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CUPRINS

THE EVALUATION OF THE MILK HEALTH AND CONFORMITY LEVEL ON A PROCESSING COMPANY CHAIN WITH CLOSE AND OPEN CIRCUIT

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Abstract

The development of the safety and traceability standards of dairy products has led to the formation of a real commercial milk chain of production, processing and exploitation. This development is also sustained in this study, based on the comparative monitoring of the health and conformity indices of milk acquired from traditional collecting centers and from close circuit farms belonging to a processing company. The quality of the milk derived from the two collecting sources situated in the central area of Transylvania was analyzed in comparison, based on the data obtained in the auto-control tests performed by the processing company. The analysis of this investigated parameters indicated very high level of conformity for the freezing point (99.67%) and contamination degree (100%), regardless the source of collection. In contrast, for the aerobic plate count (APC) and for the somatic cell count (SCC) data showed a very high degree of compliance to standards in case of milk collected from farms with closed systems (95.38% - APC and 95.00% - SCC), in comparison with milk acquired from traditional centers (43.40 % - APC and 54.28 – 73.34% for SCC). The results confirmed the quality level of the feed floristic composition, welfare and health of lactating cows in closed circuit farms, in comparison with those found in traditional collecting centers. The global analysis of the obtained values reveal high level of cellular and microbial content in the milk mixture subjected to processing, showing a low level of compliance to standards (56.37% -APC and 74.21% -SCC).

Key words: feed base, lactating cows, milk conformity, processing, dairy products

Introduction

The increase of feed quality, safety, security and traceability of dairy products requires the integration of the production, processing and development procedures in the current concept of the milk chain (Râmniceanu, 2005). The implementation of these complex forms of management in the commercial company is based on the functioning of integrated systems for health surveillance of the animal- dairy product –consumer chain (Ognean, 2002; Farnworth, 1993). These kind of chains offer advantages regarding the integrated monitoring of feed quality and health of lactating cows, evaluated on the basis of hygienic and sanitary parameters of the collected milk and processed products (Turek *et al.*, 2009; Râmniceanu, 2005). A large part of commercial farms are fitted with automatic systems, which verify cellular content and electric conductibility of milk, in order to monitor the conformity and health of the produced milk (Carey, 2001; Harmon, 1994; Ognean *et al*., 2003, Nielen *et al.*, 1995).

Along with the adherence of Romania to European Community (EC), our country adopted the new hygienic and sanitary standards for raw cow milk, which mention a APC lower or equal to100,000/ml and a SCC lower or equal to 400,000/ml. This study is also focused in this direction, for evaluating the conformity degree of milk collected from different areas with the European standards, regarding the fact that in our country the proportion of conform milk is still low (Giurcaet *et al.*, 2008; Turek *et al.*, 2009).

Materials and methods

Data obtained at the auto control tests, performed by a processing company on milk samples, collected from traditional centers and commercial farms, situated in the central area of Transylvania, was the basis for health and food security monitoring of the processed milk and of the commercialized dairy products. The research coincided whit the study period of strategies and agricultural policies of the European Institute in Romania, allowing the comparison of our results with national data (Giurcaet *et al.*, 2008).

***Milk collection network*.** In the investigated period, the company processed high quantities of milk, collected from several centers of traditional network situated in the central area of Transylvania with low degree of classification in the European standards. In addition, milk obtained in the company farms chain with closed system represented a source of fit milk. By exploiting these sources, composed of 12 collecting centers and 2 farms with closed circuit, the processing capacity of the company reached 70,000 L of milk/day, selling 31 dairy products.

The chain with closed circuit. The population of cows from two farms belonging to a processing company, including 450 Romanian Spotted Cattle (RS) and 269 Holstein Friesian Cattle (HF) were integrated in the milk chain. In order to obtain the fit milk in these farms the condition of feed, zoo-hygiene and surveillance of milking and mammary health were optimal Feeding, based mainly on grazing half-hay and concentrates, assured a medium level of lactation of 6500 L for RS and of 6700 L for HF. The appropriate requirements for loose housing were technologically assured, with sandwich like shelters, fitted with individual bunk on permanent litter, natural ventilation and illumination, maternity and nursery for calves. Milking conditions were assured by using the centralized system „Stranco", equipped with computerized monitoring of the basic indices of mammary health, production and reproduction and a small laboratory for the milk control and disinfection. The related facilities were at the same standards: veterinary practices, lockers, bushing whit separate milking facility.

