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CLARIFICATIONS REGARDING THE TOPOGRAPHICAL LOCATION OF THE VASCULAR, LYMPHATIC AND NERVOUS FORMATIONS FROM THE THORAX APERTURE IN PIGS AND SHEEP

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Abstract

The purpose of the studies is to contribute with some clarifications to the topographical location of the vascular, lymphatic and nervous formations from the thorax aperture in pigs and sheep.

The literature data is little relevant because they depict other formations from the anterior mediastinum, without making a correlation between them.

The study was conducted on 20 pig corpses from production farms, with digestive, not respiratory disorders in general, so as not to affect the studied area, and on sheep corpses used by students for dissection. The vascular formations were injected with a mixture prepared in the laboratory of anatomy.

The paper shows pictures from several dissections, determining as accurately as possible the topographical location of the anatomical formations, and it has a strong applicative character for human medicine, since the closest species to man as experimental morphological model is the pig.

Keywords: *mediastinum, lymphatic duct, cranial vena cava, caudal cervical ganglion*

Introduction

The fundamental research on the topography of the vascular nervous formations from the aperture of the thorax cavity in animals is approached by many researchers, but the data are presented separately, either for the vascular formations, or for the nervous formations, or for the lymphatic formations (1, 2, 4). These data are a real support to interpret the physiological phenomena and to clarify several aspects regarding the way of approaching the formations during surgery on the anterior mediastinum. The morphology of the species resembles that of the man, which recommends it as an experimental model, provided the European legislation of the experimental animals is observed (4).

Materials and methods

The studies were conducted in the laboratory of anatomy of the Faculty of Veterinary Medicine, on 20 pig corpses from a production farm. Before dissecting,

the aorta and the veins were injected with a mixture of substances prepared in the laboratory of anatomy. The nervous formations were treated with a solution of acetic acid 10%. The lymph formations were injected with methylene blue. The lymphatic anatomy of 5 pigs was studied and classified and a new technique for lymphatic cannulation was developed. The cannulation success rate was 55%.

Results and discussion

Formation anatomical approach is at chest level as having first milestone coast. It protects the right apical pleural recessive and dissect contained septal formations precardiac mediastinal. In relation to the first rib to show the skull mediastinal lymphonodes who are willing and medial to this axilar lymphonode of the first rib that is located in relation to the edge of the skull. Vegetative plexus is located between cervicotoracic formations located superficial venous and arterial located medial formations (fig. 1).

Fig. 1. *Mediastinal aperture approach*

The right caudal cervical ganglion, joined in 15 animals with the thoracic paravertebral ganglion 1 and 2 forms a pericarional aglomeration located on the median face of the first rib in the dorsal side of the anterior mediastinum, being placed dorsally in relation to the long neck muscle, laterally in relation to the vertebral artery, ventrally in relation to the right subclavicular artery and on the right of the bicarotic trunk. In all studied cases we have identified the middle cervical ganglion which is attached to the caudal cervical ganglion through the subclavicular loop (fig. 2).

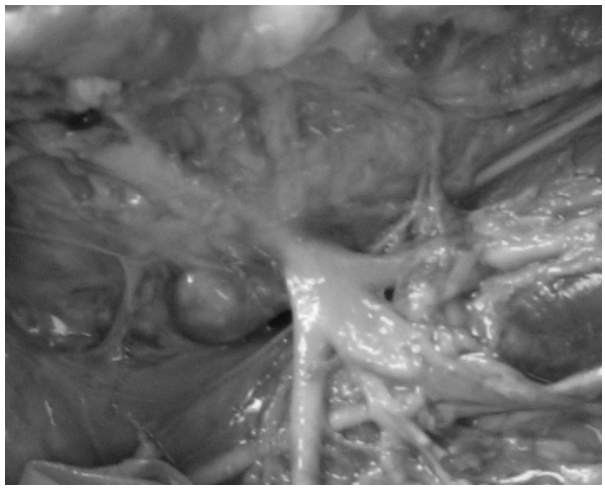

Fig. 2. *Cervical-thoracic*

The right lymph duct passes at a distance of 2 cm ventrally from the cervical-thoracic plexus formed around the cervical-thoracic ganglia, running thereafter sideways vento-cranially, descending from the right side of the aorta towards the cranial vena cava into which it pours. Before pouring in the cranial vena cava, the duct displays a branching which, after passing the aorta-pulmonary ligament, joins again the main duct (fig. 3).

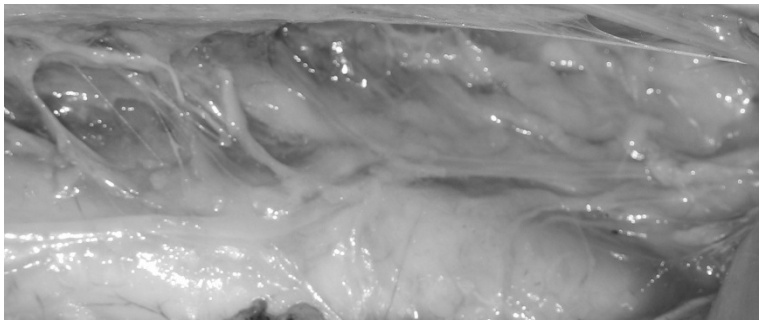

Fig. 3. *Lymphatic duct*

Cardiac lymph is the most direct medium for analyzing metabolic changes in the myocardial cell. Currently, sheep are the animals used for investigation of myocardial lymphatic function. However, questions arise when comparing and interpreting the human system to the experimental model, since the sheep coronary anatomy is different from human anatomy and pulmonary lymph contamination is found in up to 81% of the cases. Swine, having similar coronary anatomy to humans, are a proven model for cardiovascular research. The purpose of this study was to investigate the cardiac lymphatic anatomy of the swine and to develop a reliable cannulation technique to collect the lymph (fig. 4).

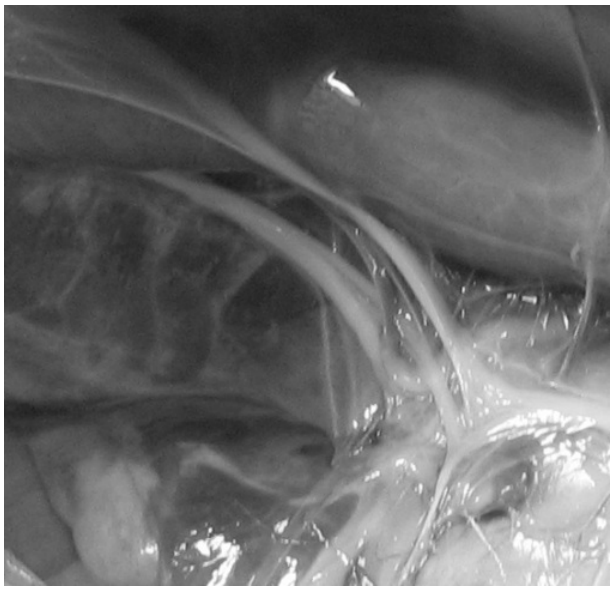

Fig. 4. *Lymphatic duct cannulated*

Conclusion

We conclude that porcine myocardial lymphatics can be successfully cannulated for the investigation of myocardial lymphatic function.

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IDENTIFICATION AND DIFFERENTIATION OF *HAEMOPHILUS PARASUIS* SERO-NONTYPEABLE STRAINS USING A SPECIES-SPECIFIC PCR AND THE DIGESTION OF PCR PRODUCTS WITH HIND III ENDONUCLEASE

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Abstract

Fifty-three sero-nontypeable strains, thirty-two serotypeable, and seven standard strains of Haemophilus parasuis had been examined with a species-specific PCR to confirm the identifications. The PCR amplicon of each strain was further digested with an endonuclease (Hind III) to produce DNA fragments, which allowed the fifty-three sero-nontypeable strains to be divided into 8 distinct restriction fragment length polymorphism (RFLP) patterns. The PCR-RFLP combination adopted in this study provided us with a molecular approach to the identification and differentiation of H. parasuis sero-nontypeable field strains.

Brief communication

Haemophilus parasuis has emerged as one of the most important pathogens in isolated and immune-naïve high health status pig herds during the past decade. So far, 15 serovars are described, and the most prevalent serovars in the United States are 2, 4, 5, 12, 13 and 14 [1]. A rather high percentage of nontypeable serovars was also noted[1,2]. However, very little has been known about the virulence factors of *H. parasuis* field strains isolated from sick pigs. According to some previous studies, the virulence and antigenicity of *H. parasuis* field isolates might vary between different serovars and even between different strains of the same serovar[2]. For this reason, fully relying on serotyping may not be adequate in an attempt to design appropriate immuno-prophylactic measures.

Identification and confirmation of *H. parasuis* field strains can be difficult and cumbersome if the field isolates cannot be serotyped and further biochemical tests are needed. Recently, a species-specific PCR test for detection of *H. parasuis* has been described by Olivereila et al[3], which will greatly improve the diagnosis of *H. parasuis* sero-nontypeable strains.

In this study, 53 sero-nontypeable strains, 32 serotypeable strains, and 7 standard strains of *H. parasuis* were examined with the species-specific PCR assay to confirm their identification. This PCR assay allowed the amplification of an 821 bp product, which was further digested with Hind III endonuclease to produce a DNA fingerprint. The reference strains were seven *H. parasuis* isolates of known serovars that were obtained from Dr. Rapp-Gabrielson. The field strains were isolated from pigs with systemic infection and sent to MVP Laboratories for diagnosis. All field strains were serotyped as described[1,4].

DNA extraction: Briefly, *H. parasuis* strains were grown on Frey chocolate agar

for an appropriate time to ensure purity. Single colonies were boiled in 25 ul of sterile PBS (pH 7.2) in a screw capped tube for 10 minutes and then placed at –20˚C for 10 minutes. The tube is then centrifuged at 14,000 × g for 3 minutes and the supernatant is removed and used as the DNA template.

PCR assay: The oligonucleotide primers that were used in this study are listed in Table 1. The PCR reaction mix for one test sample contains 5 ul of 10× PCR buffer, 4.8 ul of dNTP (each at a concentration of 2.5 mM), 1.0 ul of forward and reverse primers (each at a concentration of 40 uM), 4.0 ul of 25 mM magnesium chloride, 0.5 ul of Taq DNA polymerase (5U/ul), 29.7 ul of deionized water, and 5.0 ul of DNA template.

T a b l e 1

Species-specific primers used to amplify DNA from *Haemophilus parasuis*

HPS-forward 5'-GTG ATG AGG AAG GGT GGT GT-3'
HPS-reverse 5'-GGC TTC GTC ACC CTC TGT-3'

The PCR conditions are as follows: One cycle of 5 minutes at 94˚C, followed by 30 cycles of 0.5 minute at 94˚C, 0.5 minute at 59˚C, and two minutes at 72˚C. Then one cycle of 5 minutes at 72˚C. Amplified products are analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and recorded by using UV transillumination and Polaroid film.

DNA fingerprinting: Purification of the PCR product is performed using Wizard PCR Preps DNA.

Purification System (PROMEGA, Madison, WI), as described by the manufacturer. About 15 ul of the purified PCR product was incubated with 1.5 ul of Hind III (15U/ul) at 37˚C for two hours. The digested products were analyzed by electrophoresis in 2% agarose gel as described.

All of the 85 field strains and 7 standard strains were found to have the expected 821 bp fragment of the *H. parasuis* species-specific gene.

The pattern of the DNA fragments produced by the digestion of purified DNA amplicon with Hind III (Takara Shuzo, Shiga, Japan) allowed the 53 sero-nontypeable strains to be divided into 8 different restriction fragment length polymorphism (RFLP) patterns. They are composed of one to 4 major bands, with sizes between 260 bp and 821 bp (Table 2). The 32 serotypeable strains and 7 standard strains were divided into 7 RFLP patterns (Table 3).

The test results in Table 2 indicate that the PCR-RFLP can be used as a useful method for identification and differentiation among the *H. parasuis* sero-nontypeable strains, while the present serotyping protocol cannot give a confirmation diagnosis.

Table2

PCR-RFLP patterns of 53 sero-nontypeable strains of *H. parasuis*

PCR-RFLP pattern	Serotype	Number of field strains
1A	NT	20
1B	NT	3
2A	NT	4
2B	NT	6
2C	NT	1
3A	NT	8
3B	NT	9
4	NT	2

NT:

The test results in Table 3 also indicate that the PCR-RFLP can be used as an alternative method for studying the genetic relatedness among the strains of the same serotypes. In conclusion, the PCR-RFLP combination we adopted in this study provided us with a molecular approach to the differentiation of sero-nontypeable strains of *H. parasuis.*

Table3

PCR-RFLP patterns of 32 sero-typeable strains and 7 standard strains of *H. parasuis*

PCR-RFLP	Serotype	Number of field strains
1A	2	2
1A	4	6
1A	7	1
1A	12	1
1B	4	1
1B	7	1
1B	12	1
2A	4	1
2B	4	4
2B	12	1
2B	13	2
3A	4	1
3A	7	3
3A	12	1
3B	4	1
3B	13	1
4	4	1

4	**7**	**2**
4	**12**	**1**
1B	**Standard 2**	**1**
2A	**Standard 4**	**1**
2A	**Standard 5**	**1**
2A	**Standard 7**	**1**
2A	**Standard 12**	**1**
1B	**Standard 13**	**1**
3A	**Standard 14**	**1**

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BIOCHEMICAL EVALUATION OF MINERAL STATUS IN SWINE

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Abstract

For swine, calcium and phosphorus are two minerals with the highest percentage of useful minerals necessary for normal operation of the animal organism.

The research took place in a pig farm which is part of a growing household - annex of semi-intensive type, belonging to an enterprise – SC Central Foundry Orion SA – the city of Campina, Prahova County. The investigation lasted for 6 months (August 2011-January 2012).

The purpose of the research was to estimate the correlation between the physiological condition of sows and the phosphocalcic status.

Comparing the calcium and phosphorus values obtained in the group of pregnant sows and lactating sows group with values obtained from 1.08.2011-05.01.2012, it was appreciated that there is a positive correlation between the concentration of serum calcium and phosphorus not only before the period of pregnancy and lactation, but also during those two periods.

The gestation period is marked by greater losses of phosphorus than calcium, whereas during lactation, calcium losses are higher than those of phosphorus.

Keywords*: swine, minerals, pregnancy, lactation, correlation*

Introduction

It is known that the phosphocalcic metabolism knows permanent fluctuations influenced by many factors acting upon the animal organism (age, sex, physiological status, stress, microclimate conditions, genetic factors, feeding and exploitation condition of pigs). Depending on the quantity and biological role and production, nutrients are divided into macronutrients or major nutrients and micronutrients or trace nutrients.

The need of the biochemical assessment of the mineral status of the swine herd (especially pregnant and lactating sows), comes from the negative consequences of nutritional deficiencies upon animal health. Serum concentration of minerals is a very important parameter that must be taken into account when determining feed rations especially for certain categories of pigs (sows pregnant, lactating, infants, etc.) (Girard C.L. et al., 2006).

Material and methods

The research took place in a pig farm which is part of a growing household - annex of semi-intensive type, belonging to an enterprise – SC Central Foundry Orion SA – the city of Campina, Prahova County. The investigation lasted for 6 months (August 2011-January 2012).

Investigations were conducted on two groups of animals during August 2011-January 2012:

- a group of 15 pregnant sows in the IIIrd and IVth months;
- a group of 15 sows in the third week of lactation.

The experimental protocol was performed during the following phases:

- information, documenting and methods;
- selecting animals to assess calcium and phosphorus levels correlated with the physiological state;
- optimal timing for delivery of metabolic profile;
- processing samples and organizing information.

The instruments and equipment required: special venisection needles, ordinary tubes, ordinary plastic bottles, glass rod, rack, room thermometer, Beckman centrifuge.

Blood collection from the jugular vein was performed under the conditions of optimal harvesting so as to prevent haemolysis of blood that leads to wrong values. Blood was allowed to flow onto the tube walls, thus avoiding foaming and breaking erythrocytes. The amount of blood required in this case was 15-20 ml. Calcium and phosphorus biochemical determinations were made on blood serum using the following procedure for sampling and processing of samples:

Methodology in determining serum calcium and phosphorus:

1. Determination of serum calcium was achieved by using EDTA volumetric method.
2. Determination of serum phosphorus was achieved by emission spectrometry.

Results and discussion

Metabolic evaluation of the 15 pregnant sows in the Third and Fourth month has determined the following phosphocalcic status changes as it follows (Table 1).

a. **Sows – third month of gestation:**

- 9 pregnant sows showed deviations of physiological serum calcium value for this category of exploitation (10 ± 1 mg / dl) under normal representing 60% of the total of 15 sows included in the study;
- hypocalcaemia ranged from the minimum of 7.00 mg/100 ml serum to maximum values of 8.67 mg/100 ml serum, thus hypocalcaemia was ranged into mild, moderate and severe;
- regarding status phosphate, it knows deviations from normal and more pronounced, thus modifying serum phosphorus level below the minimum allowed (7 ± 1 mg/dl) occurred in almost all the investigated group, respectively in 12 sows (80.00%).

Table 1

Calcium and phosphorus values in sows in the fourth month of gestation

No. crt.	Ca mg/100 ml serum/(3rd month)	Ca mg/100 ml serum/ (4th month)	P mg/100 ml serum/ (3rd month)	P mg/100 ml serum/(4th month)
1.	9.00	9.00	6.75	6.50
2.	7.62↓	7.98 ↓	5.46 ↓	5.6 1↓
3.	9.00	8.23↓	6.20	5.91↓
4.	8.98	7.00 ↓	5.00 ↓	5.00 ↓
5.	8.88	7.58↓	5.36 ↓	5.23 ↓
6.	7.99 ↓	7.45 ↓	5.75 ↓	5.25 ↓
7.	7.88↓	8.23↓	6.23	6.43
8.	7.99 ↓	8.45↓	4.56 ↓↓	4.50 ↓↓
9.	8.56↓	8.75 ↓	5.00 ↓	4.71 ↓↓
10.	8.92	8.67 ↓	4.98 ↓↓	4.98 ↓↓
11.	9.00	7.98 ↓	4.51 ↓↓	4.51 ↓↓
12.	8.67 ↓	7.32 ↓	5.20 ↓	4.29↓↓
13.	8.00 ↓	7.00 ↓	5.45 ↓	5.45 ↓
14.	7.67 ↓	9.00	4.70 ↓↓	4.70 ↓↓
15.	7.00↓	7.98 ↓	4.90 ↓	5.00 ↓

b. **Sows – fourth month of gestation:**

- for this period of gestation, 13 pregnant sows showed deviations of physiological serum calcium value (10 ± 1 mg/dl) under normal, representing a percentage of 86.66% of the total of 15 sows included in the study;
- hypocalcaemia varied as in the previous case, the minimum value of 7.00 mg/100 ml serum to maximum values of 8.75 mg/100 ml serum; hypocalcaemia was ranged into mild, moderate and severe;
- the phosphate status suffered – in terms of percentage of affected sows – an almost common situation as the third month of gestation, the change of serum phosphorus level being at the minimum permissible limit values (7 ± 1 mg/dl) in a number of 13 sows (86.66%).

Analyzing the calcium level for the 15 sows, there was a slight but steady decrease serum calcium value in the fourth gestation month compared to the previous month (month of gestation III) for 11 sows (73.33%) from the total of 15. For one sow (n = 1), serum calcium levels remained in the normal range (9 mg/ml serum); for 4 sows (n = 2, 7, 8, 9), serum calcium levels slightly growth, but is considered insignificant especially since all values obtained are included in a state of hypocalcaemia. The trend line of the evolution of serum calcium levels for the

months of gestation III and IV had a linear path and based on the value of R^2 = 0.0068, 0.1718, it is estimated that there are correlations statistically insignificant between the two stages of pregnancy and calcium levels; thus we conclude that each of the two periods of gestation affects differently both calcium absorption and metabolism. (Fig. 1)

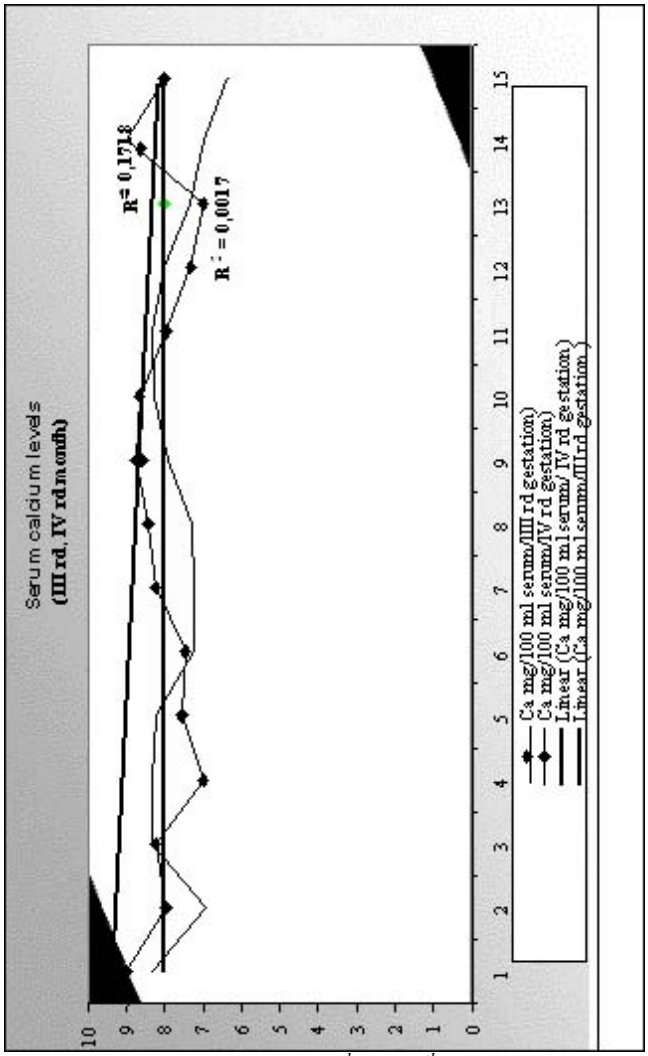

Fig. 1. *Serum calcium levels in the 3rd and 4th months of gestation*

Serum phosphorus level has declined slightly in the fourth month of gestation for 7 sows (46.66%) of the 15 tested (n = 1, 3, 5, 6, 8, 9.12). For two sows (n = 7.15) serum phosphorus levels ranged in an upward trend (n = 7, P = 6.23 mg / ml serum – month III, 6, 43 mg / ml serum – fourth month) (n = 15, P = 4.90 mg / ml serum – month III, 5.00 mg / ml serum – month IV). In both cases, however the phosphorus level is at the minimum permissible limit (7 ± 1). The trend line of

evolution for the serum phosphorus levels in months III and IV of gestation, had a slightly downward path. Based on the value of $R^2 = 0.3648$, 0.3959, it is estimated that there are significantly weak statistical correlation between the two stages of pregnancy and the phosphorus levels (Fig. 2).

