,32
	g/dl	0,63±0,02	0,72±0,06	0,75±0,01	0,70±0,12
	CV%	8,36	9,25	8,42	8,25
γ-globulin	%	26,42±1,79	26,97±1,20	27,45±1,21	26,36±1,24
	g/dl	1,92±0,07	1,97±0,12	2,10±0,29	2,09±0,23
	CV%	3,25	4,21	3,46	3,52

From the comparative analysis of data presented in table 1 and 2, an increase of α_2-globulin concentration can be seen at T_2 and T_3 in the experimental batch (T_2=0,79±0,32g/dl; T_3=0,78±0,15g/dl) in comparison with the control batch (T_2= 0,48±0,09g/dl); T_3=0,52±0,03g/dl).

The average values of β-globulin presented a slight increase in the batch B, inoculated at T_1 (0,72±0,06g/dl), T_2 (0,75±0,01g/dl) and T_3 (0,70± 0,03g/dl) in comparison with batch A, unvaccinated (T_1=0,63±0,03g/dl; T_2=0,61± 0,02 g/dl; T_3= 0,60± 0,03g/dl).

The level of γ-globulin presented high values in the batch B, inoculated at T_2, representing two weeks after the booster vaccination (2,10±0,29g/dl in comparison with 1,90±0,03g/dl from batch A).

The high level of γ-globulin concentration is maintained also at T3 (65 days after the first vaccination). Thus in batch A, the values of γ-globulin were 1,89±0,04 g/dl, and in batch B were 2,09±0,23 g/dl).

The coefficients of variability (CV%) were calculated for the analyzed batches.

The presented experiment shows that the tests results are constant and they are providing the data obtaining that fit in the unitary scale of values (table 1 and 2).

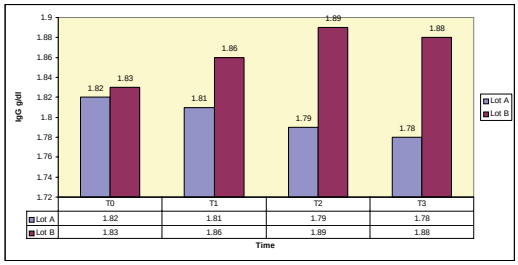
The non-specific humoral response was evaluated by determining in dynamics of the G immunoglobulin (IgG), the total protein and the lysosyme, in the control batch A and the experimental batch B. The results obtained on IgG dosing are showed in table 3 and figure 1.

Table 3.

The average values of immunoglobulin (g/dl) in control batch A (A) and in experimental batch B (B) and the statistically meaning (significance) of the difference between the batches.

Period	Batches (x ±ds)	
To	1,82±0,30	1,83±0,73
	p< 0,5*	
T1	1,81±0,43	1,86 ± 0,55
	p< 0,05**	
T2	1,79±0,21	1,89±0,92
	p< 0,001***	
T3	1,78±0,29	1,88±0,89
	p< 0,001***	
* = insignificant difference; ** = strictly significant difference; *** = highly significant difference.		

Figure 1. The average values of G immunoglobulin in sheep from the unvaccinated batch A and from the vaccinated batch B



When the experiment began (T_0), the average values of IgG were 1,82±0,30 for batch A and 1,83±0,73g/dl for batch B, the difference between the batches was insignificant (p< 0,5). At 21 days after the first vaccination (T_1) the IgG concentration raised in batch B (1,86±0,55 g/dl) in comparison with batch A (1,81±0,43 g/dl), the difference between them was distinctly significant (p< 0,05). At T_2 and T_3 the difference between the unvaccinated and the vaccinated batch was highly significant (p< 0,001) which shows that after the booster vaccination (T_1) the IgG concentration achieves a high level that persist for a long period of time.

The total protein concentration at T_2 (booster vaccination) in batch B (9,22±0,91g/dl) increased highly significant (p< 0,001) in comparison with batch A (7,91±0,78g/dl). At the last harvest (T_3), the difference

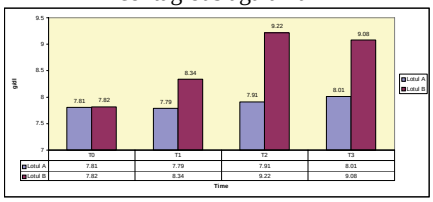
between the vaccinated batch (9,07±0,72g/dl) and the unvaccinated one (8,01± 0,65g/dl) remains highly significant (table 4 and figure 2).

Table 4.

The average values of the total protein (g/dl) in the control batch (A) and in the experimental batch (B) and the statistically significant difference between the two batches

Period	Batch (x ±ds)	
To	7,81±1,25	7,82±0,97
	p< 0,5*	
T1	7,79±1,05	8,34 ± 0,83
	p< 0,05**	
T2	7,91±0,78	9,22±0,91
	p< 0,001***	
T3	8,01±0,65	9,08±0,72
	p< 0,001***	
* = insignificant difference; ** = strictly significant difference; *** = highly significant difference.		

Figure 2. The values of the total protein in sheep from unvaccinated batch A and from batch B vaccinated against contagious agalaxia



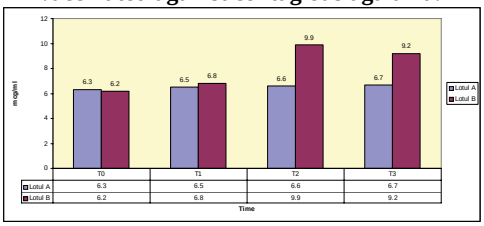
The average values of the serum lysosyme at T_0 and T_1 were insignificant (p< 0,5) in the control batch and also in the experimental one. At T_2 and T_3 has been a highly significant difference (p< 0,001) between the batches (T_2: batch A=6,6± 0,23µg/ml; batch B=9,9±0,92 µg/ml; T_3: batch A=6,7± 0,08µg/ml, batch B=9,2±0,37µg/ml) (table 5 and figure 3).

Table 5.

The average values of the serum lysosyme (µg/ml) in control batch (A) and in experimental batch (B) and the statistically significant difference between the batches.