Milking procedure involved the use of background music to stimulate the lacto-ejection, and measures of hygiene and monitoring of mammary health: removing the first jets and their examination by Contrast tests (Rotaru and Ognean, 1998); cleaning and disinfection of the udder before milking (individual pads); monitoring the electric conductibility of the milk (mS) and disinfection of the udder after milking with protective film-forming solutions. Using collection tanks with automatic programming and automatic systems for wash and disinfection, the condition of hygiene and cooling of the milked milk were also assured.

The strategy for detection of mammary infections based on the computerized monitoring of milk electric conductibility or based on the results to Contrast and Waikato tests had a major impact (Ognean, 2002; Rotaru and Ognean, 1998). The cows diagnosed with mastitis during lactation were mainly treated with intra-mammary antibiotic suspensions (Mastiject, Sinulox) and milked separately during the waiting period. Relevant measures were also applied in the transition to the non-lactating period: sudden weaning in association whit a selective diet for 24 hours and intra-mammary treatment whit retard antibiotics, followed by the obstruction of the teat canal whit a disinfectant gel. For the health surveillance of the lactating cows and milked milk we resorted to periodic evaluation of the ketone bodies level, using Ketostiks bands and the individual determination of basic milk physico-chemical parameters (fat, protein, dry matter, density, freezing point, titratable acidity), with Ecomilk equipment (Sabău and Rotaru, 2006).

The structure of the cow populations varied according to the stage of lactation and the physiological status of the cattle, thus data were recorded separately from 4 different category of cows: in the first stage of lactation (within the first hundred days post-partum); the second stage of lactation (within the second hundred days post-partum, respectively the first stage of gestation); the third stage of lactation (within the third hundred days post-partum, respectively the second period of gestation, including the transit to non-lactating and peri-parturient period (a week before parturition and two weeks after gestation).

Performed investigations. Representative samples were collected from the raw milk and dairy products and were subjected to the physico-chemical, microbiological and cytological testing. The standardized procedures and methods approved by the national and European legislation were adopted for the collection and investigation of raw milk and dairy products (Mihaiu and Rotaru, 2007). These methods were based on automatic system for counting milk cells (SomaScoper MKII and Fossomatic) and standard methods for microbiologic investigations (Carey, 2001; Harmon, 1994; Ognean, 2002; Mihaiu and Rotaru, 2007). In order to perform the tests for the udder health surveillance of cows and for the safety of the sold products, the processing company appealed to the service of some specialized laboratories, partners of the auto-control program. Individual testing on a group of very productive RS (n=22) and one of HF (n=18), whit an daily average production of 20.7 respectively 33 L of milk, was employed in parallel, in order to obtain additional data in farms with closed circuit.

Data obtained to the performed testing were interpreted in correlation whit the results recorded in the official controls, adding that the processing company apply the HACCP system for the food security and safety.

Results and discussions

The evolution of the physico-chemical parameters value recorded to milk testing didn't show any differences between traditional collecting centers and farms with close circuit, the recorded oscillations being unimportant. To support this development the average values recorded at the tests performed on a sample of 118,317 liters of raw milk are relevant: 3.6% fat, 16.8ºT acidity, 5.7ºC temperature, 1.0269 density, 1.8 degree of contamination. In contrast, the dynamic of the hygienico-sanitary indices showed important differences between the two categories of collection sources, revealing a high conformity level of milk produced in farms with closed circuit. In this regard we consider significant the recorded low average levels for APC ($85.5x10^3$ respectively $77x10^3$/ml) but also for SCC ($48.7x10^3$ respectively $37.4x10^3$/ml milk) in the group of high productive RS and HF cows.

The analysis of the physico-chemical parameters and the comparison of these results whit those reported by other researchers in the field, reveal the positioning of the determined values within the physiological ranges, whit oscillations compatible whit processing standards, including temperature and contamination degree (Ayala-Hernandez *et al*, 2009; Mihaiu and Rotaru , 2007).

On the other hand, low levels of the microbiological and cytological content of the milk derived from farms with closed circuit confirmed the remarkable conditions for feeding, maintenance, hygiene and healthy assured on the milk chain. To ensure high levels of quality and health indices of milk produced in these farms, also contributed the automatic mastitis detection through monitoring the electrical conductivity of milk. In addition, data regarding

heat expression**,** the quantity of milk production and the ablactation were also provided. The high degree of effectiveness of the chain whit close circuit was reflected by the remarkable technical level of the equipment used for collecting and cooling milk, washing and disinfection of the milking installations and especially for the preliminary testing of milk at farm level.