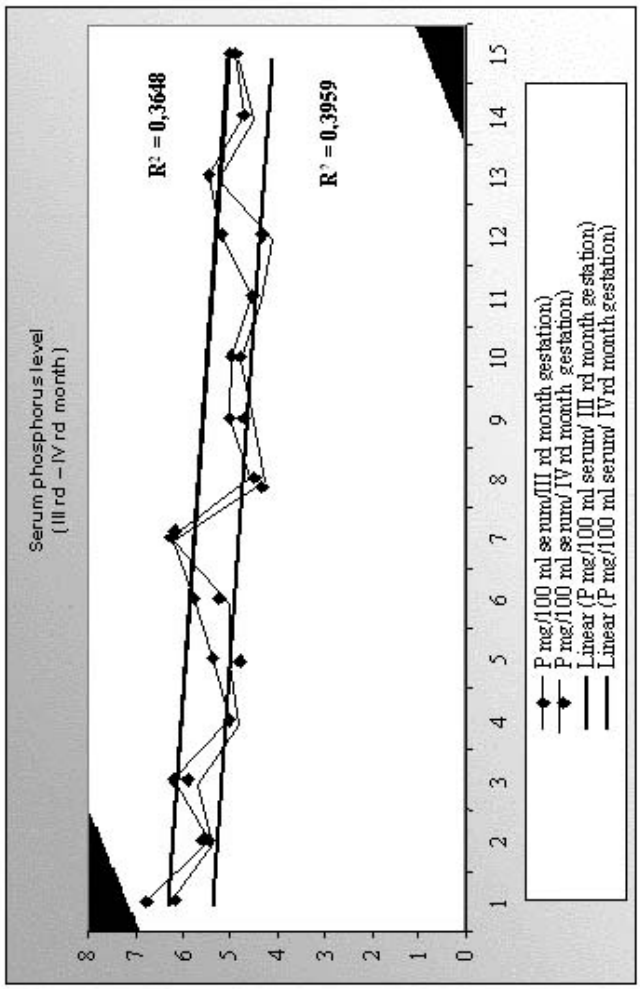

Fig. 2. *Serum phosphorus levels in the 3rd and 4th months of gestation*

Metabolic assessment of a group of 15 sows in the third week of lactation was made in order to establish the levels of serum calcium and phosphorus.

The third week of lactation was chosen to survey the two parameters, knowing that the lactation time is approximately 2 months, the milk production varying with the breed and number of piglets, from 2-3 l milk/day in the first two weeks up to 6-8 l milk/day in the next two weeks, after which it begins to decline (Stoica I. et al., 2001) (Table 2).

During this period calcium and phosphorus requirements are based on minimum feeding level of 1.8 -1.9 kg food / day and 4.4 to 5.3 kg during gestation

of food / day. If the amount of food is less than 1.8 kg food / day during gestation, the ratio should be made so as to ensure sufficient calcium and phosphorus to meet daily requirements (Pârvu Gh.et al., 2003).

Table 2

Calcium and phosphorus values in sows in the third week of lactation

No. crt.	Ca mg/100 ml serum	P mg/100 ml serum	Ca/P ratio
1.	**8,23 ↓**	**5,99**	**1,37**
2.	**8,51 ↓**	**6,14**	**1,38**
3.	**8,41 ↓**	**6,89**	**1,22**
4.	**8,69 ↓**	**6,21**	**1,39**
5.	**7,89 ↓**	**5,12 ↓**	**1,54**
6.	**7,34 ↓**	**5,23 ↓**	**1,4**
7.	**7,76 ↓**	**4,70 ↓**	**1,65**
8.	**8,00 ↓**	**5,54 ↓**	**1,44**
9.	**8,76 ↓**	**5,29 ↓**	**1,65**
10.	**8,78 ↓**	**4,58 ↓**	**1,91**
11.	**8,92 ↓**	**4,68 ↓**	**1,9**
12.	**9,56**	**6,23**	**1,53**
13.	**7.85 ↓**	**5,34 ↓**	**1,47**
14.	**8,89 ↓**	**6,54**	**1,35**
15.	**7,98 ↓**	**5,36 ↓**	**1,48**

During lactation compared to gestation, the following observations could be made:

- the serum calcium levels for the 15 sows declined steadily in 14 sows (93.33%);
- a single sow (6,66%) maintained serum calcium levels within specific physiological levels (n = 12; 9.56 g Ca/ml serum);
- the phosphorus levels in lactating sows showed deviations from the physiological values for 9 sows (60%), with limits that ranged from 4.58 mg P/ ml serum to 5.54 mg P /ml serum.

A comparative analysis of serum calcium and phosphorus levels for the entire group tested were found as follows (Fig. 3):

➢ for 9 sows (60%) during this period have been established both diagnosis of hypocalcaemia hipophosphoremia with varying degrees of severity (n = 5, 6, 7, 8, 9, 10, 11, 13,15);

➢ sows (n = 1, 2, 3, 4) were diagnosed only with hypocalcaemia, serum calcium values ranging from 8.23 mg Ca/ml serum and 8.69 mg Ca/ml. Serum phosphorus values are included in physiological limits (P 5.99 mg/ml serum and 6.89 mg P/ml serum).

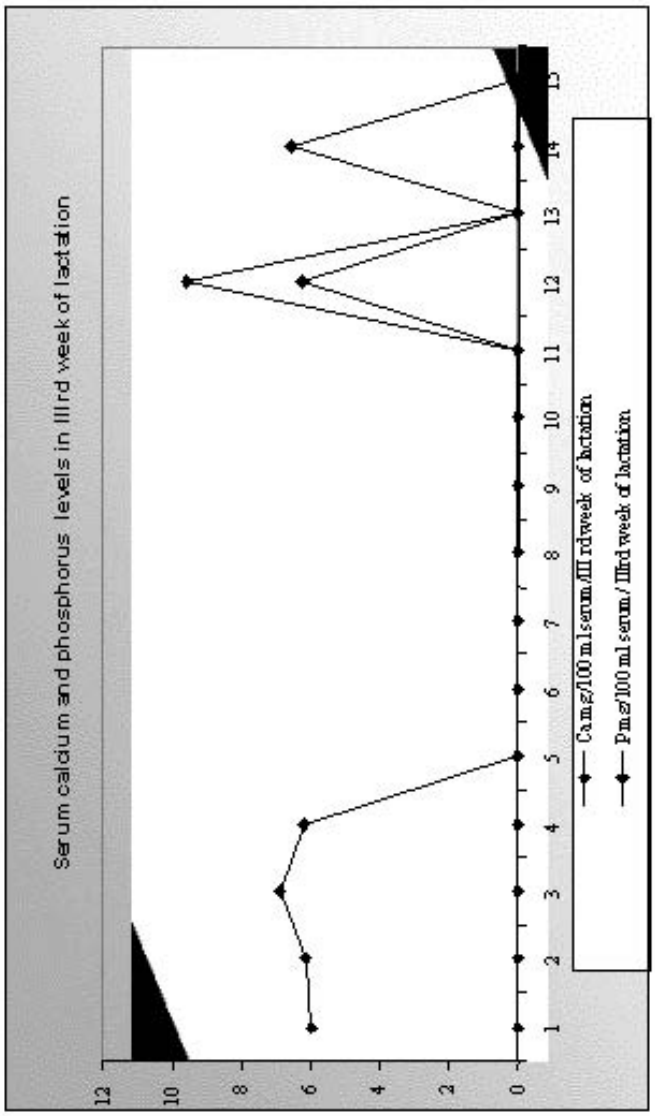

Fig. 3. *Serum calcium and phosphorus levels in the third week of lactation*

During lactation there have been more cases of severe hypocalcaemia (33.33%) than the gestation period in which depending on the stage of gestation

which has been monitored – there were 4 sows with severe hypocalcaemia in the fourth month gestation and one case (6.66%) in the third month of gestation. Therefore, we can say that the calcium requirement should be supplemented during this time, especially since it is known that the sow lactation begins on the 75th day of gestation, sow's milk having in composition 2.20 g Ca/l and 1.50 g P/ l (Simon V., 2009)

Conclusions

Based on the results obtained by both laboratory tests and especially the statistical processing of data, it was shown that there are some nutritional deficiencies regarding both the quantitative report and especially the phosphocalcic balance of pregnant and lactating sows.

Following the metabolic tests of the pregnant sows batch in months III, IV calcium and phosphorus values varied depending on the stage of gestation; cases of hypocalcaemia with three degrees of severity and hipophosphoremia with 2 degrees of severity have been recorded.

Gestation period is marked by greater losses of phosphorus than calcium, 53.33% of sows in the fourth month of gestation and 46.66% of sows in the third month of gestation were diagnosed with severe phosphorus loss, whereas during lactation, calcium losses are higher than those of phosphorus; thus 33.33% of lactating sows were cases of severe hypocalcaemia.

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DAIRY COW BEHAVIOUR AND THEIR WELFARE

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Abstract

The purpose of this research was to establish the manner in which the flooring type may influence the welfare levels in dairy cows by assessment of animals' behaviour. 34 dairy cows were grouped based on the shelter floor surface: concrete with straw bedding, asphalted concrete with straw bedding and concrete plus shavings. The behaviour was assessed through direct observation.

The behavioural displays of the cows suffering from laminitis were different from the healthy ones, as their resting behaviour outside the stalls was more prevalent (17,6% compared to 8,8%) while the feeding behaviour was less present (10,1% compared to 14,7%). Likewise, the socializing behaviour was more active in these animals, compared to the healthy cows.

Keywords: *dairy cows, behaviour, welfare*

Introduction

Special attention is paid today in commercial farms to the welfare of dairy cows raised in free stabulation systems, which give them the opportunity to express their natural behaviour – including the social one. [1, 2]

The welfare represents the way by means of which the production system caters for the physiological and behavioural needs of the animals as well as their health condition. The selection of welfare indicators and the assessment methods reflect the fundamentals of the approach to the welfare of farm animals. A physical activity frequently observed in dairy cows is the standing up and lying down behaviour. This can be assessed based on the time period and the manner of achievement, and associated to discomfort, especially in cows suffering from laminitis. Display of resting behaviours on other premises than their stalls may indicate the fact that they consider them uncomfortable. Flooring type may increase the risk of lesions and injuries, and at the same time restricts certain behavioural displays. [1, 5, 7] Good practices for cows with laminitis should ensure an appropriate resting surface, sufficient and adequate fodder while they should not be separated from the rest of the animals, as it might negatively affect them. [3, 4, 6]

Materials and methods

The study was carried out during one year, in three dairy cow farms in the southern part of the country. 34 cows were monitored multiparous, and the animals were raised in free stabulation systems, consisting in shelters organised in two and

four rows of stalls respectively. The area destined to animal movement was the same with the waste disposal one. The shelter facilities were identical. The cows were grouped based on the flooring area resting in: A lot, cows (n: 11) housed in stalls with concrete floor and straw bedding; B lot, cows (n: 11) housed in stalls with asphalted concrete floor and straw bedding and C lot, cows (n: 12) housed in stalls with concrete floor and shavings bedding.

The behaviour was assessed by direct observation rumination, feeding, resting and sleeping activities; body position (standing, lying/standing, and walking) for each animal over the course of 15 minutes once a week at different times during the first four weeks of the lactation period.

The data obtained were statistically processed using the t test in order to compare the three lots monitored in the study.

Results and discussion

The behavioural displays of cows diagnosed with laminitis (fig. 1) were different from the healthy ones, with a more prevalent resting behaviour outside the stalls (17.6% compared to 8.8%) and feeding behaviour less displayed (10.1% compared to 14.7%). Likewise, these animals showed a more active socialising behaviour, compared to healthy cows.

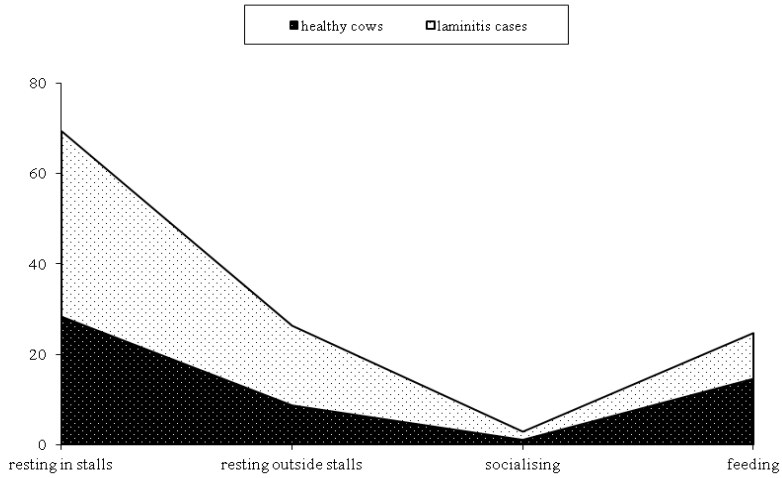

Fig. 1. *Percentage of some behaviour displayed in the monitored cows*

The comfort of dairy cows means ensuring the conditions that would answer their needs and necessities. Permanent supply of fodder and water, a good microclimate, flooring type in the movement area and comfort in the resting area have a 25% influence in production performance.

Placing cows in the movement area reduces the resting time and increases the incidence of mastitis and cloven hoof diseases. If cows are placed in a movement

area that is dirty and humid, total resting time is cut short with negative consequences on the cloven hoof health condition. Inappropriate hygiene of the udder constitutes an increased risk factor for mastitis. Moreover, there is the danger that the animals that are lying down would be injured by the moving ones.

The cows must be able to lie down without difficulty, using all their four legs. Discomfort due to the stall as such and particularly to the floor leads in most cases to decubital lesions or to the fact that animals will no longer lie down in their stalls but choose to do so on the walking lanes. Extended resting time is very important for the productivity of dairy cows because vascularisation of udder in decubitus improves by 30%, while ruminating is 70% more intense. This means also that the cloven hoofs will be less exposed mechanically, chemically and from the infectious perspective, thus meeting one of the first conditions to have healthy, productive animals.

Conclusions

The results of the study have shown the effects of laminitis on the social and individual behaviour of dairy cowsLaminitis cases are the manifestation of pain behaviour which negatively impacts the health and productivity of animals affected.

Cows diagnosed with laminitis must be housed on an appropriate resting surface, have sufficient and adequate fodder and must not be separated from the rest of the animals as this might negatively impact them.

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EMERGING ISSUES ABOUT TWO ZOONOSES, LYME DISEASE AND CANINE EHRLICHIOZA

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Abstract

This study highlights the characteristics of two canine zoonoses Lyme disease and ehrlichiosis and whose global incidence has increased significantly in recent years. Nonspecific symptoms and insidious development lead for most of the time, the late diagnosis of the two morbid entities increasing the risk of contamination of pet owners.

They studied a total of 10 pet dogs who have general status changes, loss of appetite, adynamic joint pain.

Rapid diagnostic tests currently marketed are effective in identifying these infections, their use as close to the onset of disease led to the creation of their early treatment.

Keywords: *Lyme disease, ehrlichiosis, dogs*

Introduction

Canine ehrlichiosis is known as tropical canine pancytopenia or rickettsioza. It was first described in Algeria in 1935, and later, during the Vietnam War (1959-1975) became much better known since many service dogs died after developing infectious process [1, 2]. It seems that of all breeds of dogs, Alsacian Shepherd exhibits increased sensitivity. Rhypicephallus mite infection is through sanguineous, which is contaminated with Ehrlichia canis from sick dogs in the acute phase of the disease, which is short. Massive infestations of mites favour disease transmission. The etiologic agent is transmitted to dogs when bloodsucking ticks from or where blood transfusions are running dogs taken from asymptomatic carriers. Once steeped in the dog, germs multiply in blood mononuclear cells in spleen, liver and lymph nodes.

In sick dogs notes splenomegaly, uveitis („blue eyes") with retinal damage and blindness, epistaxis and cahexie. [3, 4]. Approximately 60% of dogs with ehrlichiosis bleed in various locations, due to thrombocytopenia installed.

Treatment requires administration of tetracycline or doxycycline for a period of approximately 21 days. It is noted that if the animal is cured, the infection may recur anytime as postinfectious immunity is not lasting.

Aspects of human ehrlichiosis. The disease is transmitted to humans from the bite caused by ticks belonging to the genus Rhypicephallus. The first clinical signs

of disease develops in 5-14 days after the infective bite. Usually asymptomatic disease progresses in most cases. In the literature are described the following clinical signs: headache (migraine), high temperature up to 40ºC. Some patients present maculopapular lesions and / or petechiae on the chest and extremities. Also, in serious cases mentioned occurrence of abdominal pain accompanied by vomiting and diarrhea, disseminated intravascular coagulation (DIC) and installation irreversible comatose state [5, 6].

Lyme disease is an infectious disease in humans, cats, dogs and other domestic animals and wild species produced by Borella burgdorferi, transmitted by ticks and mainly manifested by dermatitis, arthritis, meningoencephalitis and general disorders.

In human medicine, is called “the great imitator” or “disease with 1000 faces” as affecting the entire body, presence of symptoms mimic many other diseases. Individual response to the pathogen is very different from person to person, hence the multitude of clinical manifestations. Lyme disease is a serious zoonotic disease, whose incidence and importance is steadily increasing in recent years worldwide. The etiologic agent of Lyme disease was identified by Johnson et al. in 1984 in the U.S., as Borrelia burgdorferi. Subsequently, it was shown that etiologic agent isolated from the U.S. and Europe, there are differences in the chemical composition of the outer membrane lipoprotein and their nucleic acid structure, so it was decided the taxonomic reclassification. Thus, for all 4 groups involved in infectious B. burgdorferi in the U.S., Europe B. garinii, B. afzelii, B. japonica in Asia in Japan proposed the generic name of B. burgdorferi sensu lato [4]. Germs are spirochetiform type and transmitted by ticks in particular, thereby natural focal disease.

The first symptoms occur 2-5 months after infection and consist syndrome bite fever (39.50 C-40, 50C) and impaired general condition, manifested by anorexia, lethargy and Adynamic. Explore and limb joints lymph nodes are enlarged in volume. Because the arthritis pain, the animal tried to avoid possible movement, which becomes tedious and obvious lameness. Renal disorders are also common in Lyme disease, accompanied by azotemia, hyperphosphatemia, uremia, proteinuria, edema peripheral and progressive weight loss [4]. Around the site of tick bite made, it forms a macula, more discreet than at man where it disappears in a few days. Other symptoms that may occur are neurological disorders and cardiac arrhythmias. Treatment is done by long-term administration of antibiotics [4]. Germ not trigger postinfectious immunity of the animal, which is why dogs can become infected again at another carrier tick bites, developing a new infectious process.

Lyme disease in humans. Most patients respond to treatment, especially if initiated early in the disease, but a small percentage will experience symptoms after years: muscle pain, arthritis, fatigue, insomnia and cognitive disabilities. Treatment is with a “cocktail” of antibiotics (rifampicin, doxycycline, etc.) for a period of 30 days or more, only under medical supervision.

Its purpose was to bring attention to the characteristics of two bacterial zoonoses aetiology diagnosed in dogs, Lyme disease and canine ehrlichiosis and whose incidence in our country recorded a permanent growth in the last 10 years.

Materials and methods

The research was conducted on a total of 10 pet dogs that were presented at a veterinary clinic in Bucharest in 2011 with hyperthermia, altered general condition and history of external parasitism ticks.

The research was conducted in several stages:

− Information from the literature (bibliographic study);

− Epizootological inquiry, which revealed that the animals were infested with ticks, 1-2 months ago;

− Clinical examination of the animals under study.

Clinical examination was performed by dogs semiological methods: inspection, probes, percussion, auscultation and thermometry.

Confirmation of the presence of two emerging diseases was made by rapid diagnostic tests and haematology.

A. *Diagnosis of canine Lyme disease (borreliosis)*

To confirm the diagnosis of borreliosis was used "diagnostic kit for detection of antibodies that indicate the presence of the Borrelia burgdorferi in the blood or serum of dogs" VetAll manufacturer.