Period	Batch (x ±ds)	
To	6,3±0,32	6,2±0,31
	p< 0,5*	
T1	6,5±0,82	6,8 ± 0,72
	p< 0,5*	
T2	6,6±0,23	9,9±0,92
	p< 0,001**	
T3	6,7±0,98	9,2±0,37
	p< 0,001**	
* = insignificant difference; ** = highly significant difference.		

Figure 3. The average values of the serum lysosyme in sheep from the unvaccinated batch A and from the batch B vaccinated against contagious agalaxia.



The specific humoral immune response evaluation was achieved by quantifying the antibodies level of anti-Mycoplasma agalactiae. The DO average values increased gradually at the experimental batch after the second harvest (T_2).

The highest value was obtained at T_2 (DO=1,337±54,03 in batch B, in comparison with 0,296±76,17 in batch A. The antibodies concentration stays high also at T_3 (DO=1,301±74,15 in the vaccinated batch, in comparison with DO=0,282±23,07 in the unvaccinated batch (table 6 and figure 3). These data show that the antibodies level of anti-Mycoplasma agalactiae increase after the first vaccination and is amplified after the booster immunization.

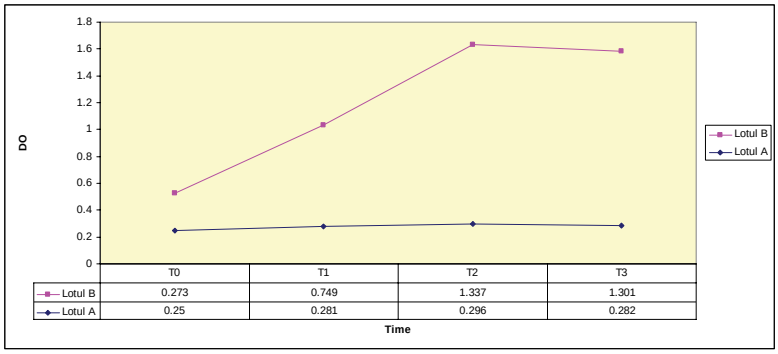
Table 6.

The quntification level of specific anti-Mycoplasma agalactiae antibodies in the unvaccinated batch A and in the vaccinated batch B using the immunoenzimatic test

Batch		DO/period			
		T_o	T_1	T_2	T_3
A	x±ds	0,250±12,03	0,281±25,07	0,296±76,17	0,282±23,07
	CV%	30,21	31,25	27,42	33,74
B	x±ds	0,273±28,32	0,749±71,32	1337±54,03	1301±74,15
	CV%	43,76	35,17	17,25	21,49

Figure 4.

The specific anti-Mycoplasma agalactiae antibodies concentration in sheep from the unvaccinated batch A and from the vaccinated batch B against contagious agalaxia determinated by ELISA test.



3. CONCLUSIONS

3.1 Humoral immunological parameters were studied (serum proteic fractions, G immunoglobulin, total protein, lysosyme) in sheep

included in two batch: A – unvaccinated and B – vaccinated against contagious agalaxia.

3.2 The experiment lasted 65 days, during this time four blood samples were taken: when the first vaccination took place (T_0), 21 days before the second vaccination (T_1), 45 days after the first vaccination (T_2) and 65 days after the first vaccination (T_3).

3.3 The unspecific immunological parameters studied induced a statistically high significant humoral response by increasing the concentration in proteic fractions, IgG, total protein and lysosyme after the booster vaccination (T_2).

3.4 The quantification of the specific anti-Mycoplasma agalactiae antibodies using ELISA test revealed high values after the first vaccination (T_1) that are maintained high until the end of the experiment (T_3).

3.5 The study of the humoral immunological effectors can provide correct evaluation criteria of the vaccination effectiveness against contagious agalaxia.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

EXPERIMENTAL RESEARCH ON THE HEALING EFFECT OF STERILE POWDER OF CORN SMUT(USTILAGO MAYDIS) IN DECUBITUS AND TRAUMATIC WOUNDS

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Key words: corn smut, plage, scarred.

SUMMARY

It has been studied evolutionary stagies of wound healing in decubitus and traumatic wounds and skin tissue remodeling effect of lesion, using a sterile product in powder form obtained out of corn smut spores (*Ustilago maydis*).
The assessment of healing effect of sterile powder obtained out of corn smut (*Ustilago maydis*) on restoring damaged skin tissue was performed by macroscopic and histological observations by comparing changes that occurred during wound healing.
The results were compared individually, within batches of animals, observing the character of uniformity of the therapeutic effect in conditions in which the differences were minimal.
Herbal components showed besides stimulating effect on the evolution of healing, analgesic and antipruritic effects, reducing animals'concern for their own lesions.
Treatment with sterile powder of corn smut ensures rapid wound healing leading to a functional and aesthetic scar.
The product was well tolerated,showing reduced antigenic reaction and biocompatibility with no complications or evolution to pathological scarring.

Smut is a common corn disease, originated in America, from where it was spread to Europe, being first reported in Italy(1809) and France (1815) and many other countries after that. Pathogen agent. The disease is produced by *Ustilago maydis* from Ustilaginaceae family, order Ustilaginales. The main symptom of disease is the presence of bags filled with chlamydospores on all aerial organs of plants, sometimes on adventitious roots. Most frequently it is attacked stems and whole cobs, and leaves less (5).

Whole cobs can be totally or partially destroyed. Tumors raise more frequently on the top or at basis of cobs, growing on account of bracts and ovaries hypertrophy. Tumors can reach 10 cm long and 5-6 cm in diameter and can reach 2 kg in weight. They are filled with a mass sporifera looking wet and greasy, which becomes powdery, being covered with a yellowish white thin membrane.

At ruminants *Ustilago maydis* toxin once ingested with contaminated feed produced abortion (6).

In homeopathic medicine, corn smut is used as a uterine tonic for pregnant women to control postpartum bleeding. Also it can treat alopecia and some rash (2,3,7).

1. MATERIAL AND METHODS

Studies were conducted on a group of 20 laboratory animals - rabbits aged 6 months who had traumatic wounds and decubitus.

During the experimental period, the influence of environmental factors has been limited, by providing a suitable habitat and a balanced diet, the animals were housed in individual boxes, depending on the category of lesion.