The maintaining low annual rates of mastitis (5-10%) in the investigated group of cows represented other major advantages of the close circuit system. In the first hundred days, mastitis was diagnosed in 16 cases (4%), in the second hundred days in 9 (2.2%) cases and in the third hundred days 5 (1.2%) cows.

The proportion of mastitis was maintained at a low level in the colostral period (1%), including 4 clinical cases whit hemorrhagic feature. It should be noted also that none of the cows whit mammary infections presented changes of the general status, although in some cows mastitis was clinically expressed.

The evolution of the values recorded at the basic auto-control, was largely confirmed by the results of the official controls. In this regard, particular relevance had the compliance with the accepted standards for the values of the microbiologic indices at the basic products: APC (2400-11000/ml milk for consumption- 3.5%, respectively 3500-22,000/ml milk for consumption - 1.8%), coliform bacteria (0-100) and E.coli (0-10).

The analysis of all the data obtained on a total number of 20,336 samples, reveal the development of very high levels of situation in the fit category for the freezing point and contamination degree: 99.67%, respectively 100% (Tab.1).

Table 1. The mean values and standards for main microbiological and cytological parameters of milk taken from farms with closed circuit, routes of reception and collection centers

Parameters	**Type of milk**	**Results**			
		Farms	**Collection centers**	**Routes of reception**	**TOTAL**
Freezing point	*Samples number*	*10,452*	*7465*	*2419*	*20,336*
	fit milk (%)	99.68	99.37	99.96	99.67
	unfit milk (%)	0.32	0.63	0.004	0.33
Contamination degree	*Samples number*	*10,452*	*7465*	*2419*	*20,336*
	fit milk (%)	100	100	100	100
	unfit milk (%)	0	0	0	0
Aerobic plate count (APC)	*Samples number*	*50*	*77*	*85*	*212*
	fit milk (%)	95.38	43.40	30.32	56.37
	unfit milk (%)	4.62	56.60	69.68	43.63
Somatic cells count (SCC)	*Samples number*	*41*	*52*	*38*	*131*
	fit milk (%)	95	73.34	54.28	74.21
	unfit milk (%)	5	26.66	45.72	25.79

This evolution indicate that despite the poor roads quality and prolonged time for collection, due to the high number of collection points on the most routes, cooling requirements and the milk transport period have been complied. As is showed in the table 1 and the figure 1, the situation evolved differently in the case of hygyenico-sanitary parameters, revealing very high level of conformity of the milk collected from the close circuit farms: 95.3% for the APC and 95% for SCC. In comparison, in the case of milk collected from the routes and collection centers a significant decrease of conformity degree was recorded, situated at 30.3-43.4% for APC (assessed on 85 samples of milk collected from routes and 77 samples of milk from collection centers) and at 54.2-73.3% for SCC (on 38 respectively 52 samples). However, more relevant were the results after processing the data gathered from 3 sources, from a total of 212 samples, which showed that 56.3% from the investigated milk samples were situated in the fit category for APC and 74.2% for SCC.