Description rapid diagnostic test. Lyme disease is caused by bacteria called Borrelia burgdorferi which is transmitted to dogs by ticks of the genus Ixodes (deer tick). For bacteria to be transmitted, the tick must remain attached to the dog's skin for at least 24 hours. Lyme disease is a multisystem disorder with symptoms that include fever, loss of appetite, lymphadenopathy, paralysis. If untreated, Lyme disease can cause heart problems, kidney and joints, and in rare cases, can lead to neurological turmoil. Principles of diagnostic rapid test borreliosis

It operates according to the imunocromatography principles. After being absorbed into the cellulose layer, anti-Borrelia burgdorferi combined with colloidal gold complex containing antigens of Borrelia burgdorferi attached layer, forming Ag-Ac complex. This complex forms direct connections sandwich, Ag-Ag-Ac with Borrelia burgdorferi antigen of nitrocellulose membrane.

The test results appear on the control line (C) and Test (T) of test boxes. The operation of diagnostic rapid test. Detection of antibodies to Borrelia burgdorferi in the blood or serum of dogs is done in 10 minutes.

The test includes: test cassettes, buffer bottle, disposable pipettes PVC. The box contains 10 rapid tests (kits) diagnosis.

B. *Diagnosis of canine ehrlichiosis*

In order to achieve canine ehrlichiosis diagnosis were performed two examinations: haematology, followed by rapid diagnostic test to confirm "SNAP 4DX" produced by IDEXX Laboratories.

Haematology. By Grümwald-May-Giemsa staining were observed in monocytes intracytoplasmic inclusions colored red, purple or dark blue, depending on the stage of development of germs.

4DX SNAP diagnostic test based on immunoassay technique for detection of specific anti-Ehrlichia canis, anti-Borrelia burgdorferi, Anaplasma phagocytophilum and anti-Dirofilaria immitis antigen ("heartworm") in whole blood or serum of dogs.

Results and discussion

Of the ten pet dogs presented for investigation, 4 dogs confirmed the result of Lyme disease and 3 dogs confirmed the presence of Ehrlichia canis infection.

In the treatment of Lyme disease in dogs receiving clarithromycin (0.005 to 0.01 g / kg, intravenously in the first stage and then orally every 12 hours) and erythromycin per os, 1 hp to 12 hours for 30 days.

The treatment of dogs with ehrlichiosis to use the following regimen:

• Minocycline: 50 mg/12 hours – specific human antibiotic use for Ehrlichia canis (and Anaplasma spp), adm. for 1 month (made in Germany) in his absence is effective and doxycycline;

• Marbofloxacin: 0.4 ml / day for 14 days;

• Sulodexid: 1cp/zi associated with due Vessel – antithrombotic agents;

• Curcumin – supplement that works in several ways: protects against free radicals, it is a powerful antioxidant, reduces inflammation by lowering histamine levels and, apparently, by increasing production of natural cortisone by the adrenal glands, liver protects toxic compounds and last but not least, reduce platelet aggregation, which improves circulation and support cardiovascular health;

• Silymarin – hepatoprotector;

• Ursofalk – product responsible for the dissolution of cholesterol gallstones;

• Milgamma N – product containing B complex used in inflammatory neurological diseases and painful muscle contractions painful facial paresis, recovering and geriatrics;

• Synoquin – nutritional supplement for dogs;

• Ultra EFA – product used to treat skin diseases (dermatitis, ulcers, erosions, keratoses), kidney (nephrosis, renal failure), inflammatory (acute intestinal inflammation, asthma, epilepsy, severe arthritis, discoid lupus, amyloidosis) Auto disease -immune, hepatitis and pancreatitis acute cardiovascular disease (endocarditis, arrhythmias, hypertension, heart failure, cardiomyopathy), anti-inflammatory and supplier of essential fatty acids Omega 3, 6 and 9, to adjust operational balances at the cellular level.

• Decaris – immunostimulating effective in RA;

• Tea Hepatocol – choleretic, cholagogue, antispasmodic, liver, biliary tract inflammatory and disinfectant;

• infusion of NaCl / soil. Ringer daily to supplement fluid losses – electrolyte specific hipotine and isotonic dehydration, this rich moisturizing treatment is an important element in eliminating toxins produced by seed;

• Administration of rations balanced by healing and return of appetite;

They presented their respective owners contamination with etiologic agents, both diseases are zoonosis and recurrence prevention methods reinfection in pets, insisting on the importance of regular external parasite.

Conclusions

1. If suspicions these morbid entities, ehrlichiosis and Lyme disease in dogs pet must be used as early as rapid tests to confirm the diagnosis

2. Treatments, although often established early due care pet owners to present the veterinary services immediately noticed changes in health status have limited effectiveness, because animals can then reinfect

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GRAVIMETRIC DETERMINATION AND CYTMORPHOLOGICAL CHANGES IN EXPERIMENTAL STRESS IN YOUNG AVIAN BREEDING

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Abstract

For carrying out the work animals of economic interest (conventional chicken, heavy breed – hybrid Rosso) have been used. There were 4 groups, each group containing 10 birds, which were subjected to immunosuppression drugs, stress, starvation and restriction of light, vaccination against avian infectious bursal disease (IBD) and control infection. Within each phase, blood samples were taken for serological tests. The experiment was conducted over a period of 42 days. At the end of the test chickens were slaughtered in white and samples were collected for histological examination.

A series of gravimetric and histological parameters were followed.

Based on research conducted a decrease in body weight and also in the weight of some lymphoid organs (cloacal bursa, spleen) was noticed.

Histologically, chickens subjected to starvation stress and restriction of light and those treated with cyclophosphamide present involutive phenomena of the cloacal bursa.

Keywords*: poultry, cloacal bursa, immunosuppression*

Introduction

Structural and functional parameters of the immune system are influenced by a series of endogenous and exogenous factors that can determine stimulation, amplification, suppression and even the annulment of the immune response (1, 3).

By numerous experimental data, the action of diverse exogenous factors concerning the immunological defence capacity of animals was demonstrated. The most significant and important are environmental, nutritional, microbial, and stress factors (2, 4).

Investigation of the gravimetrical and cytomorphological changes determined by the action of several suppressive factors, represented by maintaining the conventional chickens in unfitting microclimate and nutritional conditions, was conducted through this research.

Materials and method

The research was conducted on 40 conventional chickens, heavy breeds (Rosso Hybrid), aged 50 days, distributed in 4 lots (A, B, C, D), each one containing 10 chickens that were subjected to drug induced immunosuppression, starvation stress, light restriction, were vaccinated against Infectious Bursal Disease and also subjected to control infection.

Lot A was used as witness, lot B was vaccinated against infectious bursal disease, lot C was subjected to drug induced immunosuppression and lot D was subjected to starvation stress.

During each step blood was drawn for haematological and serological tests. The experiment was conducted over a period of 42 days.

Cyclophosphamide administration (Endoxan – 200mg active ingredient/phial) was given by intramuscular inoculation (i.m.), 3 days in a row, and repeated at 2 and 4 weeks.

Starvation stress consisted in reduced daily intake (21/2) by half, and light restriction was done by reducing the light period to 8 hours a day.

Vaccination against avian infectious bursal disease was attained with a live lyophilized vaccine, PA strain embrioadapted I – 93 ($10^{3,5}$ DIE_{50} / vaccine dose), administered subcutaneous, in 2 rounds. The first vaccination was done at the beginning of the experiment and was repeated after 2 weeks.

The control infection was done with the standard pathogenic strain of the IBD, 52/70 (2,50 X 10^2 CID_{50}), inoculated i.m.

The chickens were slaughtered in the 8 day post infection and the cloacal bursa were harvested. Individual gravimetrical determinations were conducted on chickens and on the cloacal bursa, then the organs were fixated in saline neutral formaline 10%. The cloacal bursas were paraffin embedded and then sliced at 6-7 μm.

Quantified parameters:

1) body weight;
2) fabricius bursa's weight;
3) spleen weight;
4) changes that appeared at tissular levels of some immunocompetent organs.

Results and discussions

Determining body and some lymphoid organs weight

The results regarding body weight are synthesized in table and figure 1. It has decreased *distinctly significant* in cyclophosphamide treated lot ($p<0,025$, being $1,29 \pm 0,077$) in opposition to the witness lot that was unvaccinated and *highly significant* in the lot submitted to starvation stress ($p<0,005$, being $1,18 \pm 0,145$) in opposition to the witness lot that was unvaccinated and had the final weight of $1,46 \pm 0,061$.

Table 1

Body weight values – statistical evaluation

Lot	A	B	C	D
Average ± ΔS	1,46 ± 0,061	1,51 ± 0,039	1,29 ± 0,077**	1,18 ± 0,145***

** = distinctly significant difference; *** = highly significant difference.

ΔS – standard deviation

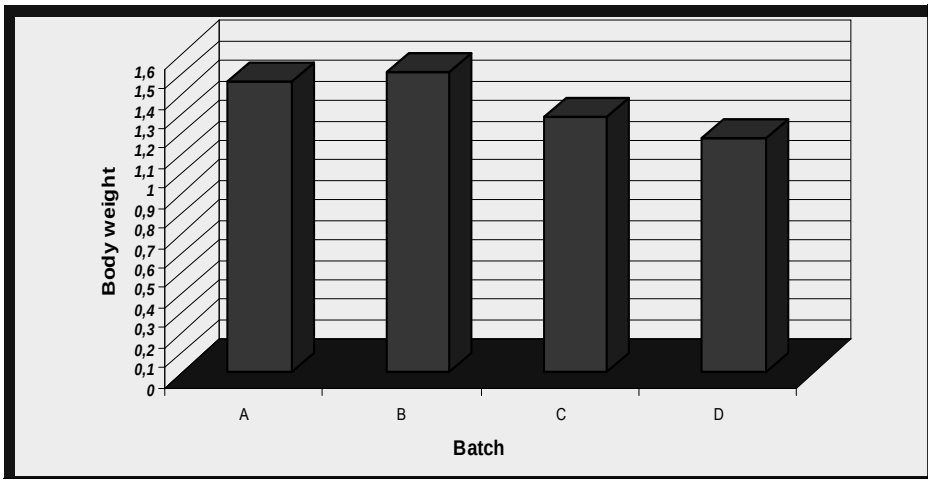

Fig. 1. *Body weight at the end of the experiment*

Gravimetrical determinations were conducted on the spleen and cloacal bursa.

Spleen weight (table and fig. 2) has decreased *highly significant* in lot C ($p<0,005$) and *distinctly significant* in lot D ($p<0,01$) opposed to the whitness lot, the evolution being similar to the one of the body weight.

Cloacal bursa's weight (table and fig. 2) has decreased *distinctly significant* only in C lot, that was treated with cyclophophamide ($p<0,01$) compared to the witness lot.

Table 2

Average weight of the cloacal bursa and the spleen – statistical evaluation

Lot	A	B	C	D
Spleen's weight ± ΔS	3,48±0,50	3,62±0,60	2,34±0,53***	1,98±0,34**
Cloacal bursa's weight ± ΔS	2,06±0,63	1,98±1,15	0,74±0,31**	1,40±0,99

** = distinctly significant difference; *** = highly significant difference.

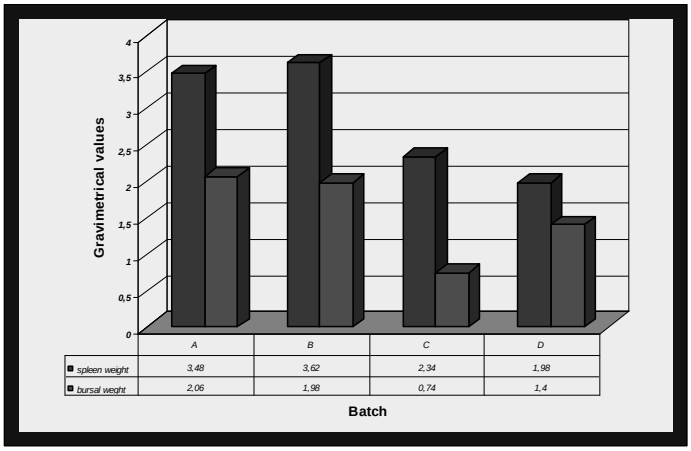

Fig. 2. *Weight of the spleen and the cloacal bursa at the end of the experiment*

Changes that appeared on tissular levels

In lot A, the cloacal bursa, at this age, presented a well organised mucous membrane, constituted by the epithelium and chorion. The bursal epithelium is continuous, but appears interrupted with other types of epithelial cells in the communication and contact spot of the lymphatic follicles with the epithelium surface (fig.3).

In lot C, the bursal epithelium suffers an intense hyperplasia process, followed by folding (fig. 4). Cyst formation is an intrafolicullar process that happens by the destruction of the bursal lymphocytes. In lot D, the cloacal bursa's cytoarchitectural changes are similar to the ones met in the cyclophosphamide action.

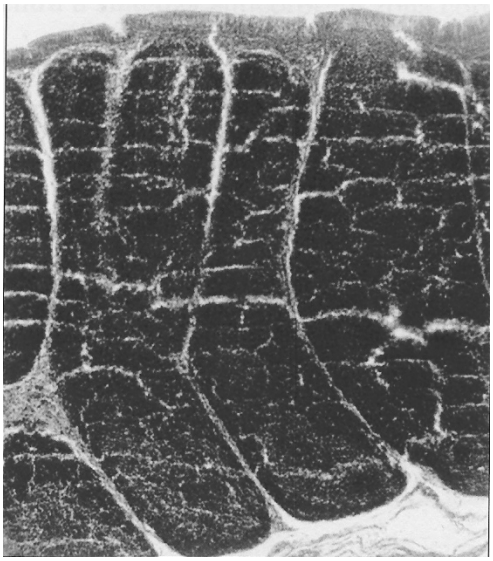

Fig. 3. *Cloacal bursa mucous structure. Bursal follicles of pyramidal shape and reduced chorion between the bursal follicles. Stain. H.E.; Ob. 6x*

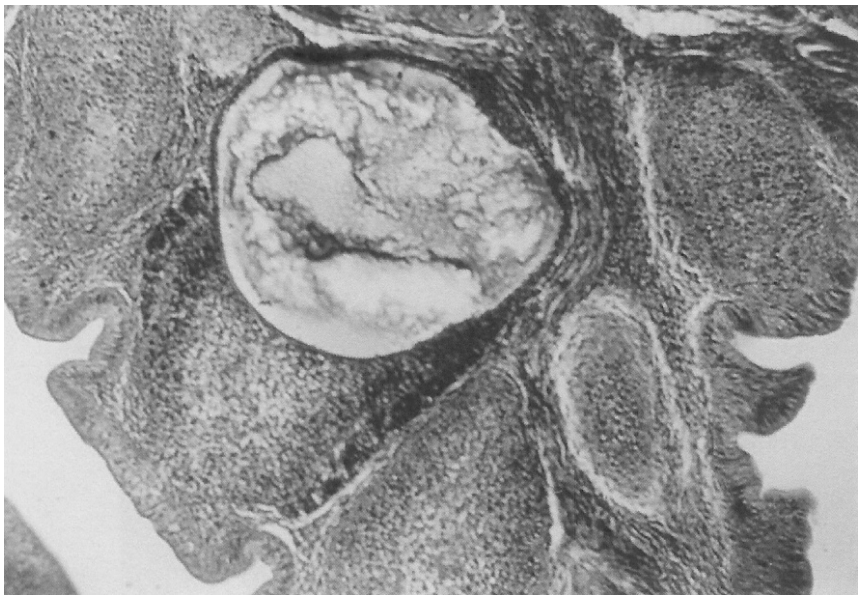

Fig. 4. *Intrafolicullar cyst after cyclophosphamide treatment*
Mallory trichromic stain; Ob. 10x

The main changes registered in this lot, in the cloacal bursa, are the mucous levels. The epithelium of the bursa is subjected to a hyperplasia of the calceiform mucous cells, but without the formation of cysts or intraepithelial glands.

In front of the contact surface between the lymphoid follicles and the epithelium associated to the lymphoid follicles appears an increase in numbers of the epithelial cells.

Conclusions

1. Suppression on the immunological response capacity in chickens was done by the administration of cyclophosphamide and through maintaining the poultry in stress conditions (reducing the food intake, exposure to poor lighting).

2. The induced suppression determined the decrease in body weight and of some lymphoid organs (cloacal bursa, spleen).

3. Histologically, the chickens submitted to starving stress and light restriction present involutive phenomena of the cloacal bursa, similar to those bursectomised by cyclophoshpamide.

4. The histological changes of the cloacal bursa are present in the mucous levels, affecting the epithelium and also the lymphatic follicles from the chorion, with consequences on lymphopoiesis and antibody genesis.

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HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN CATTLE ENTERIC SYNDROME

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Abstract

The study was aimed at determining the aetiopathogenesis and some paraclinical diagnostic elements of the post enteric syndrome renal dysfunctions of calves. Enteritis cases were recorded on a cattle farm at the end of the housing period (38-55% prevalence). The enteric syndrome invariably induced various degrees of dehydration, consequently followed by renal dysfunctions caused by the increased blood viscosity, the activity of various endogenous toxin and other pathogenic factors.

The packed cell volume (PCV), haemoglobin and total serum proteins were higher in a group of 28 diarrheic calves aged 1-42 days. Blood, urea was 38,3 ± 28,5 mg/dl (reference limits 20-38 mg/dl), while creatinine was 3,05±1,24 mg/dl as against the maximum normal value of 2 mg/dl. There existed a positive correlation among the PCV and serum urea values ($r = 0,69$, $p<0,001$), as well among the urea and creatinine ones. At 42% of the 19 urine samples collected by spontaneous urination were detected proteinuria.

These results indicate the evolution of paraclinical changes in calves subsequent to the enteric syndrome that are attributable to the renal and liver dystrophic lesions.

Keywords: *renal dysfunctions, enteritis, calves*

Introduction

Baby calves reared under farm conditions such frequently recorded digestive disease: dyspepsia, neonatal specific diarrhea or nonspecific enteritis, which can cause high mortality losses. In many episodes of enteropathy in calves, it was shown that there is a complex etiopathogenesis, the pregnant cow is often the "starting point" (3, 4, 5, 11, 12, 13, 16).

Enteric syndrome invariably produce dehydrated calves, which is manifested in complex disorders, haematological and biochemical changes: increased haemoglobin, hematocrit, serum total proteins, urea and creatinine, change in the acid-base balance, electrolyte etc. (6, 9, 10, 12, 15). Increased blood viscosity, recorded usually from enteric syndrome evolution is followed by disruption of renal filtration function. Renal function may be affected by the action of various endogenous toxins which are absorbed in the digestive enteric consecutive states. Were established, in fact, different correlations between diarrhea and kidney function in calves. For fast assessment of the degree of dehydration in calves, some

quick techniques have been developed (1), along with other biochemical parameters, determinations of serum urea and creatinine were performed (1, 8, 18). In dogs and foals have been performed to evaluate renal clearance (2, 6, 7) and more recently to determine urinary N acetyl-β activity-d-glucosaminidaza in cow urine (14,17).

Renal disorders commonly found in enteric syndrome in calves must be timely detected and diagnosed correctly in order to take appropriate measures for prevention and therapy.

Aim of this study was to determine the etiopathogenesis and paraclinical diagnostic elements of renal dysfunction in calves, after progress enteric syndrome.

Materials and methods

Farm. The research was conducted in a dairy farm with intensive farming system, which often evolved in calves enteropathy with varying degrees of dehydration.

Animal tests, phases

Clinical examinations were performed regularly in cows (pregnant and lactating) and their calves. Examined calves were divided, randomly, into two groups:

I – animals displayed various diarrheal bouts between the ages of birth to 42 days (n = 28);

II – clinically healthy animals had no diarrhea by the age of 42 days (n = 22).

Calves were always examined clinically and were blood sampled in several stages, depending on whether enteritis, groups in relation to age: 1-2 days, 3-4 days, 5-8 days; 9-12 days, 13-16 days, 21-28 days, 40-42 days. Blood samples were collected in anticoagulant (Na_2EDTA powder) and serum. Occasionally, in both groups, urine samples were collected by spontaneous urination.

Analyzes, methods

From samples taken anticoagulant degree of dehydration was determined by the method of blood spots on filter paper (table 1) on the basis of their own interpretation schemes. Laboratory were determined: hematocrit by micromethod, serum urea by urease colorimetric method, serum creatinine by the picric acid colorimetric method.

On 10 calves (one 5 in each group) was performed exogenous creatinine clearance test. The test consisted of an intravenous solution of creatinine concentration of 50 mg / l saline, which was injected 1.76 ml / kg body weight. Blood samples were taken before and after creatinine 1h, 2h, 3h and 4h. Serum creatinine was determined using the picric acid colorimetric method.

Table 1

Conditions for dehydrated interpretation

Droplet drying time (min)	Drying spot diameter (mm)	Spot color when dried	Calf status (degree of dehydration)
3 – 20	13 – 20	light red	without diarrhea (normal)
15 – 32	11 – 14	dark red	early dehydration (mild dehydration
30 – 40	10 – 20	red cherry	medium dehydration
35 – 45	9 – 11	red claret	severe dehydration
45 – 50	3 – 9	dark red	very serious dehydration

Urine samples were tested immediately, using for this purpose a kit with reactive strip. The test has revealed the presence of protein in urine (proteinuria). Necropsy examinations were performed in calves dead all subjects in the experimental period to determine the pathological picture, especially renal lesions. For most parameters, the results were statistically analyzed by calculating the „t" test and correlation coefficient.

Results and discussion

In the period under review, the incidence of infant diarrhea in calves was 38-55%, the highest proportion of disease was recorded by the age of 14 days.

The main causes of enteritis were deficiency and toxicity, the combination of opportunistic pathogens.