All animals were anesthetized by neuroleptanalgezie (NLA) before the start of the experiment, avoiding their brutal handling, and increased feeling of stress, by providing a resting period before producing lesions. It has been used:

- acepromazina (Trankilrom-Romvac) dose of 0.3 mg/kg G.V i.m;
- ketamine (Ketalrom-Romvac) in dose of 70 mg/kg G.V i.m.

Sterilization of smut corn was achieved by dry heat at 170-180°C for 40 minutes.

2. RESULTS AND DISCUSSION

Clinical signs showed the existence at the limb distal calf region of large wounds that cover the entire plantar surface of the tibio-tarso-metatarsal joint (fig. 1).

Fig. 1 - Decubitus wound-clinical aspect

Old wounds were at least 3-4 days, with approximately spherical shape, swollen edges, retracted outwards and slightly necrosis, lack of tissue to bone around plan.

Wound surface indicate the presence of a purulent secretion. Animals showed intense sensitivity evidenced by removing the support member in position.

Treatment consisted of regional grooming (fig. 2) and mechanical antisepsia (excision edge necrosis) (fig. 3a,b). Locally it has been applied a thin layer of sterile powder of spores complemented with the application of a protective dressing (fig. 4,5).

Fig. 2 – Decubitus wound toilet

a. **b.**

Fig. 3 a, b – Decubitus wound mechanical antisepsia

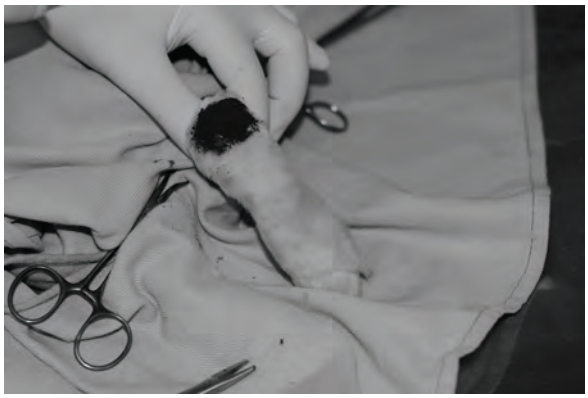

Fig. 4 – Appearance of wound after application of *Ustilago maydis* spore powder

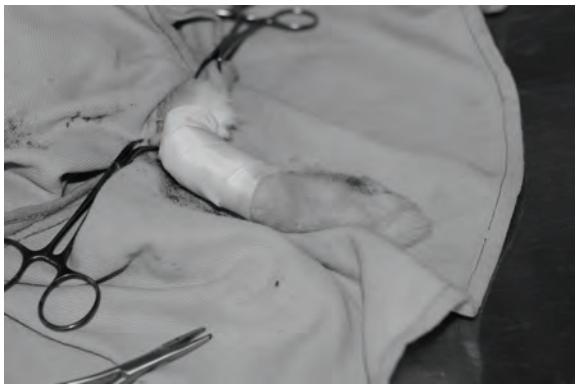

Fig. 5 – Protecting the wound with a protective dressing

After three days the patient's condition was very good, but there were some difficulties in removing the dressing, which leaves the wound bleeding, reaction was violent animals.

The wound was a decrease in secretion of purulent character, and a reduction of regional swelling. Continue to apply directly to wound a layer of sterile powder of spores.

After five days the dressing was removed more easily, the reaction of animals being absent. Limiting inflammatory edema was found only on the edges of the wound, which shows infiltrative appearance, easy bleeding ;regional sensitivity was significantly diminished.

After seven days wounds were greatly reduced in volume, and on the wound edges it has been observed red fleshy buds belonging to granulation tissue.

Fig. 6 – The appearance of decubitus wounds 10 days after application of sterile powder *Ustilago maydis* (complete healing wounds)

Clinical observations have shown that on the 10th day after mechanical disinfection and Ustilago maydis sterile powder application, a complete healing wounds accompanied by restoration of pilosity it has been obtained (fig. 6).

In traumatic wound to the lateral region of the calf ,it has been observed simple and shallow cut, with retracted edges due to regional tension, slightly infiltrated and swollenwithout local heavy bleeding.

The wound affected only the skin, without involvement of deeper tissues (wound cut, simple, single, shallow).

Fig. 7 – Appearance of a traumatic wound to 7 days after application of sterile powder *Ustilago maydis*

Treatment consisted of local grooming and mechanical antisepsia. The resulted wound was treated by local application of a layer of sterile powder of spores.

After five days the wound edges had no hemorrhagic infiltration, being flexible, elastic and in contact indirectly through a consistent granulation tissue.

After 7 days the wound was limited to only one third of the original surface being entirely covered by a dark brown rind, smooth and dry (fig. 7).

Histological examination carried out on biopsy samples from subjects treated with sterile powder of *Ustilago maydis* after 7 days treatment revealed a complete regeneration and neoformation epidemic dermal scar tissue, without this specific exudative inflammatory cell infiltrate (polymorphonuclear and mononuclear leukocytes) (fig. 8,9).

Fig. 8 - Traumatic wound rabbits treated with powder *Ustilago maydis* after 7 days treatment; Col. trichomes Mallory; Ob. 20x
1.epidermis; 2. papillary dermis.

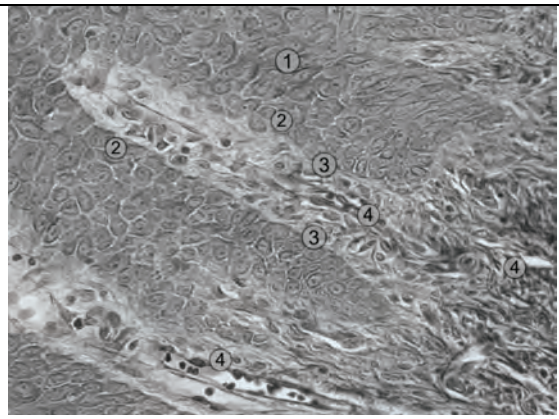

Fig. 9 - Traumatic wound rabbits treated with powder *Ustilago maydis* after 7 days treatment Col. trichomes Mallory; Ob. 40x
1. spinous layer (polyhedra); 2.basal layer; 3. basement membrane; 4. papillary dermis.

At the level of neoformative skin it could be observed a supranumerous disposal of keratinocytes that had a polymorphous appearance and basal cell layer showed an intense activity of protein synthesis, present in the keratinocytes with numerous nuclei and also many cells in mitosis, which showed an accelerated healing process (fig. 10).