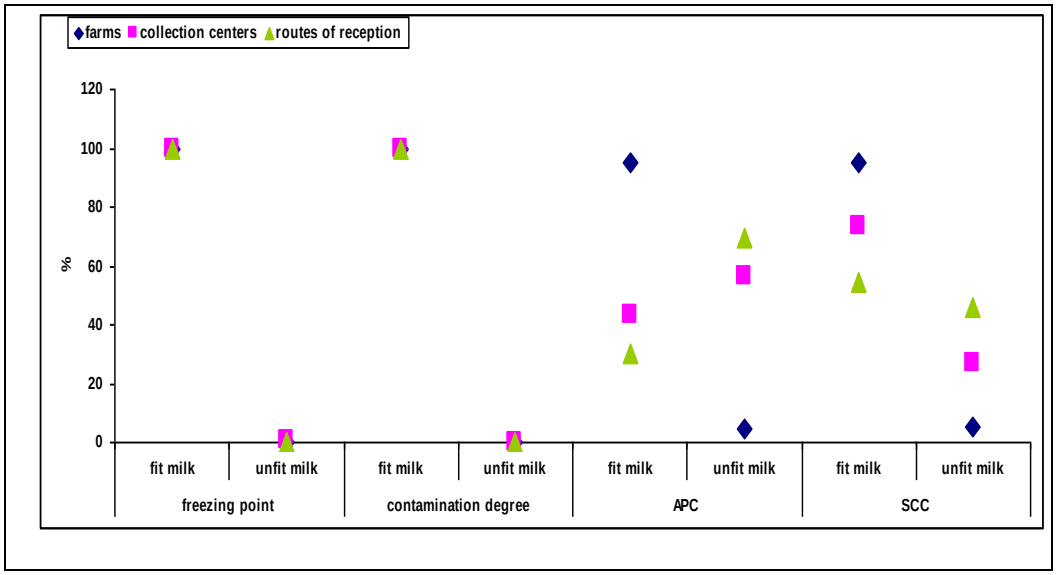

Fig. 1. Comparative analysis for main microbiological and cytological parameters of milk and observance of standards (fit and unfit milk)

The analysis of the results obtained in this study reflects the evolution of the implementation process in the Community program in order to increase the level of fit milk produced and processed in our country, integrating in the context approached by many researchers in the field (Giurcaet *et al.*, 2008; Turek *et al.*, 2009; Râmniceanu, 2005). This process started whit a period of implementation, in which the processors, generators of restructured and modernization programs, benefited from a transition period regarding the processing of non-fit milk for commercialization of products on internal market (completed at the end of 2009). These restrictions determined processing units for external market to acquire separate processing lines of fit milk. The insufficient quantities of fit milk available for units in accordance with the Community standards required the prolonging of the transition period for 2 more years (2010-2011), while the non-fit milk processing continued for the internal market.

Particular relevance for extension of the commercial companies integrated in milk chain was attributed to the internal increase of milk processing and marketing capacity. In this regard the data promoted by the actual statistics are relevant: in 2010 our country had a production of 4.4 billion L, from which only 1.35 billion were processed by the dairies, although their processing capacity was of 2.67 billion L. A large part of produced milk was sold directly on the market (1.2 billion L) and a significant quantity remained in farms for internal consumes (1.7 billion L). We also mention that at the negotiation with EU, Romania received the right to produce 3 million tones of milk per year, from which 2 million have been allocated for direct sales and another million for direct delivery to the processing factory. In the last years, according to some ministerial and patronal sources, 60% from the milk obtained in our country was classified in fit category. In contrast, in the opinion of the producers the proportion of fit milk doesn't exceed 30%. In these conditions, a new extension for the period of processing non-fit milk was requested and obtained from the European Commission, until the end of the year 2013. This European measure will support the solving of a social problem for Romania, by supporting many beneficiaries focused on the marketing of milk and also the 82 fit and non-fit milk processing companies, from which 2 are equipped whit separate processing lines. According to data synthesized by the Giurcaet *et al.*, (2008), starting from the year 2002 the imports of milk and dairy products of our country were very important, the highest level being reached after adhesion (situated at about 112000 thousands euro in 2007); with the exception of cheese category whit a positive balance in the period 2002-2005.

Conclusions

1. The average recorded values at the auto-control tests performed on 118,317 L of milk, were within the permissible limits for the following compositional and microbiological parameters of raw milk: fat (3.6%), acidity (16.8ºT), density (1.0269), temperature (5.7ºC), contamination degree (1.8), coliform bacteria (0 -100) and E.coli (0 -10).
2. The evolution of the hygienico-sanitary indices of the milk produced in close circuit farms showed a high level of conformity for APC (85.5 - $77x10^3$/ml) and SCC (48.7 – $37.4x10^3$/ml), verified on SR and HF high productive group of cows (20.7 - 33 L milk/day).
3. The evolution of a low mammary infection level in the cattle population from closed circuit farms (5-10%) reflected the efficacy of the surveillance measures for mammary health based on the use of the Stranco system, mammary disinfection, treatments in ablactation period and elimination of cow whit chronic mastitis.
4. Tests performed in milk collection sources indicated very high level of conformity for the freezing point (99.67%) and degree of contamination (100%), respectively variable for the hygienico-sanitary indices, being very high in the case of close circuit farms, 95.3% for APC and 95% for SCC, respectively low in the case of collection centers, 43.4% for APC and 54.28 – 73.34% for SCC.
5. Global analysis of the results obtained in milk samples testing in the auto-control program of the processing company indicated high level of the cellular and microbial content, revealing a low degree compliance to the European standards for APC (56.3%) and SCC (74.2%).