In all blood samples collected on EDTA initially appreciated calves state in terms of the degree of dehydration, using blood spots on filter paper and their own scheme of interpretation (table 2). From total samples analyzed in the group with diarrhea mild dehydration occurred in 58% of calves, and severe average dehydration 27% to 15%.

Comparative results on the groups in dynamic values of hematocrit, serum urea and creatinine were summarized in Table 1 were highlighted following main aspects: the group II hematocrit values decreased progressively with age calves from 31.36 ± 5 71% (group 1-2 days) to 29.23 ± 4.91% (group 3-4 days), 27.55 ± 4.06% (group 9-12 days), 28.91 ± 5, 18% (group 21-28 days). Note that PCV in calves below is 29%, indicated by such nutritional anaemia (1, 3, 11). Calf serum urea dynamics in the control group was within the limits of 15-40 mg/dl (table 2). Coefficient of variability reached values up to 74.71% Ia (samples from calves in group 9-12 days), which shows high dispersion of individuals values.

Table 2

The result compared between groups regarding hematocrit values, serum urea and creatinine

Age lot	Dynamic age calves (days)	lot I (n=28)	lot II (n=22)	Test t	lot I (n=28)	lot II (n=22)	Test t	lot I (n=28)	lot II (n=22)	Test t
1	1 – 2	40±5,11	31,36±5,71	p<0.001	34.55±14.74	23.50 ±6.68	P<0.01	2.37±0.78	2.02 ±0.49	0.1>p>0.05
2	3 – 4	36±6,85	29,23±4,91	p<0.001	34.24±15.46	23.63 ±6.88	P<0.001	2.24±0.57	1.96 ±0.48	0.1>p>05
3	5 – 8	38,23±7,14	28,14±4,16	p<0.001	30.68±13.35	22.52 ±6.61	0.01p<0.005	1.97±0.51	1.62 ±0.41	0.025>p>0.01
4	9 -12	36,58±8,33	27,55±4,06	p<0.001	38.33±28.53	19.48 ±7.17	0.005>p>0.001	1.99±0.66	1.73 ±0.46	0.2>p>0.1
5	13 – 16	34,75±6,97	27,64±4,17	p<0.001	26.54±10.74	21.61 ±7.40	0.1>p>0.05	2.03±0.44	1.77 ±0.39	0.05>p>0.025
6	21 – 28	32,20±4,43	28,91±5,18	0.25<p>0.01	26.97±16.38	21.09 ±6.76	0.2>p>0.1	1.95±	1.62 ±0.57	0.1>p>0.05
7	40 – 42	34,40±5,85	30,76±4,25	0.25<p>0.01	28.54±9.58	22.48 ±4.92	0.01>p>0.005	1.42±0.54	1.39 ±0.44	1>p>0.5

Creatinine values in calves in the control group were enrolled in general within the reference range of 1-2 mg/dl, excluding animals from group 1 and age 2, the limit value is exceeded maximum creatinine. It does not exclude the possibility of the existence of renal function in calves immediately after birth, whether or not they show clinical signs of diarrhea. The coefficient of variability of serum creatinine was higher in group I, varying between 21.92%-38.4%. Values of the three parameters analyzed were significantly higher in calves with diarrhea, compared to the control group for most groups analyzed.

Hematocrit was 40 ± 5.11% (lot I, group 1), compared to 31.36 ± 5.71 (lot II, group 1), (p – <0.001), urea from the same group was 34 , 55 ± 14.74 mg / dl (lot I) or 23.50 ± 6.68 mg / dl (lot II) etc. Differences between groups were less obvious in creatinine: there were still significant differences in creatinine values in groups 3 and 5. After calculating the coefficient of correlation between different parameters, there was a positive correlation between PCV intense and serum urea in calves of both groups. Thus, "r" ranged from 0.4 to 0.69 (p <0.001) in lot I and 0.36 to 0.48 (p <0.001) in lot II.

Positive correlation existed also between urea and creatinine values and between hematocrit and creatinine values. Exogenous creatinine clearance test was summarized in table 3.

Table 3

Exogenous creatinine clearance test in calves with diarrhea and dehydration (lot I) and clinically healthy calves (lot II)

Serum creatinine (mg / dl)	Lot I experimental	Lot II control
Before administration	2,1 ±1,2	1,7 ± 0,9
At l h after administration	13,1 ±4 8	5,1 ± 2,1
At 2 h after administration	8,4 ± 2 4	*5,6* ± 2,2
At 3 h after administration	5,7 ± 2,1	4 8 ±1 8
At 4 h after administration	5,6 ±2 2	4 3 ±1 6

In batch of calves with diarrhea and dehydration bouts after l h of creatinine administration (exogenous creatinine clearance test), its value increased serum over

5 times from baseline (before administration solution); creatinine gradually decreased to 5.6 ± 2.2 mg/dl at 4 h after administration. In controls creatinine increased to 3 times to 2 hours after administration creatinine from baseline. The test shows disruption of renal function in calves with diarrhea and dehydration and can be used to assess renal function disorder, its evolution and effectiveness of the treatment applied.

The study tested urine samples from 19 calves, of which 9 clinically healthy animals and 10 animals with diarrhea and dehydration. In calves with diarrhea and dehydration, while urine samples, blood samples were taken for determination of urea nitrogen and serum creatinine.

Urine samples were tested for the presence of protein by reactive strip. Clinically healthy animals revealed no proteinuria. Protein in the experimental group was identified in 6 of the 10 samples (60%). Protein was present in higher concentrations (+ +), especially evidence that serum urea and creatinine values were above normal maximum (table 4).

During the experiment were dead calves followed by the development of enteric syndrome after in advance, they were subjected to sampling for determination of urea and serum creatinine and proteinuria identification.

Urine samples were taken from the bladder after death of the animals. Three of the dead calf serum urea values were very high, exceeding 100 mg / dl, the highest value recorded was 342.6 mg / dl. Increased serum creatinine was evident in 7 of the 10 calves. Proteinuria was present with variable intensity in 9 of the 10 urine samples analyzed. On 7 of the 10 bodies examined found dystrophy liver and kidney. Could establish a correlation between elevated serum urea, creatinine and proteinuria, on the one hand, and the presence of liver and kidney lesions, on the other hand (table 5).

Table 4

The result of comparative tests of urine and serum samples blood from 10 calves with diarrhea and dehydration

No. registration of the calf	Test result proteinuria	Blood serum examination	
		Urea mg/dl	Creatinine mg/dl
1	+	39,80	2,96
2	-	24,10	1,85
3	+	54,20	3,05
4	++	84,20	3,24
5	+	38,10	2,85
6	-	21,40	1,80
7	++	74,20	2,95
8	-	29,40	2,20
9	+	42,30	3,02
10	-	32,10	1,84

Table 5

Urea, serum creatinine and proteinuria values correlated with picture necropsy from 10 calves died from enteric syndrome

Nr. registration	Urea mg/dl	Creatinine mg/dl	Proteinuria	Necropsy picture
1	6,38	2,0	+	hepato-renal dystrophies
2	79	3,6	++	hepato-renal dystrophies
3	88,9	3,1	+++	hepato-renal dystrophies
4	177,2	4,6	+++	hepato-renal dystrophies
5	342,6	4,7	+++	hepato-renal dystrophies
6	110,8	3,9	+++	hepato-renal dystrophies
7	85,9	2.9	+	hepato-renal dystrophies
8	65,4	2,2	+	without hepato-renal lesions
9	75.8	1,9	-	without hepato-renal lesions
10	81,4	2,2	+	without hepato-renal lesions

Conclusions

1. Enteric syndrome in calves invariably caused varying degrees of dehydration and renal function due to increased blood viscosity, the action of endogenous toxins and other factors.

2. Renal disorders in calves were manifested by serum urea, serum creatinine and proteinuria.

3. There was a positive correlation, highly significant between hematocrit values and serum urea, serum creatinine respectively, and between those of urea and creatinine.

4. Exogenous creatinine clearance test provides data for assessing the probative value of renal function abnormalities in calves with enteritis.

5. We demonstrated a strong correlation between state postenteric dehydration, elevated serum urea, creatinine, proteinuria and renal and liver dystrophic lesions in calves dead after enteropathy.

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THE RIGHT VENTRICLE HYPERTROPHY AND DILATATION IN CHRONIC PULMONARY HEART DISEASE

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Abstract

This study sought to identify changes in adaptive hypertrophy and dilatation in chronic pulmonary heart compensated occurred in 10 dogs, which were presented to the clinic Faculty of Veterinary Medicine and Clinical Vet Doctor's universe, with clinical signs of respiratory failure. ECG showed: right axial deviation, pulmonary P wave, deep S wave in DII and aVF, microvolt, tachycardia. Mean blood pressure was 160/10 mmHg. Echocardiography were recorded: a moderate increase in diastolic and systolic diameters of the right ventricle, thinning of the right ventricular free wall apparent, septal hypertrophy, fraction shortening, increased by 4% of normal, ejection fraction remained unchanged from normal.

Keywords: *dog, adaptive changes, chronic pulmonary heart*

Introduction

Chronic pulmonary heart (CPC) as offset, is a condition common in breeds brachycephalism as Chihuahua, Maltese, Pomeranians, Poodles, Yorkshire and the West Highland Terrier medium white Cocker Spaniel. Also affected are the older adults and middle-aged, obese or dental or oral disease.

The most common causes of the CPC are pulmonary disease and *Dirofilaria immitis* infestations.

The pulmonary disease is a generic term for a group of respiratory tract diseases resulting from airway obstruction: nasal obstruction, pharyngeal, laryngeal, traheobronhic, bronchiolar, chronic bronchitis, bronchiolitis and emphysema plus other lung diseases such as: bronchiectasis, cystic fibrosis, asthma. Although these diseases account for 70% of CPC cases however, 20% of these cases remain without significant hemodynamic (CPC offset) because pulmonary arterial hypertension (PAH) is shown only moderate or significant effort (Fox et al., 1999).

If infestation with *Dirofilaria immitis*, CPC occurs either due to migration of microfilariae (L1) in the pulmonary arteries with pulmonary heart attack occurrence (thromboembolism) and eosinophilic infiltration of the parenchyma (parenchymal necrosis and fibrosis) or due to the location of adult (L5) in pulmonary artery and right ventricle. But, the frequency of the parasites in our country is about 10% (Coman et al., 2007) and therefore CPC development from *Dirofilaria immitis* infestation is low.

Regardless of the type involved in the etiology of lung disease CPC, the common element is the chronic PAH to install due to a reflex mechanism of pulmonary vasoconstriction. Alveolar hypoxia occurring in regions hipoventilate pressor response induced directly or indirectly arteriolar muscle cells by stimulating the secretion of vasoactive substances (serotonin, bradykinin, prostaglandins, angiotensin, etc.). By neuroendocrine lung cells. Serotonin is a mitogenic factor causing hypertrophy and hyperplasia of arteriolar media, swelling of endothelial cells, thereby enhancing vasoconstrictor to alveolar hypoxia. The end result of these structural and functional changes is the increase in pulmonary vascular resistance and thus increased pulmonary pressure above 25 mmHg. As a result pressure right ventricular overload occurs (VD) with repercussions on the dynamics of right atrium (AD) and finally RV hypertrophy and dilation to help preserve ejection fraction, cardiac output and the RV end diastolic pressure to normal levels at rest and effort.

Right ventricular hypertrophy and dilatation of the CPC are adaptive phenomena that install usually slow, as a consequence of increased afterload in chronic PAH. As long as the right ventricle is hypertrophied and dilated possibly able to compensate by increasing afterload, CPC is accompanied virtually varying degrees of respiratory failure. Thus, a significant number of cases remaining undiagnosed CPC in the absence of laboratory tests, only respiratory failure.

Overcoming time adaptive capacity to create VD worsening PAH, the RV end diastolic pressure initially increases and then at effort and rest, ejection fraction and cardiac output to decrease and there are clinical signs of right heart failure.

The aim of this study was to identify adaptive changes in right ventricular hypertrophy and dilatation and characterize structural and functional cardiac changes in compensated chronic pulmonary heart.

Materials and methods

The study was conducted during 2012, at the Clinic Faculty of Veterinary Medicine and the veterinarian's office, Doctor’s Vet Universe, on a total of 10 dogs aged between 2 and 15 years were we identified manifestations of respiratory failure: tachypnea, dyspnoea, coughing, cyanosis gums, anorexia, depression.

In these dogs were recorded ECG changes on contractility, excitability and cardiac rhythm were then correlated with their adaptive efficiency. Also echocardiography (MINDRAY, DP-2200Vet) established structural and functional changes of the heart. Pulsoximetry was used to determine oxygen saturation.

Results and discussion

Gaynor et al. (2005) stated that although there is an index of increased mortality in patients with chronic PAH, however, very little is known about the effects of chronic pressure overload VD, AD and VD and the dynamics of adaptation response to AD maintain RV filling pressure.

Voeller et al. (2011) have shown that severe overloading pressure (70 mmHg) right ventricle, achieved experimentally by connecting the pulmonary

artery to right ventricular circumferential parietal tension decreased, suggesting its impaired diastolic and AD increased contractility to maintain pressure ventricular filling. As a result of AD and VD blood volumes and RV ejection fraction were unchanged from normal. Gaynor and Voeller concluded that these changes are compensatory mechanisms that occur early chronic pressure overload VD, before developing ventricular dilation and clinical failure of PAH or chronic.

PAH syndrome has untypical clinical signs or appear only at higher values than 30 mmHg pulmonary artery (4) have pursued so common clinical manifestations in patients with lung diseases, which are the first in the aetiology of CPC.

Seven of the ten dogs, clinical signs lung were accompanied by a decrease below 90% of SO2. ECG (Fig. 1) showed: right axial deviation, pulmonary P wave, deep S wave in DII and aVF, microvolt, tachycardia. Mean blood pressure was 160/10 mmHg. Track indicators were: fraction shortening (FS%), right ventricular end diastolic diameter (DTDVD), right ventricular end systolic diameter (DTSVD), right ventricular free wall thickness in systole and diastole, septal thickness in diastole, interventricular septal systolic motion the left ventricle, the rate of thickening parietal general aspects morphological cardiac dimensions.

Fig. 1. *ECG, DII, 10 mm/mV, 25 mm/s*

Echocardiographic changes occurred, dilatation and hypertrophy of the cavitated structures and adjacent walls. Thus they found that adaptive changes: an increase in end diastolic and systolic diameters moderate right ventricular shortening fraction increases right ventricle with 4% thinning apparent right ventricular free wall, septal hypertrophy leading to a value over-unit ratio interventricular septum /posterior wall of the left ventricle in response to pressure overload (Fig. 2).

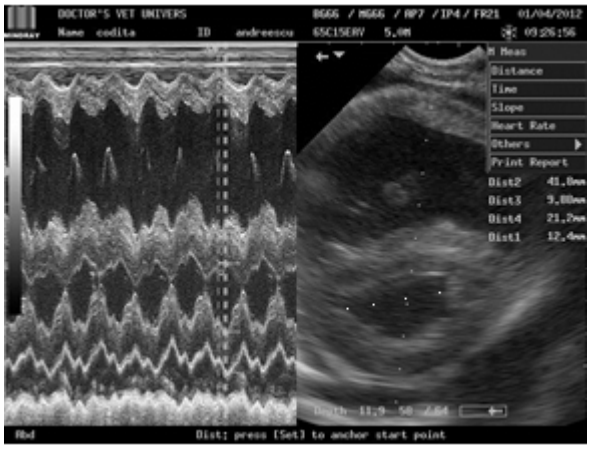

Fig. 2. *Right parasternal view, short axis, the section on papillary muscle (dilated right ventricle)*

Ultrasound image (Fig. 2) shows increasing value of right ventricular diameters at end diasolei that sisolei (RVEDD and RVESD) and interventricular septum thickness in systole or diastole (IVSD and IVSS), and the ratio right ventricle / left ventricle of 2/1.

Assess the degree of displacement of the interventricular septum to the left ventricular cavity with its deformation was achieved by measuring the 2D cross-sectional, longitudinal and transverse diameters of the left ventricle – eccentricity index (Ryan Index), who had a subunit value. Also dilated right ventricle is accompanied by dilated right atrium (Fig. 3).

Fig. 3. *Right parasternal view, short axis, section of pulmonary artery*

Moderate PAH, right ventricular overload pressure led to its adaptive hypertrophy to maintain constant cardiac output (Fig. 4).

Fig. 4. *Right parasternal view, short axis, section valves the aorta*

Hypertrophy of the right ventricular free wall, led to changes in the ratio of left atrial diameter / aortic ring whose appraised value is less than 1.5 (Fig. 4).

Bogaard et al. (2009) stated that although the first reaction triggered by ventricular adaptation to increased afterload in PAH is compensated hypertrophy, however there are other cellular and molecular mechanisms that neurohormonal signaling, oxidative stress, inflammation, ischemia, which contribute to the development of ventricular dilatation and right heart failure.

Regarding the other three dogs they've showed fatigue and syncope associated with lower effort than 90% of SO2, PAH associated CPC specification was difficult. They were dogs of breeds brachycephalism, which, due to the anatomy of cones are favoured air of upper airway obstruction. These obstructions can develop for 2-3 years, hypoxic pulmonary vasoconstriction in pulmonary hypertension installation (PAH) or chronic postcapillary precapilare (Kellihan and Stępień, 2010). In these dogs have not found changes in ECG or echocardiography allowing diagnosis of CPC.

Schober and Baad (2006), Kellihan and Stępień (2010) have shown the importance of Doppler echocardiography in the diagnosis predictive of PAH (tricuspid without regurgitation) by estimating the following parameters: time and speed of the RV outflow tract, volumes and flows systolic artery pressure lung and right ventricle.

Guglielmini et al (2010) have shown that elevated cardiac troponin I (cTnI) serum than 0.20 ng / ml are associated with elevated systolic pulmonary pressure so that cTnI can be used as a biomarker of PAH precapillar and postcapillary dogs.

Conclusions

1. Particular attention should be paid to respiratory dysfunction brachycephalism breeds can develop PAH at pressures greater than 30 mmHg in the pulmonary artery, but not accompanied by ventricular dilatation.

2. In pressure overload occurring VD initial compensatory adaptive changes in myocardial contractile function of the heart to maintain cardiac output straight constant over a period of time depending on individual variability and response to treatment.

3. Hipercapneei appearance means installing a permanent pulmonary hypertension, likely to induce CPC, even if no electrocardiographic signs of hypertrophy / dilation of the right ventricle.

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STUDIES ON THE THERAPEUTIC ABORTION ON BITCH

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Abstract

This study addresses the most commonly used therapeutic methods used to induce abortion in the bitch. Interruption of gestation is often required when owners do not want a definitive break through sexual activity, in particular, the economic value of animal and their desire to have more than one or more controlled matching and get youngsters.

We studied 26 female cases, in the of USH Reproduction clinic from Bucharest, for a period of 18 months, between December 1, 2010 and May 31, 2012. The assessment considered the comparative use of several types of substances used for abortion, their effects on females, in relation to the timeliness of the effect of active substances and the influence on the general condition of the animal.

Keywords: *abortion, bitch, progesterone, prostaglandins*

Introduction

Producing abortion can be done, in the bitch, in the early stage, when it is intended to prevent embryo implantation and thus survival and advanced stage of pregnancy, when progesterone concentrations decreases are finally followed by the expulsion of the concept. The most used substances for abortion are prostaglandins, prolactin inhibitors, antagonists of progesterone and estrogen substances. Depending on the stage of pregnancy (Bârțoiu and Seiciu, 2004; Foster, 2003), as it applies to therapy, maternal effects can range from a slight modification of the general condition to adverse effects with adverse consequences for the genital mechanism and / or body in general (infectious complications, toxic changes, blood severe bone marrow toxicity, etc.).

Aim of this study was to compare the abortigene effects of therapy induced with prostaglandin (natural and synthetic), prolactin inhibitors (Bromcriotina, Cobergolina), progesterone antagonists (Aglepristona) and estrogens (dietilsilbestron, estradiol benzoate).

Materials and methods

The study was conducted on females who presented the clinical faculty to interrupt gestation resulting from unwanted breeding. We have conducted clinical and laboratory examinations of the animals (inspection, palpation, scans, blood, biochemical and hematological tests) both before and after treatment with abortive substance.

Results and discussion

There have been surveyed 26 females and four main groups of substances used for abortion. Efficacy was monitored by ultrasound and hormonal (the dosage of progesterone). If the standard therapeutic protocol proved insufficient we continued to administer medication to achieve the desired effect.

The first method used in number of cases, prostaglandin-therapy was the most requested, first-order pecuniary advantage. Prostaglandins, in addition oxytocic effect, serve to inhibit progesterone, reduce its concentration in the blood, so the corpus luteum cannot develop and, finally, takes place by lysis (Paraipan, 1982). When choosing a method of abortion is well known that in the bitch it should be made well when abortion.

The favourite is the second stage (between 22 and 40 days of gestation) before fetal skeletal ossificatuion (table 1). Dose differs from natural prostaglandins (between 0.1 to 0.5 mg / kg) than the synthetic (2.5 mg / kg). And different dosing interval in the first case (natural prostaglandins) is 12-24 hours, and in the second (synthetic prostaglandin) to 48 hours.