In stratum spinosum keratinocytes with normal morphology were also present, together with cells showing vacuolized cytoplasm and picnotic nuclei. Basement membrane is under complete regeneration activity due to their intense synthesis of connective tissue cells in the dermis (fig. 10).

Papillary dermis showed structural elements of connective tissue with a normal distribution, neoformation layer of connective fibers having a fiber different from the affected areas which also presented numerous neoformation capillary (fig. 10).

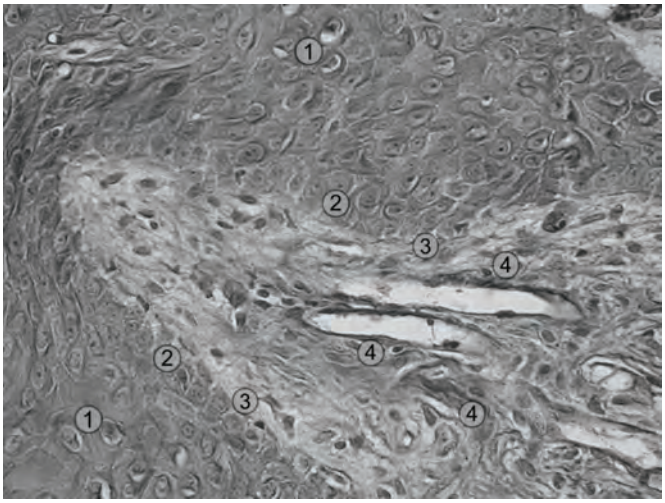

Fig. 10 - Traumatic wound rabbits treated with powder *Ustilago maydis* after 7 days treatment; Col. trichomes Mallory; Ob. 100x
1. spinous layer (polyhedra); 2.basal layer (generator);
3. basement membrane; 4. papillary dermis.

3. CONCLUSIONS

Experimental research on stimulated wound healing using a sterile powder produced by *Ustilago maydis* led to these conclusions:

3.1. Herbal components showed, besides stimulating effect on the evolution of healing, an analgesic and antipruritic effects, reducing animals'concern for their own lesions.

3.2. Treatment with sterile corn smut ensures rapid healing of wounds of castration with the formation of a functional and aesthetic scar.

3.3. The product was well tolerated, showing reduced antigenic reaction and biocompatibility with no evolution to complications or pathological scarring.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

THE INTAKE OF FOOD ADDITIVES IN ROMANIA-2009

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Key words: additives, intake, foodstuff, sodium monoglutamate

SUMMARY:

Food additives are substances added intentionally in foodstuff for technological purpose The use of food additives in Romania is ruled by specific normative. In the frame of the national activity of monitoring additives, the local public health authorities started, from 2009, the evaluation of the intake of additives. The results showed that the additives with the highest intakes are citric acid, glutamate, phosphates and cyclamate. The main food products contributing to this intake are meat products and soft drinks.

Food additives are very used in modern food processing. Actually, the modern food industry cannot be imagined without the presence of additives. They are substances, not aliments, being added on purpose for technological reasons. They are noted with the letter "E" plus a specific number. Additives are classified in several categories, some of the most frequently used being: colorants, sweeteners, preservatives, antioxidants, enzymes, stabilizing agents, etc. In every country specific laws regulate the use of additives, thus ensuring the consumer that these substances could never become a health hazard. In Romania there are also specific regulations in this field (Order 438/295/2002). However, lately more and more reports show the fear in the general population that the additive intake is dangerous. Different allowed substances are blamed for staying at the origin of different diseases, even if minute research offer no solid base for such affirmations. In this context, it is of utmost importance that the additive intake is kept under control (Saltmarsh, 2000).

1. MATERIAL AND METHOD

In 2009, as in the previous years, the use of main food additives was monitored, in the frame of the National Program II (the monitoring of

the determinant health factors from the work and life environment). Data from the Territorial Public Health Authorities were gathered and analyzed at the Public Health Regional Center of Targu Mures. A new part of the monitoring was the first attempt made in our country to evaluate the average per capita. intake of additives

In order to evaluate the food additive ingestion, it has been used the method of national food balance. There have been taken in consideration products analyzed for additives in the 2008 (meat products, soft drinks, diary products, fruit and vegetable preserves).

Because our former research showed that the maximal allowed concentration of different additives was exceeded only in 4,13% of cases, in the present evaluation we considered in each case the maximal allowed quantity of additive, as shown by laws in force. In fact, we included in our calculation only those additives that have maximal allowed limits, and ignored those for which the legislation has no maximal limit for the specific products taken in account in the evaluation. We calculated the total quantity of additives included in products sold on the Romanian market in 2009 and then divided it by the total number of Romanian inhabitants, using the figures offered by the National Institute of Statistics at the beginning of 2009.

2. RESULTS AND DISCUSSIONS

The highest intakes are for citric acid (63,63g) originating in soft drinks, for phosphates (29,92g), where 97,45% originate in meat products and only 2,54% in diary products, for monosodic glutamate (25,06g), from meat products and for ciclamate (2,45g), from soft drinks. The quantity refers to the average anual ingestion, calculated per person. It can be seen that some of the „sensitive" additives, which are frequently feard by the consumers (taste enhancerrs: glutamate; artificial sweeteners: ciclamate) are also consumed at the highest level, compared with other additives.

Citric acid is a weak organic acid. It is a natural preservative and is also used to add an acidic, or sour, taste to foods and soft drinks (Penniston et al, 2008). Monopotassium phosphate (also potassium dihydrogen phosphate, KDP, or monobasic potassium phosphate, MKP) -- KH_2PO_4 -- is a soluble salt which is used as a food additive and a fungicide. It is a source of phosphorus and potassium. It is also a buffering agent. Monosodium glutamate, also known as sodium glutamate and MSG, is a sodium salt of the naturally occurring non-essential amino acid glutamic acid. It is used as a food additive and is

commonly marketed as a flavour enhancer. It has the E number E621(Food Standard Agency, 2010). Sodium cyclamate is an artificial sweetener. It is 30–50 times sweeter than sugar and it is the least potent of the artificial sweeteners. Some people find it to have an unpleasant aftertaste, but generally less so than saccharin or acesulfame potassium. It is often used synergistically with other artificial sweeteners, especially saccharin; the mixture of 10 parts cyclamate to 1 part saccharin is common and masks the off-tastes of both sweeteners. It is less expensive than most sweeteners, including sucralose, and is stable under heating (Mitchel, 2006).