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ASPECTS CONCERNING THE MORPHOLOGY OF PASTEURELLOSIS IN ROE DEER

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Abstract
As cinegetic species, roe deer livestock is well represented in Moldavian counties. Studies performed so far proved the receptivity of the species to most pathologic entities that affect domestic ruminants. This paper aims at establishing whether the model of tissular reactivity of roe deers is different from domestic species in the case of a well studied pathological entity such as pasteurellosis in compared veterinary medicine.
Key words: roe deer, fibrinous bronchopneumonia, pasteurellosis

Wildlife pathology is one of the latest interest subjects in veterinary medicine, due to the major epidemiological and economic impact that different disease of wildlife may have on livestock and humans and, last but not least, on natural ecosystems biodiversity. (Jubb et al., 1993)

The roe deer is a wide spread species in North East Romania; its ecobiological similarities to the domestic ruminants (especially sheep and goats) make it a focus point of administrative institutions enabled to maintain animal health status. Direct contact between wildlife and livestock has become more and more frequent, and the role different vectors have in disease transmision is indisputable. (McGavin, 2007, Rhyan, 2010)

In this context, this paper aims at describing the lesional aspect of a major livestock disease, pasteurellosis, compared to the evolution described in domestic ruminants.

Material and methods

Study material was represented by organ fragments prelevated from 2 roe-deers extracted from the populations during the 2011 hunting season in Neamţ county. The fragments were examined in the Pathology Department of the Veterinary Medicine Faculty in Iasi.

Macroscopical and histological examinations were performed, prior to which tissue fragments prelevated were paraffine embedded and stained in the general orientation method *Hematoxilin – Eosin – Methyl blue.*

Results and discussions

In the case we examined, macroscopic pictures of the fibrinous bronchopneumonia were identified. Lungs were slightly increased in volume, with a very obvious lobular pattern visible under the pleura, with increased density and a dry section surface.

The etiology of pasteurellosis in roe deers involves species like *Mannheimia haemolytica* biotype A and *P. pneumotropica* (Paul, 2005).

Pulmonary lesions were characteristic to fibrinous bronchopneumonia, with the coexistence of all evolutive stages. Some of the pulmonary lobules showed the classic image of the filling stage, with more or less severe pulmonary congestion. The presence of air-filled alveolae proves the lesions were not produced consequently to the shot wound, and the

haemorrhages are not the result of blood aspiration. Capillaries in alveolar spaces are distended, filled with blood: epithelial debris and macrophagues are present in some of the pulmonary alveolae. (*Fig. 1*) (Perianu, 2003).

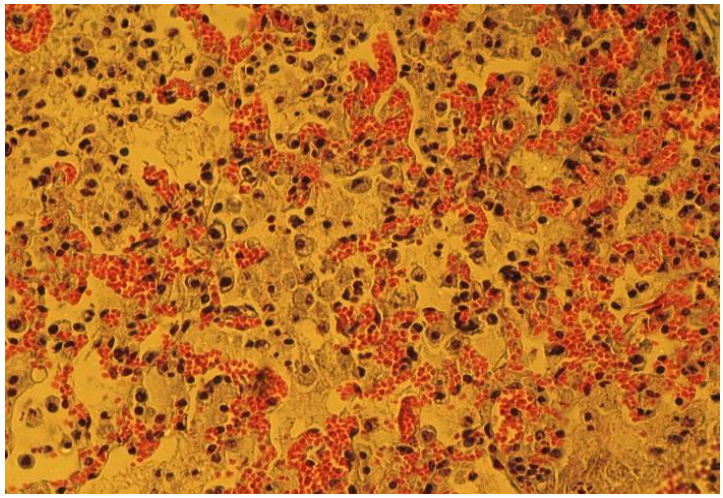

Fig. 1. Roe deer. Lung. Filling stage. Severe septal congestion. Epithelial debris and macrophagues in the alveolae. HEA, x400

The stadial evolution of the pathological process becomes obvious with the identification of territories in the exsudative phase. Alveolar spaces start to fill with fine reticular oxyphile deposits which stain orange in Azan staining method. Fibrin deposits become more and more abundant, untill they block the whole alveole. (*Fig. 2*)