Table 1

Prostaglandin administration method

Breed/ Age	Natural Prostaglandins	Synthetic Prostaglandins
7 years, Brac	2 adm. at 24 hours	-
2 years, half breed	3 adm. at 24 hours	-
2,5 years, German Sheppard	3 adm. at 24 hours	-
1,5 years, Pechinez	4 adm. at 24 hours	-
3 years, German Sheppard	4 adm. at 12 hours	-
4 years, German Sheppard	3 adm. at 24 hours	
6 years, Pechinez	-	3 adm. at 48 hours
2 years, half breed	-	4 adm. at 48 hours
6 years, half breed	-	3 adm. at 48 hours
5 years, Caniche	-	3 adm. at 48 hours

Typically, low concentrations of progesterone in 1mg/ml for more than 24 hours occurred after two to four doses, and fetal death events accompanied by abortion.

The next heat period occurs after a variable time, usually one to two months.

The second type of substances used for abortion was the prolactin inhibitors. These promoters of dopamine will inhibit prolactin secretion and, simultaneously, the activity of corpus luteum (yellow body) followed by lower levels of progesterone and abortion.

Cernescu (2004) and Drugociu (2001) showed that prolactin secretion is supported side of serotonin (serotonin is the dopamine antagonistic effect of antiprolactin).

Bromcriotine is, therefore, a dopamine promoter, a major therapeutic concentration will cancel the effect of serotonin supporter of prolactin achieved so far.

In the table below are given the most frequently encountered adverse effects related to the type of substances used (Table 2).

Table 2

Adverse effects of various abortigene substances

Substance used for abortion	Number of cases with									
	Inappetence	Hipersalivation	Anemia	Hipothermia	Vomit	Walking incoordonation	Extension of oestrus	Mydriasis	Dyspnoea	Anxiety
Prostaglandin	4	2	-	3	4	6	-	2	1	1
Bromcriptin	2	-	-	-	2	2	-	-	-	-
Cabergolin	-	-	-	-	1	-	-	-	-	-
Aglepristin	1	-	-	-	1	1	-	-	-	4
Estrogen	2	-	3	-	1	1	4	-	-	1

This substance was used on a total of three animals at a dose of 0.1 mg / kg / day orally for 5-7 days after day 35 of gestation.

Another substance used was Cobergolin, in one case, was administered orally as a syrup, the dose of 5 mg / kg / day for 5 days, between the 30th day and 35th day of gestation.

A third method was studied abortion with progesterone antagonists, such substances compete with progesterone occupies its binding sites and thus block its action.

There are theories that criminalize an involvement of these substances in inhibiting the release of LH (Foster, 2003). Substance used was Aglepriston, a number of four cases. Although the advantages are obvious (you can use anytime between the first and 45th day of gestation). The method was not applied because of the prohibitive price exceeding 15-20 kg animal.

Dose was 10 mg / kg on days 1 and 2 to install unwanted. It was observed in all four cases in the next heat cycle, the presence of nervous lactation with a abortion aetiology caused by hormonal therapy. Last group of substances used in a number of four cases was estrogen therapy. It is a method used to prevent embryo implantation, estrogens maintain the proliferative stage of endometrium, inhibits secretory stage, also have a tonic action, smooth muscle contraction oviduct and utero-tubal junction, causes degeneration of oocyte and embryo survival course.

In female dogs, implantation is performed between the 4th and 10th day of gestation, therefore abortifacient estrogen therapy is performed immediately after unwanted mounting.

They used two types of estrogen:

– Dietilsilbestron in two cases – 1 mg / kg im, 2 doses 48 hours;

– Estradiol benzoate (0.2 mg / ml) two cases at a dose of 0.01 mg / kg.

It was administered on days 3, 5, 7 after unwanted install (one of the two cases received in mating day). In case of the four treaties was observed after only one heat cycle, cystic endometrial hyperplasia accompanied by pyometer (with closed cervix), followed by ovariohysterectomy.

Side effects observed after surgery with substances used for abortion were mixed anxiety and lack of appetite, until dyspnoea, salivation or walking incoordination and have been challenged as appropriate to specific medication (vitamin therapy, prevent vomiting etc.).

Conclusions

1. The method used is prostaglandin-therapy due to low cost.
2. Progesterone antagonist therapy has the fewest side effects after administration, is the fastest, but is more expensive compared to the first method.
3. Prolactin inhibitor therapy is difficult to administer orally, there is a therapeutic dose that must be administered properly and not be given in full.
4. Estrogens are used only in case of lack of an alternative, adverse effects on the blood picture (shown toxic effects on bone marrow) are sometimes long-term or irreversible.

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SUSTAINABILITY OF HEALTH IN THE FRAMEWORK FOR SUSTAINABLE DEVELOPMENT

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Abstract

The paper treated area systems framework for sustainable development and quadrivalent operationalization of the concept of sustainability for health. It concludes that the environmental dimension is passed on multidimensional affect on all other dimensions of sustainable development framework. However, the environmental dimension of sustainable development comprises 5 sub-themes related topics, marking the procedural specificities operational framework for formalizing contributions to sustainable development.

Keywords: *health sustainability, sustainable development processes and biophysical chemistry, aggregate indicator.*

Introduction

Health, including the health and sustainability of health services, can be found among the topics for general social dimension of sustainable development.

The main dimensions of sustainable development framework refers to social practic valence, environmental issues, economic development, and the institutional construction.

It is noted that the dimensions of sustainable development framework targeted topics can be found in a matrix indicators by which to obtain an aggregate indicator composite on sustainable development.

Health, human and veterinary medicine, general health system and sustainability / sustainable development provides advance feasible / reliable in contemporary human societies.

The purpose of this paper is to formalize symbolic, equational events in sustainable development as a mathematical operating configurations affect biochemical and biophysical phenomena and processes.

Materials and methods

Study on the concept of sustainability health / sustainable development was based on the original equations and mathematical formulas presented for the first time in the literature, developed by the author of the article.

Results and discussion

Sustainability of health is taken as an organic part of sustainable development.

A sustainable development model designed locally in Romania and require consideration of the topic „health" at least subthemes' access to health services „„" access to water „and" sanitation „. These themes and sub-themes are part of a set of dimensions for sustainable quadrivalent.

According to the Commision for Sustainable Development [1] (2001, pp. 15-16), the main dimensions of sustainable development framework are: i) social (SD) ii) environmental (DM), iii) economic (DE) and iv) institutional (DI) (Fig. 1).

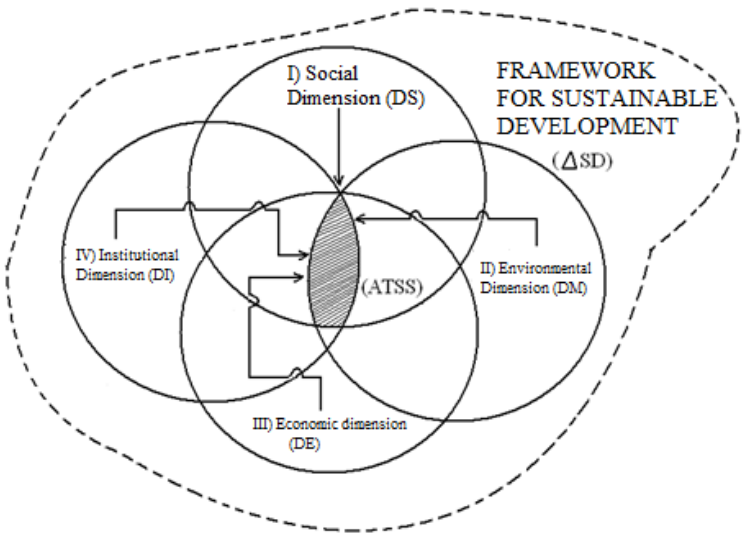

Fig. 1. *Systems framework for sustainable development and quadrivalent area operationalization of the concept of sustainability for health (ATSS)*
(source: the author)

Dimensions of sustainable development framework themes and subthemes concerns that should be combined in a matrix indicators, ultimately, provide an aggregate indicator composite weighted sustainability development. Formalizing equational original for the first time this indicator has the following configuration symbolic:

$$\begin{cases} \{ATSS\} = \{DS\} \cap \{DM\} \cap \{DE\} \cap \{DI\} \\ (\{DS\} * \{DM\} * \{DE\} * \{DI\}) \rightarrow \max(\Delta SD) \end{cases} \quad (1)$$

The intersection of positive influences of conventional dimensions should provide a maximized area of sustainability (sustainability).

Proceeding to examine in depth each dimension, we deduce that health, including health services and health sustainability issues, can be found among the themes and sub-topics related to the social dimension of sustainable development (fig. 2) {theme (I) 2 with 3 sub} :

$$\begin{cases} (DS) = \{(I)_1 * \frac{\square}{\square} \cap (I)_2 * \frac{\square}{\square} \cap (I)_3 * \frac{\square}{\square} \cap (I)_4 * \frac{\square}{\square} \cap (I)_5 * \frac{\square}{\square} \cap (I)_6\} \\ \{(I)_6\} \xrightarrow{max \square} \square (\Delta SD)_{(I)_6} \end{cases} \quad (2)$$

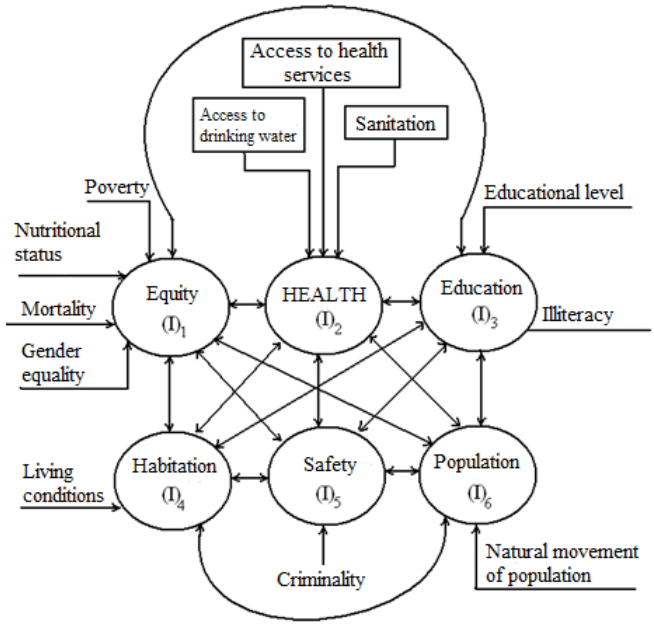

Fig. 2. *Retrieving health with sustainability issues amongst health issues and sub-topics related to the social dimension (DS) for sustainable development* (source: the author)

It appears that the environmental dimension (DM) sustainable development comprises of five sub-themes related topics, marking the procedural specificities contributions to formalize operational sustainability framework (Fig. 3).

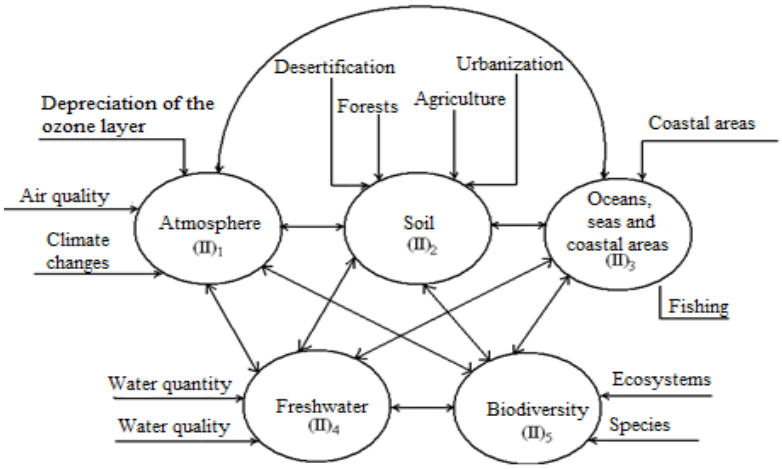

Fig. 3. *Themes and sub-themes of the environmental dimension (DM) in the sustainable development affected operating in biochemical and biophysical phenomena and processes* (source: the author)

Expression equational mentioned participation is:

$$\{DM\} = \left\{(II)_1 * \frac{\square}{\square} \cap (II)_2 * \frac{\square}{\square} \cap (II)_3 * \frac{\square}{\square} \cap (II)_4 * \frac{\square}{\square} \cap (II)_5\right\} \quad (3)$$

From equation (3) we deduce that the environmental dimension is passed on multidimensional affect on all other dimensions of sustainable development framework.

Equally, the economic dimension (DE) has constituted two significant issues related subthemes (Fig. 4).

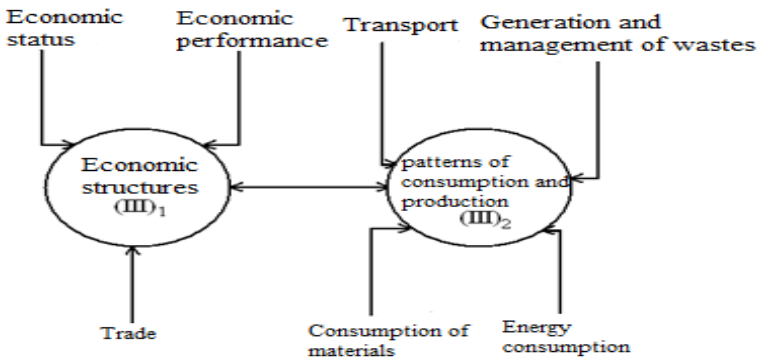

Fig. 4. *Themes and sub-themes of economic size (DE) of the sustainable development affected operating in biochemical and biophysical phenomena and processes* (source: the author)

In the same context reconfiguring equational that:

$$\{DM\} = \left\{(III)_1 * \frac{\square}{\square} \cap (III)_2\right\} \quad (4)$$

However, the institutional dimension (DI) may be retained for at least 2 main issues specific sub-themes, framework and institutional capacity for sustainable development general insurers (Fig. 5).

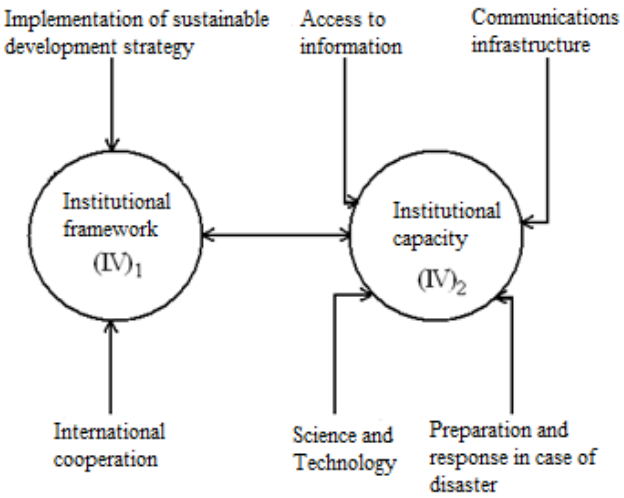

Fig. 5. *Themes and sub-themes of institutional size (DI) of the sustainable development affected operating in biochemical and biophysical phenomena and processes* (source: the author)

It concludes that:

$$\{DI\} = \left\{(IV)_1 * \frac{\square}{\square} \cap (IV)_2\right\} \quad (5)$$

In the above sense formalizatoare notice that equations / systems of equations (2), (3), (4) and (5) are deductible or are joint contributory components / composition for synthetic equational system (1), marking mode maximize aggregate indicators, composite sustainable development, which is shown separately where sanitary sustainability.

Such formalized symbolic picture with pre-indicators matrix representations are essential for the study of applied economics and management control structures to ensure sustainability biochemical and biophysical health.

In fact, each theme and sub-theme of each size listed includes elements intrinsic / default constitutive nature of participation, place, role and consequences of biophysical and biochemical phenomena and processes in ensuring sustainable plumbing, relying on economics and management control structures on regarding optimization, efficiency and feasibility of general procedural question.

C*onclusions*

1. It introduced the first model considered the initial conceptual framework for the practical expression of sustainable development, aiming for operational area concept of sustainability quadrivalent health.

2. Retrieving health with health issues is operationalized sustainability issues among and related sub-topics related to the social dimension of sustainable development.

3. Themes and sub-themes of the environmental dimension of sustainable development affected operating in biochemical and biophysical phenomena and processes back into contemporary scientific actuality.

4. Themes and sub-themes to meet the economic dimension of the sustainable development affected operating in biochemical and biophysical phenomena and processes.

5. Themes and sub-themes of the institutional dimension of sustainable development affected operating in biochemical and biophysical phenomena and processes are referential requirements for research in the field.

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SUSTAINABILITY HEALTH INSURANCE STRUCTURES – THEIR SUBSISTENCE/BIOSOCIAL EXISTENCE IN HUMAN COMMUNITIES

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Abstract

The paper deals with management systems for sustainability sanitary control structures. In conventional literature, the usual distribution and inequality issues are discussed and analyzed with high frequency, with imperative accents, especially in terms of revenues. We appreciate, however, that providing uncompromised ability of future generations to meet their own needs should benefit equally the same intensity and importance of analytical approaches and other resources of society as possible in almost their entirety.

Health sustainability has its original operationality for medical services distributed matrix alignments of bio-social subsistence needs of human communities and participate in the operational sustainability.

Keywords: *sustainability health, subsistence and existence, biosocial, sustainable development processes and biophysical chemistry.*

Introduction

Sustainable development represents the ability to meet present generation without compromising the ability of future generations to meet their own needs aces. Sustainable development is the intergenerational dimension.

Health services, together with education, equal opportunities / opportunities and human rights, subject to their distribution as resources of society / human communities.

Health sustainability has its original operationality for medical services distributed matrix alignments of bio-social subsistence needs of human communities and participate in the operational sustainability.

We find that the biochemical and biophysical phenomena and processes of natural origin and among those fibers, promoted directly or indirectly by man and his activities, are involved in the definition, namely sanitary sustainability.

Control structures (in this case, control specialized biochemical, biophysical respectively), in turn, must be organized and conducted.

Management control structures is oriented to formalize as many reactive loop (reverse side) corrections to entries, or to adjust the final ordering processors attainment synthetic characterized health sustainability ensured.

Biochemical and biophysical phenomena and processes cannot be considered exclusively "aggressive" on Biosocial and functional human condition and human communities, as long as they (the phenomena and processes in question) can be mastered, corrected, adjusted, restructured and others with specific structures / private control.

Materials and methods

Boot from structures sustainability sanitary operational by procedural algorithm followed to reach health status ensured sustainability (ensuring the sustainability of health), using control structures (biochemical and biophysical) organized and conducted efficiently under resulting efficiency / obtained in the field, based on the original equations and mathematical formulas presented for the first time in the literature, developed by the author of the article.

Results and discussion

Sustainability of health is part of the multidimensional concept of sustainable development, implicitly expressing the continuously evolving communities and entities in human society.

Gro Harlem Brundtland [1] developed under the auspices of the World Environment and Development (OMMD) document entitled "Our Common Future", known as the "Brundtland Report" (1972), which was formally adopted at the Global Summit in Rio de Janeiro (1992).

In the document it can be found the definition of sustainable development that meets the broadest agreement from all stakeholders' ability to meet present generation without compromising the ability of future generations to meet their own needs aces.

It is noted that sustainable development is an intergenerational dimension.

In fact, every generation, one after another in evolution, must be able to meet their own needs, while income distribution and other resources of society always occurs.

It appears that health services, together with education, equal opportunities (opportunities) and human rights, subject to their distribution as resources of society / human communities (Fig. 1).

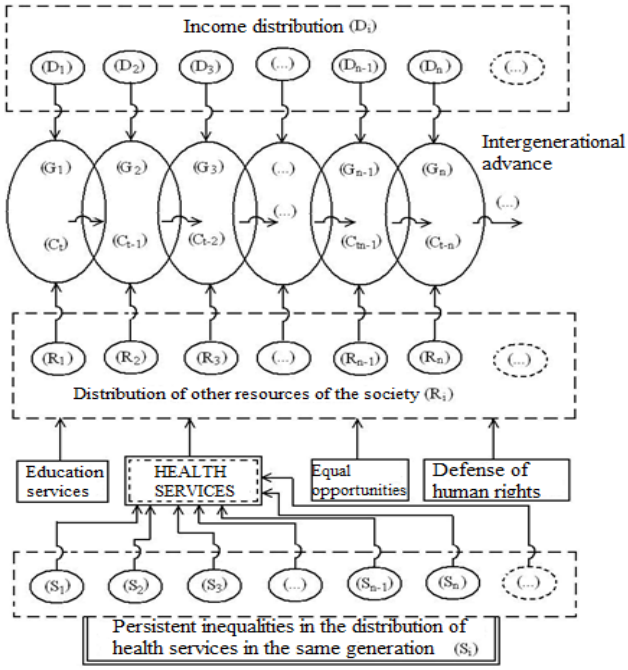

Fig. 1. *Damage sustainability through ongoing health inequalities on the distribution of health services within the same generation*
(G1), (G2), ..., (Gn) = generation;
(Ct) (Ct-1), ..., (Ct-n) = capacity of a generations to meet their own needs.
(source: the author)

The basic characteristic of the general process of distribution is given by the persistence of inequalities sharing and using the service even within a generation and more visible, intergenerational evolutionary chain.

In conventional literature, the usual distribution and inequality issues are discussed and analyzed with high frequency, with imperative accents, especially in terms of revenues.

We appreciate, however, that providing uncompromised ability of future generations to meet their own needs should benefit equally the same intensity and importance of analytical approaches and other resources of society as possible in almost their entirety.

Health sustainability has its original operationality for medical services distributed matrix alignments of bio-social subsistence needs of human communities and participate in the operational sustainability.

It is understood that health services strongly structured operational real-time streams corrective interventions and securing the people from local, regional and global, are contributory to confirgurating the sustainability of human society as a whole.

Therefore, sustainable development is impossible without sustainable operationality and effectiveness of health services.