The present calculation is estimative and unfortunately has some limitation from the following reasons:

- nine local public health authorities (8 counties + Bucharest) didn`t sent data from their area;
- we don`t know if the public health authorities included in their investigations all the local producers
- some of the producers (19,88%) did not report how much they sold from some products (9,73%), claiming that this is a company`s secret or that the product is newley introduced on the market (table 1)

Tabel. 1.

Products for which the comercialised quantity has not been reported

Category:	Nr. of products	% of the reported products
Soft drinks	36	2,34%
Meat products	94	6,10%
Diary products	20	1,30%
Total products without reported quantity	150	9,73%
Total products with reported quantity	1541	

3. CONCLUSIONS

3.1. Widely used foodstuff, meat products and soft drinks, are also main contributors to the additives intake.

3.2. Respecting the normative regarding the use of additives is a priority, being important and necessary in supervising the quality of foodstuff (ILSI 1999).

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

SALT IN ROMANIAN FOOD PRODUCTS (2007-2009)

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Key words: salt, hypertension, meat products

SUMMARY:

Natrium chloride, cooking salt, represents a compound used by man since ever. But in the contemporary world salt is overconsumed, thus becoming a health hazard. Reducing salt intake is the corner key in the management of hypertension.The monitoring of salt content in Romanian products between 2007-2009 showed that some percents of every analyzed food category has an excesive quantity. In order to follow the World Health Organisation target, to lower the salt intake under 5g/day it is mandatory a joint effort between technologues, food producers and public health authorities.

First applied on food in order to prolong its freshness, salt entered little by little in the range of alimentary ingredients sought by any of us for its specific taste. The majority of humans refuse food without salt finding it tasteless. The salty taste is a "sine qua non" of food (deserts excepted). Unfortunately, salt, or better said, the overuse of salt became also a forceful enemy of human health. Used once as exchange currency, salt is, in the contemporary world, a product frequently associated with disease end death. In most cases, the overuse of salt and its complications are underestimated.

Men and women need natrium and chloride, the two components of cooking salt. But the necessary is close to the natural content of food. Nevertheless, in civilized countries the intake rises to over 10 g/day, 75% of it originating in processed food (Hignett, 2009)

And all this though adding salt in preservative purposes is not needed any more, in the era of freezers and refrigerators. The excessive intake of salt is a reality and constitutes a risk factor for high blood pressure. From over a billion person with HTA, approximately 30% can blame the excess salt for it, 14% of strokes and 9 % of myocardial infarction appear on the hypertension background due to excess salt intake (Bibbings-Domingo *et al*, 2010;) .

Reducing salt intake is the corner key in the management of hypertension. A study carried out in Canada showed that reducing it could lower the prevalence of hypertension, from 3.5 millions, to 2.2. Reducing dietary salt by 3 g per day could reduce the annual number of new cases of CHD by 60000 to 120000, stroke by 32 000 to 66000, and myocardial infarction by 54 000 to 99 000 and to reduce the annual number of deaths from any cause by 44000 to 92000. Because most of the salt is added not at home, but during the processing, measures in this field are mandatory (Fluegel and Manger, 2009).

Up to now, in Romania we have no studies to evaluate the salt intake, but every year the National Institute of Public Health centralizes data gathered by the territorial Public Health Authorities and estimates the salt content in widely available Romanian food, in the frame of The National Health Program II. The target is to create a data bank covering a large area of products and to encourage food manufacturers to reformulate products, were this thing is possible and it is not interfering with the technological necessities.

1. MATERIAL AND METHOD

The following results were centralised during the 2007-2009 by the Institute of Public Health and came from the chemistry laboratories from the local public health authorities and from Bucharest. For meat products, the analysis was made taking in acount the order Nr.560/1271/339/210/2006, for the aprooval of the Norms reffering to the comercialisation of meat products, for the rest there have been considered the technical specifications regarding the product, or its label.

2. RESULTS AND DISCUSSIONS

The number and type of samples analyzed each year si presented in table 1.

Table 1.

Samples analyzed : number and type

Product	***2007***	***2008***	***2009***
Diary products	843	221	280
Meat products	1800	513	228
Fish products	390	148	-
Bread	-	606	573

Cattering products	-	-	240
Other	-	323	-
Total number	3033	1811	1321

The average concentration of salt in different products is presented in Tabel 2

Table 2.

Average quantity of salt in analyzed products

Product	***2007***	***2008***	***2009***
"Telemea" cheese	3.98%	-	3,6%
Mature cheese	2.14%	-	-
Melted cheese	1.4%	1,73%	-
Cremwurst	1.84%	-	-
Parizer salamy	1.91%	-	1,92%
Summer salamy	2,16%	2,08%	-
Saussages	2,25%	-	-
Liver pate	1,62%	-	-
"Caviar" salad	3,05%	-	-
Fish salad	2,66%	-	-
Ham	-	2,42%	-
"Sibiu"salamy	-	3,5%	-
Fish in oil preserves	-	1,23%	
Fish in tomato sauce preserves	-	1,43%	-
Bread	-	1,7%	1,1%
Chips	-	1,7%	-
Sticks	-	2,62%	-
Other crisps("Pufuleti")	-	1,48%	-
Catering products	-	-	1,41%

The percents of products having more salt than normatives is presented in table 3.

Table 3.

Percents of samples with a higher than admited content of salt

Products	***2007***	***2008***	***2009***
Diary products	16%	2,26%	27,86%
Meat products	3,8%	4,68%	2,6%
Fish products	4,4%	8,78%	-
Bread	-	1,3%	4,1%
Other(chips, crisps)	-	10,8%	-
Catering products	-	-	7,5%

Some differences in the above percents can be explained by the different sortiments taken into evaluation in different years. For example, for diary products, in 2008 we have a rather small percent of samples with an overload of salt, compared with the other years, but in 2008 there has been analyzed just melted cheese, and not telemea cheese.

Even though the number of samples having salt more than admited is not high, it can clearly be seen that some widely consumed food stuff, especially meat products (including fish) have frequently a lot of salt. Another product who seems to be very salty is cheese.

3. CONCLUSIONS

Frequently food with a low salt contet are described as tasteless but food technologues show that reducing with 18-20% the salt would be unnoticed by the consumers (AMA,2007). So minimizing the bad consequences of the salt overconsumption needs an effort from the food industry in order to lower salt from all processed food. The World Health Organization has as prioritary objective the reducing of salt in processed food world wide, lowering the salt intake at or under 5 g/day, and our country has to take also measures in this area (WHO, 2010). It is mandatory a joint effort between technologues, food producers and public health authorities, in order to reformulate some products without consequences on their preservation or quality and to teach consumers to limit their salt intake. These strategies will surely pay off, leading to a substantial decrease of illnesses linked to hypertension (Smith-Spangler *et a*l, 2010).

Acknowledgements: This study couldn't come to a conclusion without the help of our colleagues from the local public health authorities and from the Bucharest Public Health Center.

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Scientific works, C series LVI(1), 2010
ISSN 1222-5304

LYMPHATIC DRAINAGE OF THE CRANIAL (T1) AND CAUDAL (T2) THORACIC MAMMARY GLANDS IN THE DOMESTIC CAT

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Key words: lymphography, mammary glands, lymphatic vessels, lymph nodes, cat.

SUMMARY

The study initiated by us aims to describe, interpret and elucidate several aspects regarding the topography, the morphology and the drainage of the mammary lymphatic circulatory system in the domestic cat.

We have compared our results to data from specialized literature to determine what is common norm, individual particularity or aspects that have not been previously described regarding the radiographic indirect lymphography and the mammary lymph drainage in cats.

To identify and illustrate the topography of the lymphatic vessels, afferent and efferent lymph nodes of the mammary gland in the domestic cat, we have used as study method the radiographic indirect lymphography with a contrast agent.

The lymphographies were taken in the Radiology Laboratory of the Faculty of Veterinary Medicine, Cluj-Napoca, using a TEMCO GRx-01 type fixed radiographic equipment.

The theme of this research covers the anatomy of the mammary lymph drainage in the domestic cat, as well as its deviations. It is a well known fact that any changes of the mammary lymphatic drainage can have pathological implications of a neoplastic nature, or at best, such changes can be considered an individual peculiarity.

While researching the specialized literature for theoretical documentation, we have concluded that there are a limited number of studies regarding the lymphatic drainage of the mammary gland in the domestic cat, which constituted another reason for choosing this theme.

1. MATERIAL AND METHODS

The study was conducted on 11 European common breed cats, clinically healthy and well kept, from a rural background. The 11 cats of various ages (2-6 years), with a bodyweight range of 2.5 to 4 kg, were divided in two groups, according to the physiological state of the

mammary gland. Group I consisted of 5 pregnant cats and group II consisted of 6 lactating cats.

From all the investigative methods cited in the specialized literature, we have used indirect lymphography with a contrast agent.

The lymphographies were taken in the Radiology Laboratory of the Faculty of Veterinary Medicine, Cluj-Napoca, using a TEMCO GRx-01 type fixed radiographic equipment.

Before describing the method, we would like to mention that the cat normally has 4 pairs of mammary glands, of which two thoracic and two abdominal. Because of its topography, as described by Silver (1966) and by Christensen (1979), each mammary gland has a specific name, as following: cranial thoracic (T1), caudal thoracic (T2), cranial abdominal (A1), caudal abdominal (A2) and inguinal (I) (Raharison and Sautet, 2007).

The mammary glands that we have chosen to study in both the pregnant and the lactating cats were the cranial thoracic mammary gland (T1) and the caudal thoracic mammary gland (T2).

For each studied individual, we have monitored its behavior and its physiological parameters (pulse, respiration, temperature).

Each subject was anesthetized. Neuroleptanalgesia was induced through the intramuscular administration of Acepromazine 10%, 0.5 mg/kg body weight, and Ketamine 10%, 20 mg/kg body weight. After the anesthesia set in, the whole mammary region was trimmed, shaved and disinfected with medicinal alcohol.

Next, we continued with the injection of the contrast agent. The shots were made with a 27-G needle in the subareolar region and in the mammary parenchyma. The rhythm was slow, trying to maintain the same pressure during the injection of a mammary gland. We administered 0.5 ml of Optiray in each studied gland, with the exception of one case, which will be mentioned in the results and discussion section. To facilitate the absorption, each mammary gland was gently massaged after the injection (Kvasnicka et al., 1971; Mortimer, 1990). The administration of the Optiray 350 contrast agent was made in a single point, taking care each time not to puncture any blood vessels. This was accomplished by gently pulling on the mamelon of the mammary gland (Pereira et al., 2008).

After the administration of the contrast medium, followed the radiologic exposure, with the cats in dorso-ventral and latero-lateral decubitus and with a ventro-dorsal and latero-lateral exposure. The examination consisted of seriate exposures at 1, 5, 10, 15, 25, 30, 35 minutes from the injection of the contrast agent.

2. RESULTS AND DISCUSSION

Group I

Group I consisted of 5 pregnant cats.

In this group we have examined a total of 10 mammary glands. In each cat we have studied the cranial (T1) and caudal (T2) thoracic mammary gland from the left side of the mammary chain. Each case was examined during 3 days. In the first day, the radiologic exposure was ventro-dorsal, and in the third day, it was latero-lateral. The second day was reserved for the subjects' rest.

In the studied T1 mammary glands, we have underlined the presence of a single lymphatic vessel in 4 cats out of 5 and the presence of two lymphatic vessels in one cat out of 5. The visualization of the lymphatic vessels was possible right after the injection, because five minutes later the contrast substance remained only at the administration site (**Fig. 1**).

The lymph drainage, in 5 cats out of 5, was made cranially, towards the accessory axillary lymph node. Our study has revealed the cranial accessory axillary lymph node.