Examination sustainability identifier serves to health a) domain structure, b) the health systems, c) properties and characteristics related field, d) mission, objectives, goals and targets pursued by operationalizing health, e) results, outcomes and impacts among local and sustainable health subtopic, the framework of sustainable development and last but not least, f) quantification participation.

Delimitation operational procedural control structures to ensure sustainability biochemical and biophysical health.

Mainly sustainability of health need a) defined conceptual and content, respectively, b) provided (actually retrieved, concrete operational condition characterized by durability / sustainability).

We find that the biochemical and biophysical phenomena and processes of natural origin and among those fibers, promoted directly or indirectly by man and his activities, are involved in the definition, namely sanitary sustainability.

Alignments technical, technological, procedural, phenomenological, etc. biochemical and Biophysical invoice is found in two forms stake in sustainable development: 1) the constructive role of factors generating added value, utility /consumer uses, production, and operation of human reproduction and related infrastructure, ie a) the emotional role, disturbing, harmful, destructive, etc., human operational structures.

When the ratio of 1) and 2) recorded equivalence, then there is zero sum operationality.

However, we find that alignment 2) (the condition) is cumulative and therefore it should at least be countered, considering that it can never be eliminated.

Therefore, control of biochemical and biophysical have established specialized structures with defined practices, resulting in specificity applied.

In fact, the control scheme is essential managerial approach reactive circuit (feed-back) and, consequently, he has organized and led.

Control structures (in this case, control specialized biochemical, biophysical respectively), in turn, must be organized and conducted.

Management control structures is oriented to formalize as many reactive loop (reverse side) corrections to entries, or to adjust the final ordering processors attainment synthetic characterized health sustainability achieved (Fig. 2).

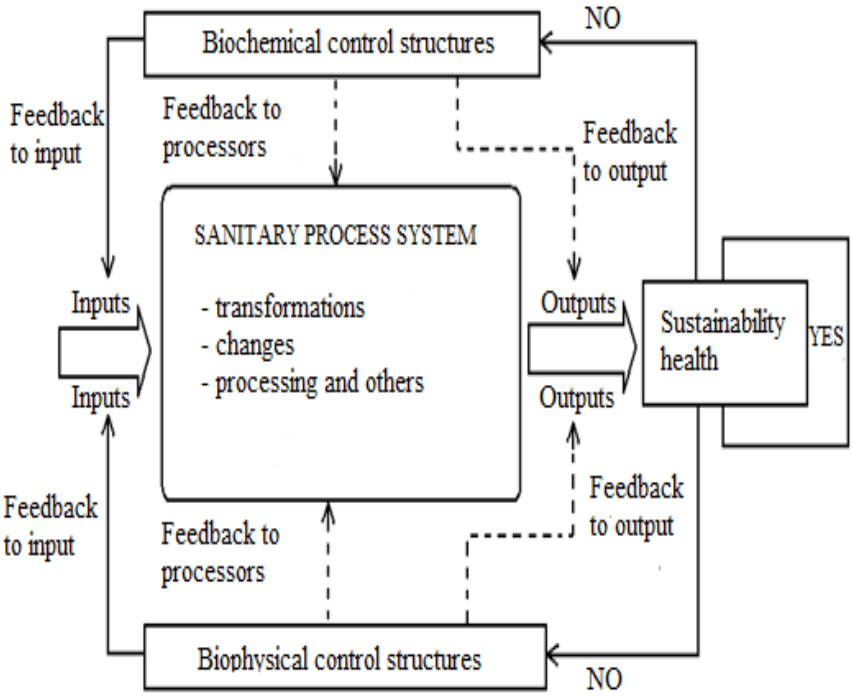

Fig. 2. *Using biochemical and biophysical control structures for correction of inputs, processing and outputs of health systems to achieve sustainability of health considered final command value*
(source: the author)

Once designed flow inputs, processing and outputs, corresponding to operational health system, prove decisive importance steps: a) determining (through taxation) value (ownership, size, quality, etc.) results in case „insured health sustainability" and b) formalizing biochemical and biophysical control structures that become agents reverse reactions operators (reactive loops) for applying corrections on input, processing and output, so the final value expected (ordered) be obtained with high net even certainty

Application flow corrective feedback in the field researched sites can be based on notional demarcation health sustainability.

Once you understand and remember „what is sustainability of health", it must be enclosed in an operational procedural algorithm to ensure the sustainability of healthcare in value order, required, expected in a dynamic, stochastic, taking into account the time factor the concerns transformative phenomena affected by biochemical and biophysical natural and / or artificial (fig. 3).

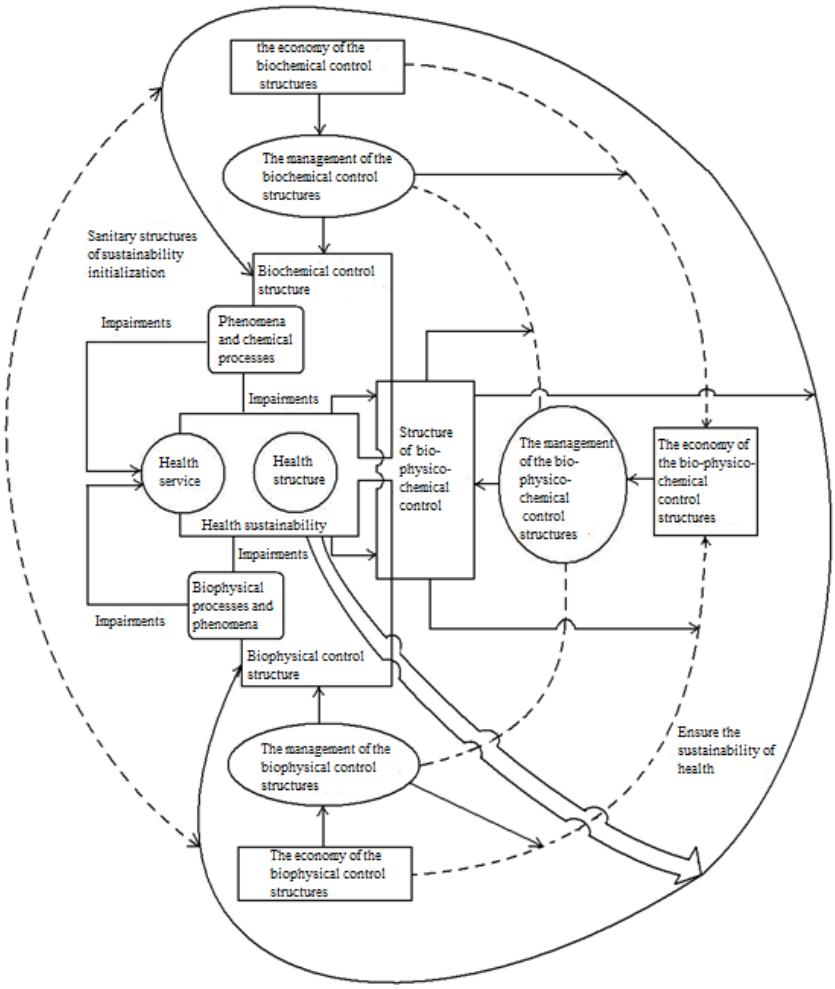

Fig. 3. *Defining sustainability sanitary and operational procedural algorithm to ensure the sustainability of health economics and management using biochemical and biophysical control structures* (source: the author)

In fact, the health service is realized (implemented) using instrumental health structures.

Health sustainability in our opinion, is hosted on composite areas of health services and structures. On the latter (services and facilities) meet (before them) damage (erosion, faults, dismantle, etc.) of biochemical and biophysical phenomena and processes.

Therefore, biochemical and biophysical phenomena and processes can be formalized (build) and dedicate specific structures custom control.

Ultimately, it is possible to formalize / configure a control composite structures bio-physico-chemical (bio-chemical-physical).

Once built layouts (set), they must be organized and managed.

It is therefore legitimate introduction procedural algorithm proposed operational management control structures biochemistry and biophysics.

Accordingly, by extension and articulation set is appropriate and formalization of management control bio-physico-chemical/bio-chemical-physic structures.

The whole conceptual construction described above is assumed only in terms of feasibility and operational efficiency.

As such, it is necessary to define economic behavior control structures designed.

Control structures economy is composed the management structures concerned.

So, from initialization structures sustainability sanitary operational by procedural algorithm followed to reach health status ensured sustainability (ensuring the sustainability of health), using control structures (biochemical and biophysical) organized and managed effectively in terms of efficiency derived / obtained in the field.

Incomplete notification aggressive role and processes of biochemical and biophysical phenomenon of Biosocial condition of man / human communities.

Of assertions described is inferred that sustainability is participating in the general health of subsistence insurer (under „minimum") and existence (under „Maximum") bio-social human and human communities (Fig. 4).

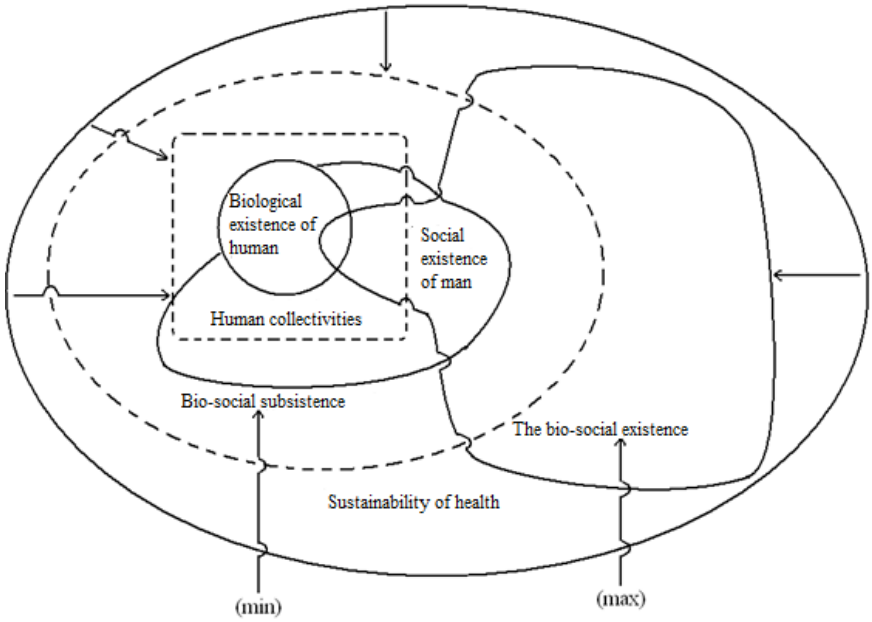

Fig. 4. *Influence of sanitary sustainability of subsistence (min) that existence (max) Biosocial human / human communities* (source: the author)

Biochemical and biophysical phenomena and processes cannot be considered exclusively „aggressive" on Biosocial and functional human condition and human communities, as long as they (the phenomena and processes in question) can be mastered, corrected, adjusted, restructured and others with specific structures / private control.

Incompleteness of aggression before it is quasi-conventional way scroll context and consequences of any natural or artificial phenomena and processes, to the extent that perfection is an ideal property to absolute, unattainable in practice never times in real life and especially in the sustainability of sanitary studied.

Instead, it recommends increasing attention, concentration and review important weights in the overall health sustainability sustainable development, given the vital precautionary consequence, essential for subsistence / bio-social existence of man / human communities.

Conclusions

- One can see that affecting sustainability are achieved through persistent health inequalities on the distribution of health services within the same generation.
- Using biochemical and biophysical control structures is for the correction of inputs, processing and outputs of health systems to achieve sustainability of health considered final command value.
- Health and sustainability delimitation algorithm to process-operational sanitation sustainability is through economic and management control structures biochemistry and biophysics.
- Influence sustainability of subsistence Health (min) or existence (max) Biosocial human / human communities has influences of the biochemical and biophysical.
- It is necessary to define economic behavior control structures designed in biochemistry and biophysics.
- Control structures economy is composed the management structures concerned in biochemistry and biophysics.

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INTERRELATION BETWEEN THE EGGSHELL QUALITY AND THE LAYING HENS BREEDING SYSTEM

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Abstract

We studied the interrelation between the eggshell quality and the breeding system of Albo and Roso laying hens. The birds were breed in conventional cages, on the floor and the traditional system. The following parameters were determined: egg weight, eggs surface area, height and shape index, breaking strength, the percentage of eggshell and shell thickness. There were significant differences observed at hens maintained on the ground (floor and the traditional system) to their breaking strength ($p \leq 0.01$) and shell thickness ($p \leq 0.05$).

Keywords: *eggshell quality, laying, breeding system*

Introduction

Since the maintenance of hens in cages will be banned in 2012, a number of researchers studied the effect of breeding system on eggs production (Sauveur, 1991; Leyendecker et al, 2001). Quality assurance shell eggs in the various systems maintenance are of major importance, because a higher percentage of broken eggs are not economical.

We aimed at carrying out a study on the influence of intensive and traditional systems maintenance of laying hens on the main parameters of eggshell quality.

Materials and methods

The experiment was conducted on hens hybrid Roso, aged 32 weeks, kept in cages (L1), on the floor (L2) and in traditional system (L3). The hens were fed with diets having the same structure and quality (standard levels). Experimental period was 4 weeks. They followed the parameters: the weight of eggs (individual weighing), eggs surface area (based on Mongin and Bonnet's equations), diameter and height, index format (the ratio between height and diameter), percentage of shell (by breaking, reporting to egg weight) and the shell thickness (using mycrometer).

The data were statistically processed.

Results and discussion

In table 1 are presented the parameters for the study in Roso hens. The eggs weight was: 60.2 g at L1, 63.7 g at L2, 64.5 g at L3. It was found that the differences between groups were not significant, but in groups maintained on the

floor and in the traditional system, values were higher by 6% and 7.1% ($p \geq 0.05$). The results are similar with those presented in literature (Sauveur, 1991; van den Brand et al., 2004).

The eggs surface was 70.2 cm^2 at L1, 71.3 cm^2 at L2 and 72.4 cm^2 at L3 ($p \geq 0.05$). The egg format has made the following values: 77.4% at L1 lot, lot 77.8% at L2 and 79.6% at L3, the differences are insignificant ($p \geq 0.05$). The breaking strength was 5.9 N / mm at L1, 7.4 N / mm at L2 and 7.6 N / mm at L3. This parameter was significantly affected by the maintenance of hens, compared with L1, being bigger with 25 and 29% at L2 and L3 ($p \leq 0.01$). Egg shell thickness has made the following amounts: 0.451 mm to L1, 0.462 mm to L2 and 0.497 mm to L3. This parameter was not influenced significantly by the maintenance of hens, being higher in groups bred on the floor and in the traditional system ($p \leq 0.05$).

Table 1

Eggshell parameters on Roso hens

Roso hens	Cages	Floor	Traditional system	
Egg weight (g)	$60.2^a \pm 3,6$	$63.7^a \pm 4,3$	$64.5^a \pm 4.4$	NS
Egg surface area (cm^2)	$70.2^a \pm 2.3$	$71.3^a \pm 2.9$	$72.4^a \pm 2.2$	NS
Egg height (cm)	$5.3^a \pm 0.3$	$5.4^a \pm 0.2$	$5.4^a \pm 0.2$	NS
Egg diameter (cm)	$4.1^a \pm 0.1$	$4.2^a \pm 0.1$	$4.3^a \pm 0.1$	NS
Shape index (%)	$77.4^a \pm 2.7$	$77.8^a \pm 2.1$	$79.6^a \pm 2.4$	NS
Breaking Strength (N/ mm)	$5.9^a \pm 1.1$	$7.4^b \pm 1.4$	$7.6^b \pm 1.2$	DS
Shell percentage (%)	$10.2^a \pm 0.4$	$10.5^a \pm 0.3$	$10.3^a \pm 0.2$	NS
Shell thickness (mm)	$0.451^a \pm 0.011$	$0.472^a \pm 0.017$	$0.497^b \pm 0.014$	S

a, b followed by different letters in the same row are significantly different at a probability level of 95%.

NS Non significant; S $p \leq 0.05$; DS $p \leq 0.01$.

Data obtained from Albo hybrid are presented in Table 2.

Table 2

Eggshell parameters on Albo hens

Albo hens	Cages	Floor	Traditional system	
Egg weight (g)	$55.6^a \pm 2.9$	$58.8^a \pm 3.1$	$60.1^a \pm 3.3$	NS
Egg surface area (cm^2)	$64.8^a \pm 2.1$	$65.3^a \pm 2.5$	$68.5^a \pm 2.4$	NS
Egg height (cm)	$4.7^a \pm 0.5$	$4.9^a \pm 0.1$	$5.1^a \pm 0.4$	NS
Egg diameter (cm)	$3.9^a \pm 0.1$	$4.1^a \pm 0.1$	$4.3^a \pm 0.1$	NS
Shape index (%)	$83.0^a \pm 3.5$	$83.7^a \pm 3.3$	$84.3^a \pm 2.4$	NS

Breaking Strength (N/ mm)	$4.8^{a} \pm 1.3$	$5.7^{b} \pm 1.2$	$6.1^{c} \pm 1.5$	DS
Shell percentage (%)	$10.3^{a} \pm 0.4$	$10.7^{a} \pm 0.3$	$10.8^{a} \pm 0.2$	NS
Shell thickness (mm)	$0.386^{a} \pm 0.023$	$0.410^{a} \pm 0.017$	$0.431^{b} \pm 0.014$	S

a, b, c followed by different letters in the same row are significantly different at a probability level of 95%.

NS Non significant; S $p \leq 0.05$; DS $p \leq 0.01$.

The weight of eggs was 55 g at L4, 58.8 g at L5 and 60.1 g at L6. Differences between groups were not significant ($p \geq 0.05$), but it was found an increase with 6% from hens maintained on the floor (L5) and 8% in traditional system (L6).

The eggs surface was 64.8 cm^2 at L4, 65.3 cm^2 at L5 and 68.5 cm^2 at L6, the differences are not significant ($p \geq 0.05$). The egg format has made the following values: 83% at L4, 83.7% at L5 and 84.3% at L6. Between groups there were not significant differences ($p \geq 0.05$). *The breaking strength* was 4.8 N / mm in L4, 5.7 N / mm in L5 and 6.1 N / mm in L6. This parameter was significantly affected by system maintenance ($p \leq 0.01$), at the hens maintained ground the values are higher with 19% (L5) and 27% (L6). *Egg shell thickness* was also influenced by the system maintenance. The values that were recorded 0.386 mm in L4, 0.405 mm in L5 and 0.418 mm in L6. This parameter was influenced by the system maintenance, with more than 6% to 12% and L5 from L6.

In literature, data concerning the breeding influence on the eggs quality are very different. Leyendecker *et al*. (2005) have found an improvement in the egg shell thickness at the hens maintained free range, compared with those maintained in batteries and furnished cages. Tumova and Ebeid (2003) have noted that the egg shell thickness is bigger in battery cages, while van Den Brand *et al*. (2004) have not noticed any difference. Casiraghi *et al*. (2005) have argued that the only parameter that is not influenced by the maintenance is the index. Leyendecker *et al*. (2005) believe that the differences of the eggs quality are determined by the possibility of bird's moves (they are maintained on the floor), and better calcium metabolism if maintained on free range.

Conclusion

Regardless of race or hybrid, the egg weight has increased with 6% on the floor maintenance and 8% in traditional system ($p \geq 0.05$). The breaking strength has increased significantly where hens were maintained on the floor and in traditional system ($p \leq 0.01$).

Egg shell thickness has been influenced by the system maintenance, being higher in hens kept in traditional system ($p \leq 0.05$).

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RESEARCH ON THE LUNG, AESOPHAGUS, INTESTINE AND KIDNEY HISTOSTRUCTURE OF SWINE FETUS AGED 60 DAYS OLD

T. PETRUȚ

Abstract

The authors have studied the histological structure of organs of the thoracic and abdominal cavity in swine embryos aged of 60 days after having used a fixing solution of saline formalin, a paraffin inclusion and after the serial division into section of the organs.

The sections have been coloured with HE methods, Mallory trichromical method, Gömöri silver impregnation method.

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

Oesophagus in 60 days is undergoing a structural organization. Oesophagus shows three tunics (mucosa, muscular and adventia), not differentiated submucosa. Muscle lacking mucosa. Oesophageal mucosa is under differentiation, being present both stratified pavement epithelium and corion soft type.

Small intestine from fetuses of 60 days shows villous extensive chorion intestinal mucosa is poorly developed and lacking Lieberkuhnn glands. The intestinal epithelium shows no caliciform mucous cells, the apical pole of enterocytes is differentially present microvilii as forming a "striated shelf".

Kidney at 60 days shows nefron differentiated morphologic structure. Malpighi corpuscles are highlighted and appear differentiated from the podocytes of Bowman membrane structure. 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.

Key words: *embryo development, lung, oesophagus, intestine, kidney*

Introduction

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

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).

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 the gastric epithelium cells (Georgieva R.K., K. Gerov, 1975).

Material and methods

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

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

Results and discussions

The lung at age 60 days shows a weak bronchial tree diferentiation 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.

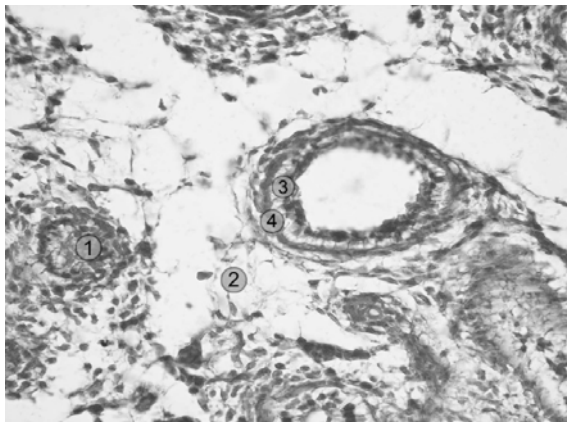

Fig. 1. *Swine fetus – 60 days, lung section, Mallory trichromic stain, Ob 40x*
1) bronchial buds; 2) mesenchymal tissue; 3) bronchial epithelium; 4) bronchial muscle

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).