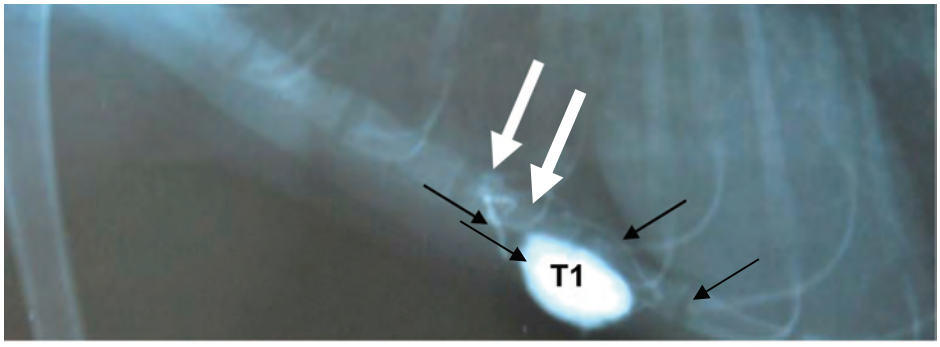

Fig. 1. Lymphatic vessels (black arrow) leading from the cranial thoracic mammary gland to the accessory axillary lymph node (white arrow) – immediate exposure after the Optiray injection.

The results obtained from the pregnant cat research group indicate that these animals present cranial lymphatic drainage, the first lymphatic relay, the accessory axillary lymph node, having been highlighted by us. The study has shown that the drainage is done through a single lymphatic vessel in 4 out of 5 cats, and in one cat we have visualized two lymphatic vessels.

The radiologic exposure made immediately after injecting the contrast medium offered a very good view of the cranial accessory axillary lymph node in all subject of this group, this node being placed

between the third and the fourth sternebrae, according to literature data (Sugimura et al., 1956).

Regarding the caudal thoracic mammary glands (T2) which we have studied, the latero-lateral exposure and the latero-lateral decubitus of the cats offers important data regarding their lymphatic drainage.

From the T2 mammary gland, one or two lymphatic vessels, according to specialized literature, drain the lymph to one or two accessory axillary lymph nodes, belonging to the axillary lymphocenter. In this research group, based on the lymphographies, we have noticed in 5 cats out of 5 the presence of a single lymphatic vessel which drains the lymph cranially from the caudal thoracic mammary gland (**Fig. 2**). In one of the 5 cats of this group we have noticed the presence of a very thin lymphatic vessel leaving the gland's parenchyma caudally. We have in this case the possibility of a double lymphatic drainage (cranial and caudal) of the caudal thoracic mammary gland (**Fig. 3**).

The collector lymphatic vessel which leaves this gland cranially does not penetrate the parenchyma of the adjacent gland (T1) and head to the accessory axillary lymph node (**Fig. 2**).

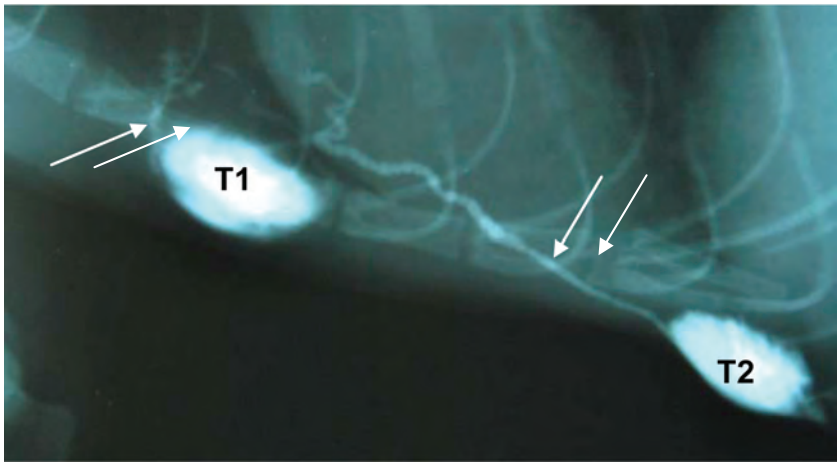

Fig. 2. Cranial drainage of the T1 mammary gland (5 min. after the injection) and T2 mammary gland (1 min. after the injection).

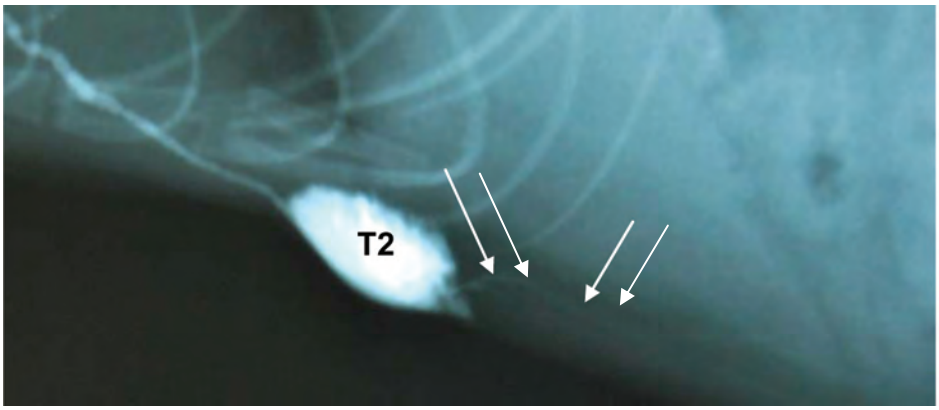

Fig. 3. Presence in one cat out of five of a thin lymphatic vessel which drains caudally.

Regarding the caudal thoracic mammary gland (T2), several authors state that the drainage is made cranially by the accessory axillary lymph node caudally by the accessory inguinal lymph node and the superficial inguinal lymphocenter (Raharison and Sautet, 2006; Vollmerhaus and Roos 1997). Other authors do not confirm this type of drainage, maintaining that it is made exclusively cranially through the axillary lymphocenter (Papadopoulou et al., 2009).

Our results based on the pregnant cats group lymphographic study show that the caudal thoracic mammary gland is drained by a single lymphatic vessel in 5 cats out of 5. In four cats this is exclusively cranial through the axillary lymphocenter. A single cat has presented a double drainage of this gland, the lymphography showing a lymphatic vessel leaving the gland through its caudal extremity, heading caudally, but with no observable lymph node relay receiving it.

Group II

The 6 cats in the second group have been chosen due to the functional state of the mammary glands, all of them lactating at the moment of performing the study.