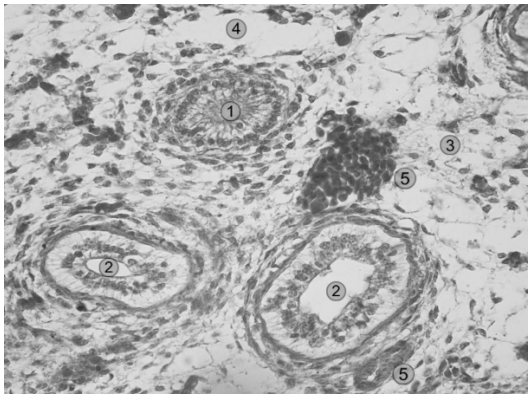

Fig. 2. *Swine fetus – 60 days; section through the pulmon lobs; Col. trichromic Mallory; Ob. 40x*
1) bronchial buds; 2) intralobular bronchi;
3) mesenchymal tissue; 4) capillary limphatic;
5) capillary vessels

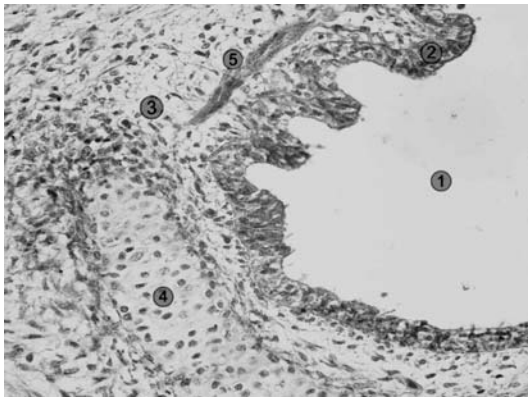

Fig. 3. *Swine fetus – 60 days; pulmon section; Col. HE; Ob. 40x*
1) extralobular bronchi; 2) bronchial epithelium; 3) fiber-muscular-cartilaginous tunic; 4) cartilaginous nuclei; 5) bronchial muscle

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 accumulation 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.

Oesophagus at 60 days is being organized structurally. The oesophagus has three tunics (mucosa, muscular and adventitia), the submucosa not being differentiated yet. Mucosa's muscular layer is missing. Oesophageal mucosa is being differentiated, the stratified epithelium and the chorion are both present (fig. 4), without the mucosa's muscular layer being formed which separates mucosa from submucosa.

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

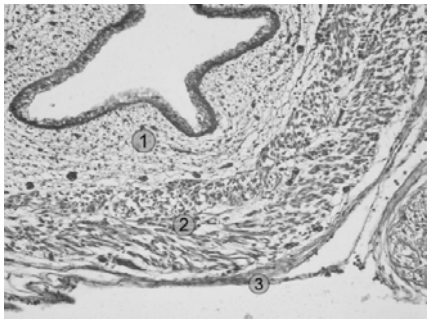

Fig. 4. *Swine fetus – 60 days; section through the esophagus; Mallory trichromic stain; Ob 40x* esophageal mucosa; 2) oesophageal muscular layer; 3) adventitia

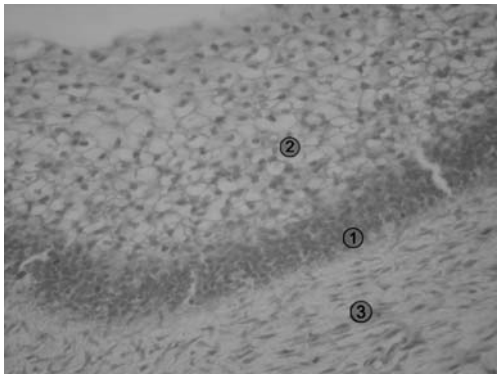

Fig. 5. *Swine fetus – 60 days; section through the esophagus; Col. HE; Ob. 40x* 1) stratum basal; 2) stratum spinosum; 3) chorion

Oepithelium 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 layer 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 60 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. Chorion intestinal mucosa is weakly developed and devoid of Lieberkuhnn glands. Submucosa is presented as a thin layer of collagen (fig. 6).

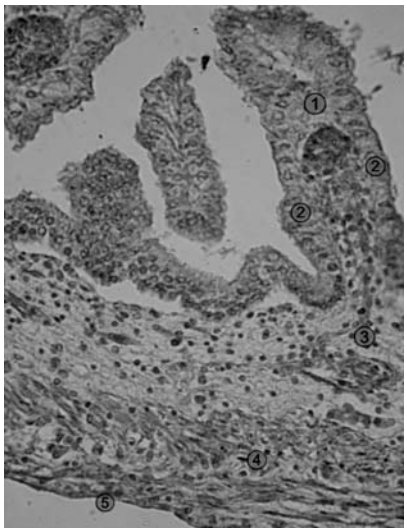

Fig. 6. *Swine fetus – 60 days; section through the intestine; Col. HE; Ob. 20x*
1) intestinal villous; 2) intestinal mucosal epithelium; 3) chorion;
4) muscular; 5) serous

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

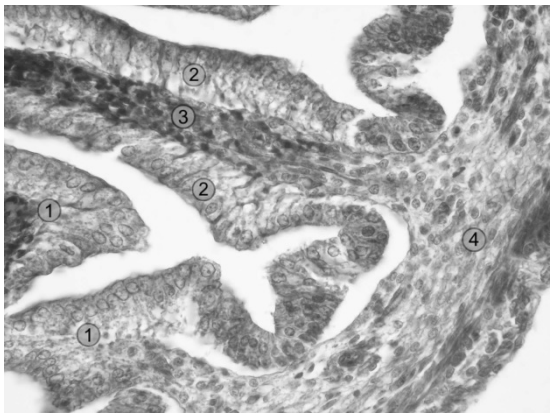

Fig. 7. *Swine fetus – 60 days; section through the intestine;*
Col. Mallory trichromic stain; Ob. 40x
1) intestinal villous; 2) intestinal mucosal epithelium; 3) axis villus;
4) submucosa

Kidney at 60 days has 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 they have the nefroread uriniferi tubules arranged on the basement membrane in the form of a simple cubical epithelium.

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

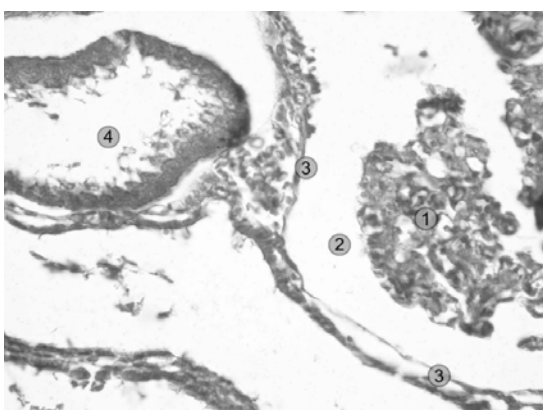

Fig. 8. *Swine fetus – 60 days; section through the kidneys; H.E. Col., Ob 20x*
1) vascular glomerule; 2) space filtering; 3) Bowmann capsule;
3) vascular glomerule; 4) uriniferi tubules

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.

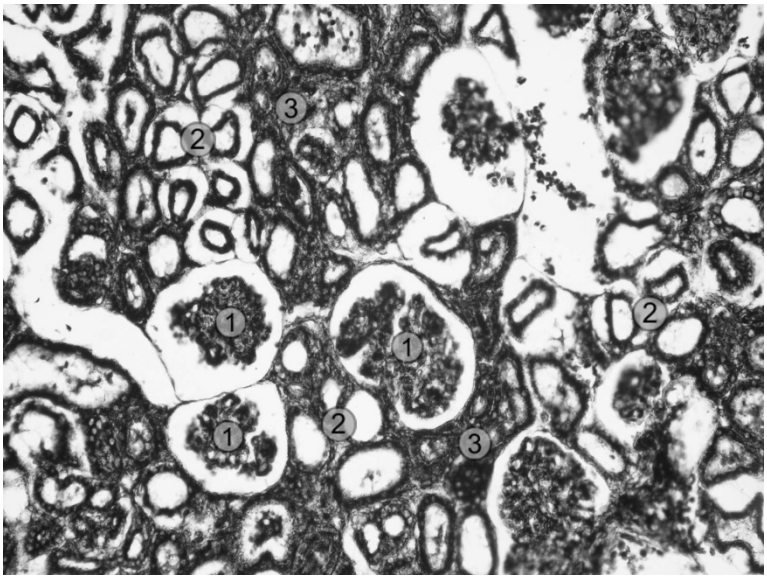

Fig. 9. *Swine fetus – 60 days; section through the kidneys; silver impregnation Gömöri method, Ob 20x*
1) Malpighi corpuscles; 2) uriniferi tubules; 3) reticulin fibers

Conclusions

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

2. Oesophagus in 60 days is being structurally organized. The oesophagus has three tunics (mucosa, muscular and adventia), not differentiated submucosa. Muscle lacking mucosa. Oesophageal mucosa is under differentiation, being present both stratified pavement epithelium and the chorion soft type.

3. Intestine from fetuses of 60 days has well developed villous, chorion intestinal mucosa is weakly developed and devoid of Lieberkühnn glands. The intestinal epithelium has not caliciforme mucous cells, the apical pole of enterocytes is different, being present microvill forming, “shelf striated”.

4. Kidney at 60 days shows nephron differentiated morphologic structure. Malpighi corpuscles are clearly differentiated and Bowmann podocits of membrane structure appear. Uriniferi tubules present functional nephrocites and at the apical pole appear willing microvill poorly differentiated form of 'edge in the brush.

Reticulin fibers are evident, structural elements of the juxtaglomerular apparatus are not present.

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STUDIES ON THE AETIOLOGY AND TREATMENT OF UTERINE SUB-INVOLUTION IN THE BITCH

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Abstract

The study was conducted over a period of eight months and consisted of twenty cases of postpartum uterine sub involution bitches age 2 years and 8 years, patients of the reproduction clinic of USH. Factors have been incriminated as of maternal and fetal origin. Treatment aimed at both hormonal therapy and surgery and was complemented by broad-spectrum antibiotics for preventing infections associated.

Keywords: *sub-involution, bitch, calving*

Introduction

Recently, at the clinic of FMV-USH several females who were in the immediate period after birth delayed uterine involution were brought. Causative polymorphism was found (Groza and Munteanu, 2002; Bârțoiu and Seiciu, 2002), so the owners were advised to always consult their doctor before gestation for a detailed consultation and advice for female-related preventive care animal before, during and after pregnancy.

Its purpose was to stimulate uterine involution remission by treatment under antibiotic protection.

Materials and methods

The study was performed on 20 cases, diagnostics were completed with inspection and palpation in all cases of abdominal ultrasound and biochemical and hematologic blood tests according to methods described by Avram and Cuca (2004). Hormone therapy aimed particularly the medroxyprogesteron acetate and oxytocin use, and the broad-spectrum antibiotics were used: Amoxicillin, Cephalexin and Enrofloxacin. In one case total ovariohysterectomy was applied, all being done with Acepromazine neuroleptanalgezia / Ketamine, monofilament absorbable sutures (PDS) and polifilament (Bicril) were used.

Results and discussions

In the aetiology of uterine sub-involution were identified two factors (Cernescu, 2004; Ronsin and Berthelot, 1997) (figures 1 and 2):

a) factors of maternal origin (fig. 1):

- Malnutrition – 2;
- Old age – 3;
- Hypocalcaemia – 3;

– Calving laborious – 3.

b) factors of fetal origin (fig. 2):

– Excess of total fetal volume / part – 4;

– Dead fetuses – 2;

– Number of fetuses – 3.

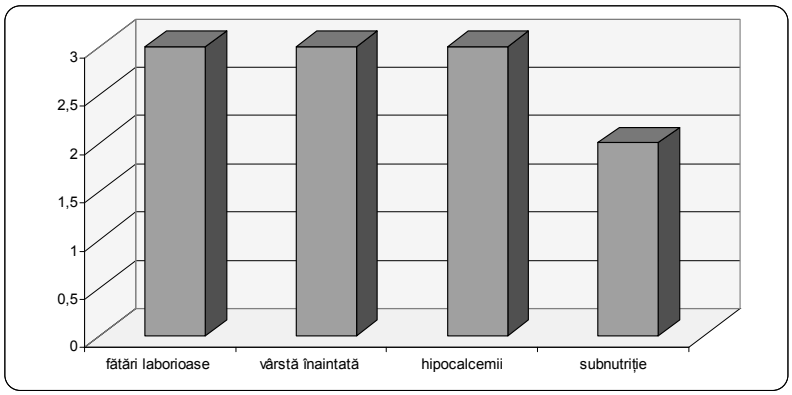

Fig. 1. *The prevalence of maternal factors in the aetiology of uterine sub-involution in the bitch*

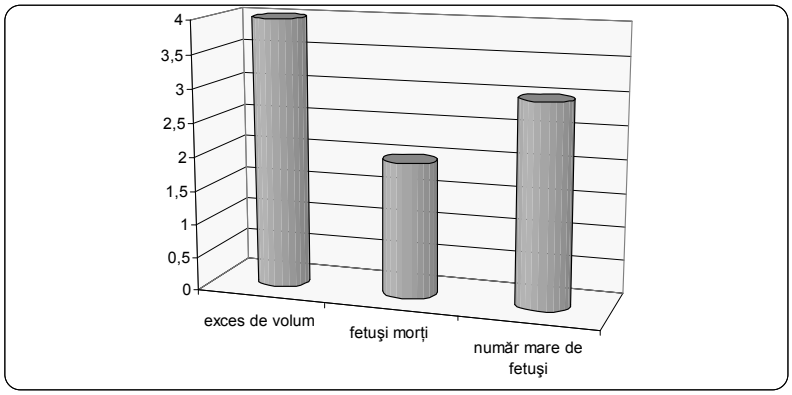

Fig. 2. *The prevalence of fetal factors in the aetiology of uterine sub-involution in the bitch*

Treatment

In all 20 cases we tried hormone therapy, as follows: 8 were treated with Medroxi-rogesteron acetate in doses of 20 mg / kg, and 12 cases were treated with oxytocin at doses of 2-10 IU / ml. Because large areas of the uterus (caused by sub-involution) antibiotherapy was necessary in all cases. Were used in 10 cases, 20 mg/kg/12h Amoxicillin in 8 cases Enroxil 5 mg/kg/24h, and in 2 cases (both with hyperthermia 39.2°, 39.4° C respectively) Cephalexin 25 mg / kg / 12 hours (table 1).

Table 1

Classes of antibiotics

Antibiotic used	Amoxicillin	Enroxil	Cefalexin
Number of cases	10	8	2

To mention that in one case, treated with medroxyprogesterone acetate, was an important uterine bleeding resistant to anti-hemorrhagic treatment. Therefore, total ovariohysterectomy was used by the classical method.

After neuroleptanalgezi with Tranchilrom and Ketamine, a celiotomy was executed at the white line, ligatures on mezoovaries and cervix with absorbable thread of polifilament Bicril.

Abdominal cavity was closed with absorbable monofilament thread (PDS) and the skin was made a dermo-dermal suture with PDS of the same thread. Postoperative treatment was three days with iv cephalexin 25 mg/kg/12 hours. In all other 19 cases where we used hormonal treatments mentioned above, uterine sub-involution remission was observed within 2-3 days without the need for supporting electrolyte and vitamin therapy.

Conclusions

1. Uterine sub-involution is a relatively common condition, occupying more than 10% of puerperal diseases in the bitch.
2. Plurifactorial aetiology emphasizes the importance of specialist advice before and during pregnancy to prevent sub-involution caused by hypocalcemia, submetrite or fetal death.
3. Medroxyprogesterone or oxytocin hormone therapy has proven very useful and often effective in disease remission, but done under medical supervision.
4. Antibiotics are necessary in all cases and should be done with great vigor because the flaccid uterus and increased in volume is a "culture medium" excellent for polymorphic bacterial flora that can cause serious infections with repercussions both on the reproductive system and the entire body.

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STUDIES CONCERNING THE DIAGNOSIS, TREATMENT AND PROPHYLAXIS OF COWS MASTITIS IN A DAIRY COWS FARM FROM ILFOV COUNTY

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Abstract

Research was conducted concerning early detecting of mastitis in cow for treatment application of appropriate prophylactic measures. Clinical exams were conducted and milk samples were harvested from 21 cows. To the 22 milk samples a quick test, R-Mastitest, was conducted to identify the infection of the mammary gland. The test helped determine the total number of somatic cells, the total number of mesophilic aerobic germs, bacteriological exams for isolation and identification of Staphylococcus aureus, "in vivo" and "in vitro" pathogenicity tests and the antibiogram. From these 22 milk samples, 13 had organoleptic modification. From the samples analysed, 8 were positive to the R-Mastitest, the number of somatic cells varying between 200.000 and 5.500.000 cells per ml of milk. Out of 8 samples, coagulase positive Staphylococcus aureus was isolated. From 8 isolated strains, 2 were resistant to ampicillin, 5 to amoxicillin, 1 to cefaclorum, 7 to erythromycin and 8 to the G penicillin. This study showed in this farm the presence of some resistant strains of Staphylococcus aureus to 2 antibiotics of election and to some large spectrum antibiotics used in therapeutic practice in the farm.

Keywords: *cows, mastitis, treatment, prophylaxis, antibiogram*

Introduction

Subclinical mastitis in dairy cows is a problem in animal and public health. Their detection is a time consuming problem and it is very important to intervene using as severe hygienic measures as possible. The damage caused by this disease can amount to up to $ 200 per cow per year (Miller et al., 1993; Rainard et al., 2003). The most common mastitis are of infectious (32-48%) and trauma (34-36%) nature (Miller et al., 1993). Bacteriological examination and antibiogram revealed a variety of bacteria present in milk, predominantly *Escherichia coli* and *Staphylococcus aureus*. Milk quality is significantly lower compared with European Community requirements regarding somatic cell counts and total aerobic mesophilic germs (Sayed and Mohamed, 2008). In our country the losses caused by

mastitis are estimated at about 11% of milk production. These losses can be both qualitative and quantitative and mainly consist in the reduction of nutrients in milk (Fiț et al., 2010, Turcu et al., 2007). The authors have isolated six bacterial strains in milk samples from cows with subclinical mastitis, the most common being *Staphylococcus species* with *S. aureus* subspecies. It has been identified in a proportion of 59.37% of the samples analyzed. The total number of germs (NTG) identified ranged from 140 CFU/ml to 7629 CFU/ml. Isolated strains were sensitive to enrofloxacin, florfenicol, amoxicillin and resistant to lincospectin. Turcu et al., 2011, have done research on some morpho-biologic characteristics of bacteria isolated from cows with clinical and subclinical mastitis. 62 bacterial strains were isolated and species such as *Staphylococcus aureus* (53.22%), *Staphylococcus agalactiae* (20.96%), *Staphylococcus dysgalactiae* (11.29%), *Staphylococcus uberis* (9.67%) and *Escherichia coli* (4.38%) were identified. *Staphylococcus aureus* strains were sensitive to vancomycin, amoxicillin, norfloxacin, chloramphenicol and erythromycin.

NTG analysis results showed higher values in milk from cows with mastitis compared with healthy, 375,000 / ml milk in samples from clinically healthy cows, 1.2 million/ml milk in samples from cows with subclinical mastitis and 2.6 million/ml milk from cows with clinical mastitis (Hromei, 2006).

Mastitis therapy should be based on bacteriological exam and especially on the antibiogram. Intensive use of antibiotics both in humans and animals has led to the selection of microbial strains resistant to some antibiotics (Nadăş et al., 2010). Studies on the sensitivity of bacteria isolated from milk from cows with mastitis showed varying degrees of response depending on the bacterial species and the generation of antibiotics. Hromei (2006) demonstrated that bacteria have been shown to be sensitive to cephapirin, benzothin, enroxil, colistin, erythromycin and less sensitive to cloxacillin, oxytetracycline, tetracycline and streptomycin. Nadăş et al. (2010) showed that the sensitivity of bacteria isolated from milk from cows with mastitis was higher to amoxicillin followed by enrofloxacin and Tetra Delta.

The purpose of this study was early detection of mastitis in cows, allowing the application of effective treatments and taking appropriate preventive measures.

Material and methods

The research was conducted on a total of 22 milk samples collected from cows with subclinical mastitis out of a farm in Ilfov County. Samples were taken from affected udder quarter after disinfection and removal of the first jet of milk. Before morning milking and evening milking, an R-MASTITEST was conducted on the stable line, for rapid identification of mammary gland infections. The reaction was performed in a plastic plate set with 4 wells (Figure No 5). In each well, 2 ml of milk were put, over which 2 ml of R-MASTITEST reagent was added (SN Pasteur Institute SA).

The mixture was mixed by rocking movements of the plate and reading and interpretation of the results was done at 3-5 minutes. It forms a detergent – DNA somatic cells complex. For determining the total aerobic mesophilic germs

(NTGMA), dilutions were performed in nutrient broth tubes (Biokar Diagnosis), and to determine the total number of yeasts and molds dilutions were performed in tubes with Sabouraud liquid medium (Liofilchem). Then, both were seeded in Petri dishes with nutrient agar medium (Biokar Diagnosis) and with Sabouraud medium with chloramphenicol (Liofilchem) and were incubated for 72 hours at 37 degrees C to determine NTGMA, and 7 days at 26 degrees C for yeasts and molds.

For isolation and identification of staphylococci, 10% blood agar petri dishes were used, along with Chapman agar (Biokar Diagnosis), Baird Parker agar (Oxoid) and Vogel Johnson medium (Biokar Diagnosis). Agar medium Giolitti-Cantoni (Liofilchem) was used to confirm the presence of the species *Staphylococcus aureus*. In vitro pathogenicity test was performed in tubes of 10/100 ml to which 0.3 ml rabbit citrate plasma was distributed (Biokar Diagnosis), 0.1 ml of which was put in broth culture for 24 hours and analysed. The tubes were incubated in a water bath set at a temperature of 37°C and were read several times in a period of between 30 minutes and 4 hours.

For conducting the antibiogram, the microtablets technique was used. On nutrient agar petri dishes, microbial suspension to be analysed were seeded and after 10 minutes the microtablets containing antimicrobials were added using a mechanical device from the kit, complying with the 30 mm distance between tablets and the 15 mm from the plate edge. Incubation was performed at 37°C, and the reading and interpretation of the results was performed at 72 hours. R-MASTITEST test was performed on the stable line. Before running the test, udder and teats were cleaned and disinfected. At the time of milk sampling, the reaction plate was always kept in the same position to easily identify quarters' appropriate evidence. In each well was harvested in early jets, 2 ml of milk over which 2 ml of solution R-MASTITEST was added. Reaction mixture was homogenized using rocking movements of the plate and after 3-5 minutes the result was read and interpreted.

Results and discussions

21 cows were examined clinically and rapid tested using R-Mastitest, and 22 milk samples were collected from cows with various mammary gland disorders: udder edema, organoleptic changed milk, diarrhea, high temperature. Table 1 shows the results obtained in determining the total aerobic mesophilic germs (NTGMA), the total number of yeasts and molds expressed in CFU/ml and total somatic cell count (SCC)/l milk.

Table 1

The results obtained in determining the total aerobic mesophilic germs (NTGMA), the total number of yeasts and molds and total somatic cell count (SCC) in milk

Sample No	Total no of germs CFU/ml	Total no of yeasts and molds CFU/ml	Total somatic cell count/ml of milk
1 S	11x104	4.3 x 10	2,000,000
1D	10x104	1x10	1,900,000
2	5.4x104	0	1,700,000
3	3x102	0	1,500,000
4	3.5x105	1 x 10	450,000
5	2x103	0	470,000
6	1x103	0	200,000
7	6x106	7 x 10	3,200,000
8	3x105	0	520,000
9	1x104	0	410,000
10	1x105	0	540,000
11	2x106	3 x 10	5,500,000
12	2.6 x 103	0	380,000
13	4.1 x 104	7 x 10	480,000
14	3.4 x 104	7 x 10	420,000
15	2.2 x 105	1 x 10	520,000
16	3.1 x 106	1 x 10	500,000
17	8 x 103	9 x 10	1,800,000
18	2.6 x 103	0	2,000,000
19	3.2 x 103	3 x 10	2,000,000
20	3.7 x 103	4 x 10	3,200,000
21	4 x 104	4 x 10	2,000,000

From the data presented in Table 1 it is observed that the results are variable, with samples showing NTGMA between 1x103 CFU / ml of milk and 6x106 CFU / ml of milk.

Total yeasts and molds ranged from 0-910 CFU / ml of milk.

Figure 1 shows the sample NTGMA 11 (A = dilution 10-3 and B = dilution 10-5).

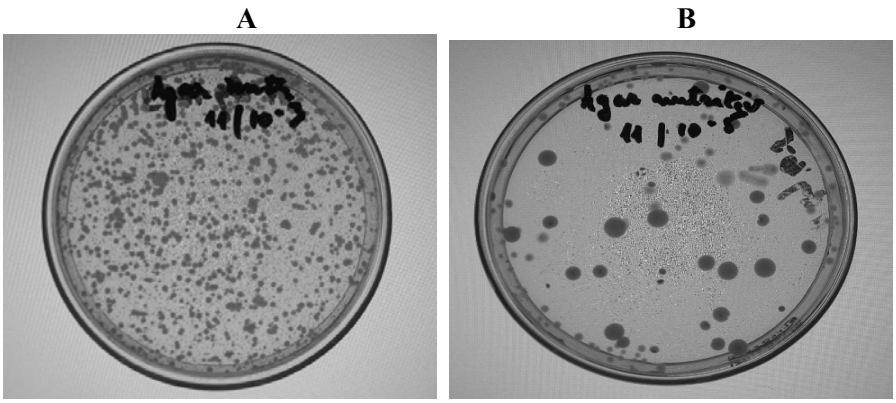

Fig. 1. *Determination of the total number of aerobic mesophilic germs from (NTGMA) 11 sample, having (A) dilution 10-3 and (B) dilution10-5*

Investigations on somatic cell count (SCC) showed values between 200,000 and 5,500,000 cells/ml of milk.

In milk sample 11, NTGMA was 5,500,000 cells/ml of milk with gelatinous aspect and not draining upon tilting the board.

Development of microbial culture on selective medium Baird Parker showed black colonies surrounded by a clear area and the environment Giolitti-Cantoni the appearance of black colonies confirmed the presence of *Staphylococcus aureus* germ. By using these media we could differentiate the coagulase-positive staphylococci from the coagulase-negative ones (Rainard et al., 2003)

Figures 2 and 3 show the appearance of colonies of *Staphylococcus aureus* on Baird Parker and Giolitti-Cantoni media.

Fig. 2. *Staphylococcus aureus on Baird Parker selective medium*

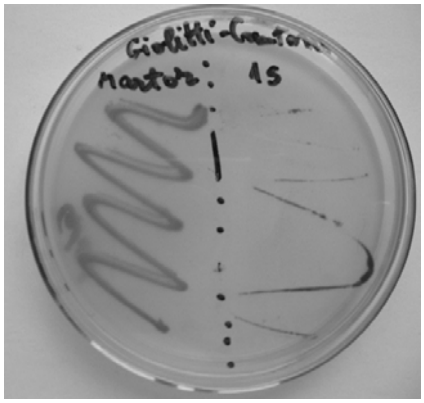

Fig. 3. *Staphylococcus aureus isolated from sample 1S on Giolitti-Cantoni*

Analysis of bacterial flora isolated from milk showed different aspects depending on the degree of impairment of mammary gland.

Strains 1, 2, 7, 11, 17, 18, 19, 20 and 21 presented on Baird Parker medium black colonies, smooth, shiny, convex, surrounded by a white-gray border, surrounded by clear zones of 2-5 mm in diameter. These strains were seeded on selective Giolitti-Cantoni medium and the presence of *Staphylococcus* has been confirmed by the appearance of black, smooth, glossy and regular edges colonies. From these data we can see that isolated strands from samples 1, 2, 7, 11, 17 and 21 were coagulase-positive and sample 17 was also hemolysis-positive (Figure 4).

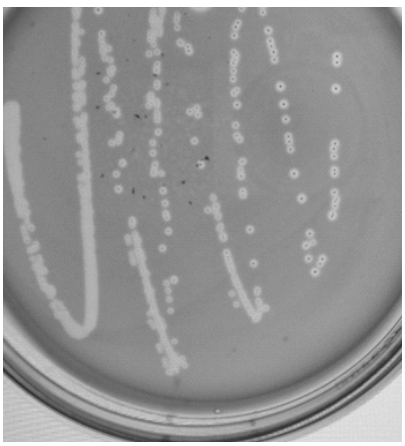

Fig. 4. *Staphylococcus aureus hemolysis-positive*

As a result of the fact that treatments were not administered on time and for an appropriate time required for the sterilization of the body, this led to the emergence of strains resistant to some antimicrobials of choice used in this milk production farm.

By analyzing the results obtained by approximating SCC / ml of milk by rapid R-MASTITEST test, 12 samples were negative and did not exceed the limit of the SCC, of 500,000 cells / ml of milk. Threshold value of 400,000 SCC / ml of milk established by Regulation EC 853/2004, conditions acceptability of raw milk to processing (Puia M., 2009).

Positive samples 1S, 1D, 2, 7, 17, 20, 21 and 22 exceeded the allowed target, containing between 1,000,000 and 2,000,000 somatic cells / ml of milk.

Figure 5 shows the appearance of milk collected from cows with mastitis examined by R-MASTITEST. On the positive samples, milk became consistent with floaters and low fluidity with blue colour.

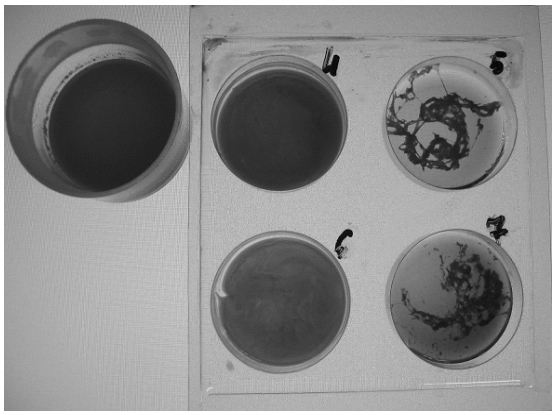

Fig. 5. *Reading and interpretation of the results obtained after the analysis of milk samples with R-MASTITEST for determinining the approximate value of SCC*

Coagulation test results in rabbit plasma citrate performed on milk samples showed that of the 22 milk samples investigated for the presence of coagulase, 8 samples were positive, which indicates that there are strains of *S. aureus* coagulase-positive with pathogenic and toxigenic potential. Coagulase activity was highlighted by Vogel-Johnson environment cultivation based on colony appearance.

Antibiotic sensitivity of strands isolated from milk of cows with subclinical mastitis are presented in Table 2.

Table 2

Sensitivity to antibiotics of strains of *Staphylococcus aureus* isolated from analyzed milk samples

Antibiotic	Micro tablet content µg	Isolated strands								
		Inhibition zone diameter (mm) / results interpretation								
		1S	1D	2	7	10	11	17	20	21
Nalidixic Acid	30	15 (R)	15 (R)	10 (R)	21 (S)	20 (S)	16 (S)	19 (S)	17 (S)	20 (S)
Amoxicillin	10	36 (S)	35 (S)	25 (S)	9 (R)	25 (S)	8 (R)	27 (S)	28 (S)	26 (S)
Ampicillin	10	29 (S)	28 (S)	13 (I)	0 (R)	15 (S)	0 (R)	10 (R)	9 (R)	5 (R)
Cefaclorum	30	28 (S)	28 (S)	20 (S)	18 (S)	19 (S)	14 (R)	25 (S)	28 (S)	27 (S)
Chloram phenicol	30	20 (S)	22 (S)	37 (S)	23 (S)	0 (R)	23 (S)	25 (S)	24 (S)	31 (S)
Ciprofloxacin	1	20 (I)	20 (I)	14 (I)	16 (S)	19 (S)	20 (S)	20 (S)	22 (S)	25 (S)
Doxycycline	30	30 (S)	25 (S)	34 (S)	15 (S)	7 (R)	16 (S)	18 (S)	20 (S)	22 (S)
Erythromycin	15	19 (I)	22 (I)	12 (R)	0 (R)	7 (R)	7 (R)	11 (R)	10 (R)	8 (R)
Gentamicin	10	15 (S)	16 (S)	16 (S)	15 (S)	7 (R)	15 (S)	18 (S)	16 (S)	19 (S)
Kanamycin	30	15 (I)	14 (I)	17 (I)	13 (R)	12 (R)	11 (R)	10 (R)	8 (R)	7 (R)
Neomycin	30	10 (R)	10 (R)	7 (R)	14 (I)	13 (I)	11 (I)	8 (R)	5 (R)	10 (R)
G penicillin	10	26 (R)	27 (R)	26 (R)	20 (R)	14 (R)	8 (R)	25 (R)	26 (R)	21 (R)
Streptomycin	10	10 (R)	9 (R)	9 (R)	11 (R)	8 (R)	7 (R)	10 (R)	8 (R)	7 (R)
Tetracycline	30	30 (S)	25 (S)	33 (S)	22 (S)	20 (S)	23 (S)	24 (S)	25 (S)	31 (S)
	S = sensitive; I = intermediate; R = resistant									

The data presented in Table 2 shows that strain 1 was resistant to nalidixic acid, neomycin, penicillin and streptomycin, strain 2 was resistant to nalidixic acid, erythromycin, neomycin, G penicillin and streptomycin, strain 7 to amoxicillin, erythromycin, kanamycin and streptomycin, strain 10 to chloramphenicol, doxycycline, G penicillin, gentamicin, kanamycin and streptomycin, strain 11 to amoxicillin, ampicillin, cefaclorum, erythromycin, kanamycin, G penicillin and

streptomycin, strain 17 to ampicillin, erythromycin, kanamycin, neomycin, G penicillin and streptomycin, strain 20 to ampicillin, erythromycin, kanamycin, neomycin, G penicillin and streptomycin, strain 21 to ampicillin, erythromycin, kanamycin, neomycin, G penicillin and streptomycin.

In the dry period, into the papillary channel there were introduced with a lengthening, antibiotics, like cloxacillin suspension or synthetic penicillin (DC Noroclox intramammary syringes). The antibiotics are no longer administered starting 28 days before calving and meat waiting time is 28 days. After administration, the udder was massaged gently with upward movements so as to spread the antibiotic deep into the tissue. After treatment, teats were disinfected by immersion in a solution of sodium hypochlorite. Disinfection shall be repeated after 10-14 days.

In lactating animals, treatments have been conducted based on antibiogram results. Penicillin was administered (Pen-Strep, bottle of 100 ml dose of 1 ml/25 kg) for 7 consecutive days, (waiting time is 10 days for meat and milk for 3 days) and amoxicillin (Noroclav, intramammary syringes administered every 12 hours). Treated quarters are emptied at the next milking and milk from cows with streptococci and staphylococci mastitis, is collected separately and is inactivated with disinfectant after each milking.

At the 21 cows with mastitis amoxicillin injection was used (Noroclav, bottles of 100 ml) at a dose of 1 ml/20 kg for 7 days). Waiting time is 42 days for meat and milk 80 hours.

To prevent dissemination of infection in the mammary parenchyma intramammary antibiotic infusions were made, repeated 8-12 hours. Breast infiltrate was absorbed faster if water and juicy feed was reduced and breast massage was performed from bottom to top. To those with chronic tendency, resolution acting ointment was applied: salicylic acid ointment, iodine ointment.

To extract stasis milk, a complete milking was done, 6-8 times per day. Massaging from top to bottom permitted crushing coagulated milk blocking ducts and sinuses, facilitating their elimination. Elimination of coagulation was obtained by introducing into the sinus of a warm sodium bicarbonate solution. Local treatment consisted of the removal, as best as possible, by milking and massaging of the affected compartment, followed by the introduction of antibiotics or sulphonamides to the affected quarter. Antibiotics have been used as solutions, ointments, emulsions or oil emulsions. After each treatment the teat was left to stand for 5-6 hours, as applied medication could act, then milked every 2 hours until the next treatment.

In case of purulent mastitis the pursue was primarily to remove purulent exudate through frequent milking, repeated every 2-3 hours. After discharge of the chamber, cloxacillin and ampicillin intramuscularly injected was infused intramammary for 7 consecutive days. Treatment results depended largely on precocity implementation.

Conclusions

Of the 21 samples collected milk, 13 presented organoleptic changes. 8 of 22 samples analyzed were positive milk at R-MASTITEST test, somatic cell counts ranged from 200,000 to 5,500,000 cells / ml of milk. Out of 8 samples, coagulase-positive *Staphylococcus aureus* was isolated, and from one sample (sample 17) hemolysis-positive *Staphylococcus aureus* was isolated. Out of 2 samples, both coagulase-positive and hemolysis-positive *Staphylococcus aureus* was isolated. Out of 8 strains of Staphylococcus aureus: 8 were resistant to penicillin, 2 to ampicillin, 5 to amoxicillin, 1 to cefaclorum and 7 to erythromycin. This study identified the presence of coagulase-positive and hemolysis-positive *Staphylococcus aureus* strains, resistant to 2 antibiotics of choice and to some broad-spectrum antibiotics commonly used in therapeutic practice. Due to the fact that treatments were not made on time and for an appropriate time required for the sterilization of the body, it led to the emergence of strains resistant to some antimicrobials of choice used in this milk production farm.

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THE HEALING EFFECT OF STERILE POWDER OF CORN SMUT (USTILAGO MAYDIS)

N. VELICU, N. BERCARU, T. PETRUȚ

Abstract

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. 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.

Keywords: *corn smut, plage.*

Introduction

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. The disease is produced by *Ustilago maydis* from *Ustilaginaceae* family, order *Ustilaginales* (1, 2, 4). 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 stems and whole cobs are attacked, and leaves less (3).

Material and methods

Studies were conducted on a group of 20 laboratory animals – rabbits aged 6 months who had traumatic wounds.

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.

Results and discussions

Treatment consisted of regional grooming and mechanical antisepsia (excision edge necrosis). Locally it has been applied a thin layer of sterile powder of spores complemented with the application of a protective dressing (fig. 1).

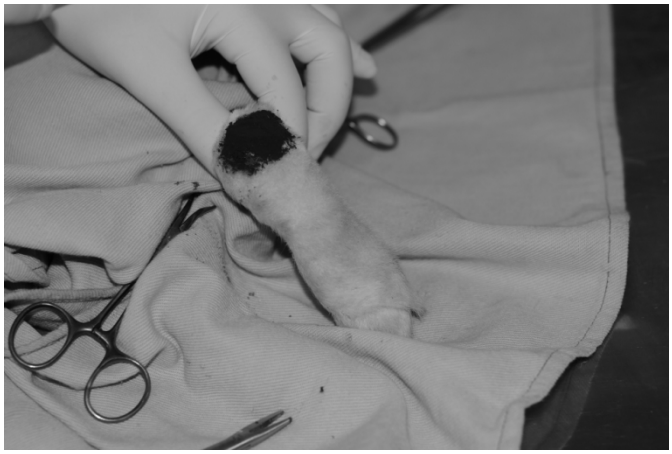

Fig. 1 . *Appearance of wound after application of Ustilago maydis spore powder*

After 5 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 7 days wounds were greatly reduced in volume, and on the wound edges it has been observed red fleshy buds belonging to granulation tissue.

Fig. 2. *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. 2).

In traumatic wound to the lateral region of the calf, simple and shallow cut have been observed, with retracted edges due to regional tension, slightly infiltrated and swollen without local heavy bleeding.

The wound affected only the skin, without involvement of deeper tissues (wound cut, simple, single, shallow).

Fig. 3. *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 5 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. 3).

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).

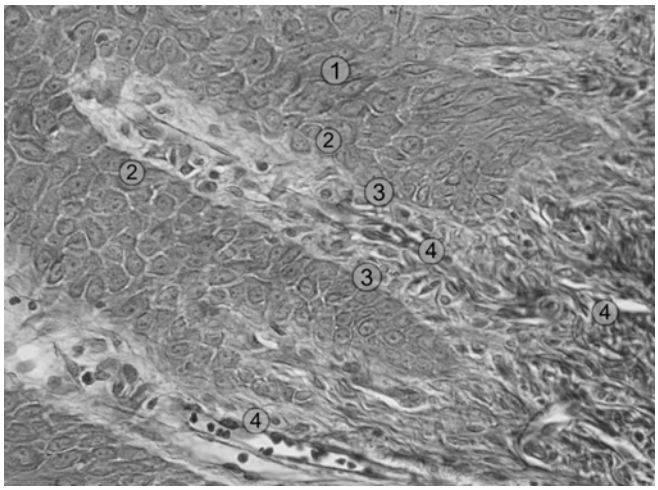

Fig. 4. *Traumatic wound rabbits treated with powder* ***Ustilago maydis*** *after 7 days treatment Col. trichomes Mallory; Ob. 40x*
1) spinous layer (polyhedra); 2) basal layer; 3) basement membrane; 4) papillary dermis.

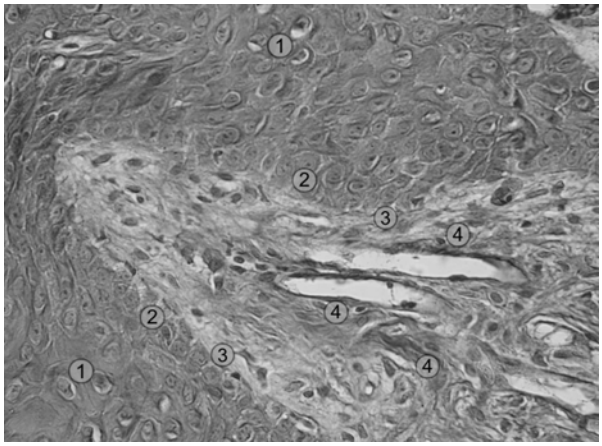

Fig. 5. *Traumatic wound rabbits treated with powder* ***Ustilago maydis*** *after 7 days treatment; Col. trichomes Mallory; Ob. 100x*
1) spinous layer (polyhedra); 2) basal layer (generator);
3) basement membrane; 4) papillary dermis

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.

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.

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. 4, 5).

Conclusions

Experimental research on stimulated wound healing using a sterile powder produced by *Ustilago maydis* led to these conclusions:

1. Herbal components showed, besides stimulating effect on the evolution of healing, an analgesic and antipruritic effects, reducing animals' concern for their own lesions.

2. The product was well tolerated, showing reduced antigenic reaction and biocompatibility with no evolution to complications or pathological scarring.

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