In this lactating cats group we have examined a number of 12 mammary glands. The research protocol was similar to the one used for the previous research group, with the difference that in both days of the experiment we made radiographies with both ventro-dorsal and latero-lateral exposures.

Regarding the cranial thoracic mammary gland, this group confirms that there is cranial lymphatic drainage in the lactating cats as well.

The ventro-dorsal radiologic exposure and the dorso-ventral decubitus of the cats has led to obtaining a lymphography in which we

have visualized immediately after the contrast medium injection a single lymphatic vessel emerging from the cranial pole of the mammary gland, heading to the accessory axillary lymph node, situated under the ventral border of the *latissimus dorsi* muscle (**Fig. 4**). This situation was similar in all 6 cats.

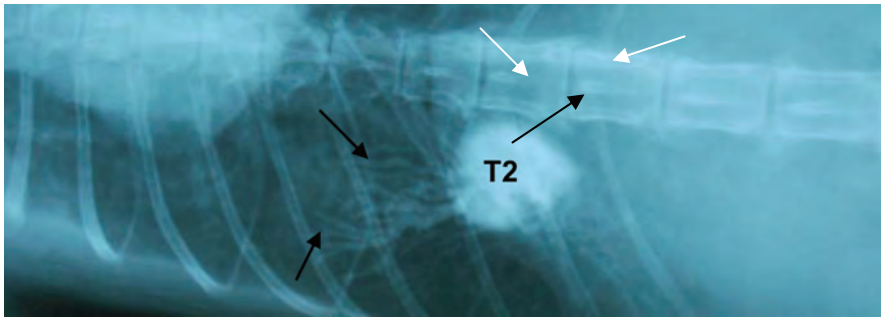

Fig. 4. Lymphatic vessel (black arrow) of the T1 mammary gland, which drains cranially to the accessory axillary lymph node (white arrows).

In the lactating cats group, 6 cats out of 6 have presented one lymphatic vessel leaving the T1 gland through its cranial extremity, becoming an afferent vessel of the accessory axillary lymph node situated at the ventral border of the *latissimus dorsi* muscle. Similar to the pregnant cats group, the best radiologic results were obtained when the exposure was made immediately after the injection of the contrast agent.

In this group we have encountered a special case regarding the caudal thoracic mammary gland (T2). While examining cat number 1, following the injection of 0.5 ml of contrast medium and the radiologic exposure of the subject in both dorso-ventral and latero-lateral decubitus, we have obtained a lymphography on which the only visible element was an opaque area at the injection spot. The peripheral zone of the injection spot presented the tendency of arborescent opacification. This has led us to repeat the injection of the mammary parenchyma with the same quantity (0.5 ml) of Optiray 350 contrast agent. After the second shot, the arborescent opacity took a concrete shape, allowing us to visualize the mammary parenchyma along with the subcutaneous lymphatic vessels belonging to the mammary gland's structure.

The other subjects from this group did not necessitate a repetition of the contrast medium injection, because after the first shot and the radiologic exposure both latero-lateral, but especially ventro-dorsal, we were able to view the mammary parenchyma accompanied by the subcutaneous lymphatic vessels and the cranial convergence of the latter

towards the axillary lymphocenter (**Fig. 5**). Their visualization was possible due to the lactating state of the mammary gland, as this aspect was not visible in any of the cats from the pregnant group.

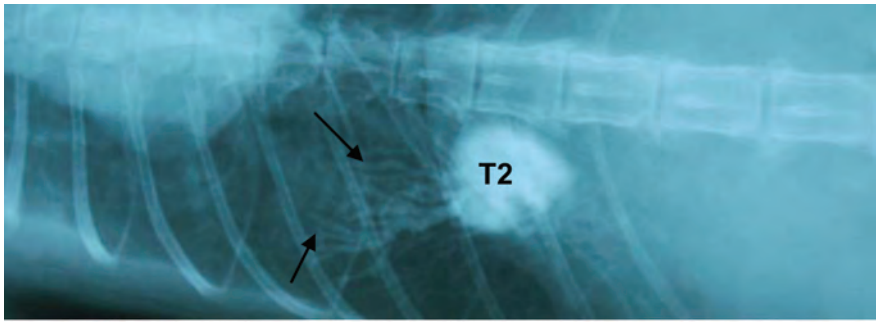

Fig. 5. Visualization of the mammary parenchyma and its adjacent lymphatic vessels.

Regarding the lactating cats research group, we must mention that the results obtained from it confirm only the cranial drainage of the caudal thoracic mammary gland (T2). The difference between this group and the pregnant cats group is that in this one we have highlighted through a single injection the mammary parenchyma and its adjacent lymphatic vessels in 5 cats out of 6.

Regarding interglandular connections between T1 and T2, some authors believe that they exist (Mailot et al., 1980), but others disagree (Papadopoulou et al., 2009; Raharison and Sautet, 2007; Raharison and Sautet, 2006). Neither of our research groups has presented lymphatic vessels connecting homolateral thoracic mammary glands.

3. CONCLUSIONS

3.1. In all mammary glands considered for this study, we have observed the cranial lymph drainage through the axillary lymphocenter, more precisely through its first relay, the cranial accessory axillary lymph node.

3.2. In T1 mammary glands, we have observed the presence of a single lymphatic vessel in 10 cats out of 11, and the presence of two lymphatic vessels in one cat.

3.3. 10 cats out of 11 presented exclusive cranial lymph drainage for the caudal thoracic mammary gland.

3.4. In only one pregnant cat, the caudal thoracic mammary gland presented double lymph drainage, both cranial and caudal.

3.5. Neither of groups presented lymphatic vessels connecting homolateral thoracic mammary glands.

3.6. Neither of groups presented lymphatic vessels connecting heterolateral thoracic mammary glands.

3.7. The Optiray 350 non-ionic contrast agent used for this study has highlighted through the means of radiographic indirect lymphography the path of the lymphatic vessels of the mammary glands and their adjacent lymph nodes.

3.8. In all subjects, latero-lateral radiologic exposure with latero-lateral decubitus of the animal yielded the best results for viewing mammary lymphatic vessels and their sentinel lymph nodes.

3.9. The Optiray 350 non-ionic contrast medium used by us has not altered the health of the cats included in this study.

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