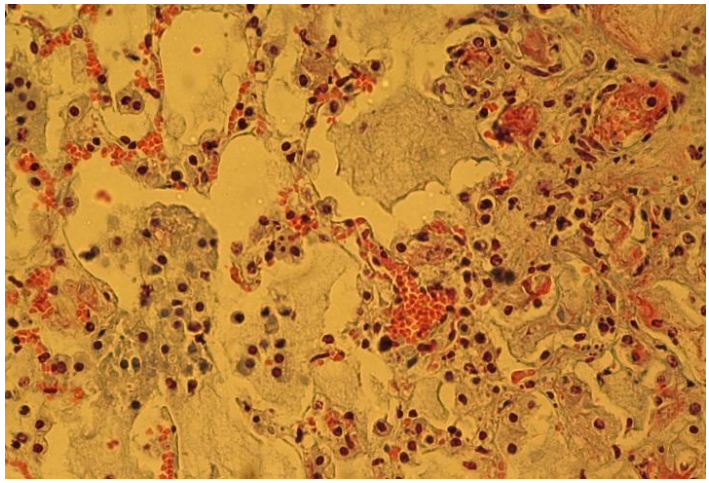

Fig 2. Roe deer. Lung. Fine reticular deposits of fibrine in the alveolae. HEA, x400

As the pathological process becomes more advanced, fibrine also accumulates in the bronchiolae, the abundant deposit blocking the airwaves. Cilliated prismatic epithelium

suffers a slight hyperplasia, with severe further changes like aplatissation and descuamation, which leave a denuded basal membrane. Interalveolar and interlobular spaces show evident congestion, since fibrin is an important chemotactic factor for neutrophiles. (*Fig. 3)*

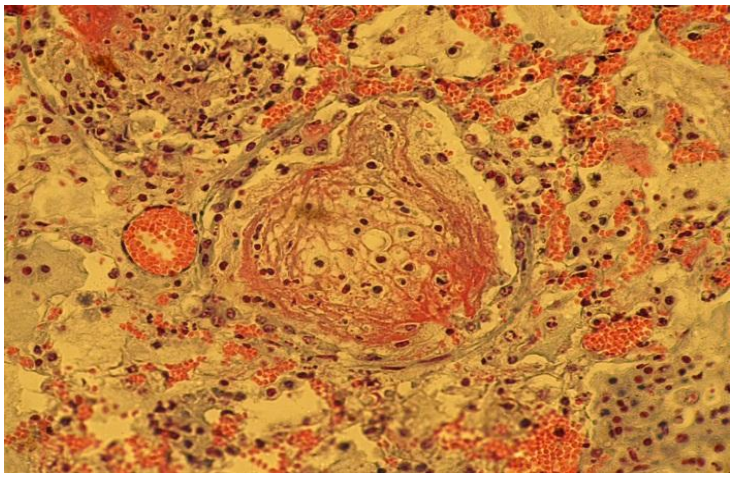

Fig. 3. Roe deer. Lung. Reticulat fibrin deposit in a bronchiola. Flattened epithelium. HEA, x400

As the pathologic process moves further, fibrin deposits accumulate in interlobular spaces as well. The same microscopic field (x400) shows coexistence of different evolutive stages of inflamation. Septal congestion persists, but the exsudate is predominantly leukocytic this time. (*Fig. 4*)

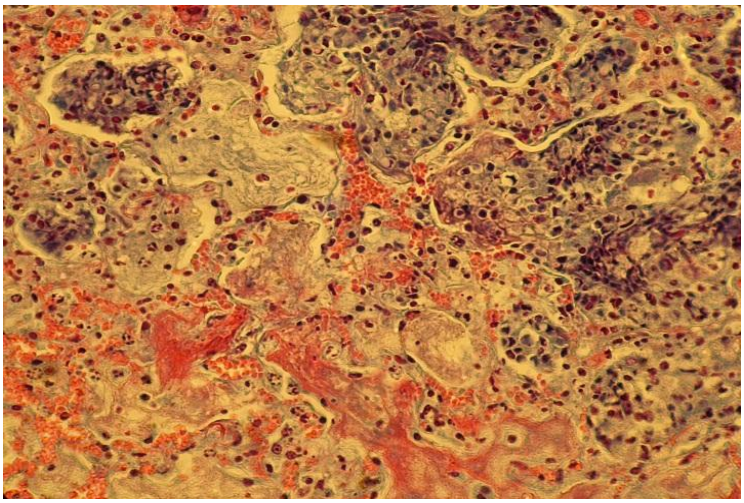

Fig. 4. Roe-deer. Lung. Sublobular areas in different stages of fibrinous inflamation: fibrin on the left, leukocytic exsudate on the right. HEA, x400

Intraalveolar leucocytes are mainly represented of neutrophiles and macrophagues; they perform a local detersion, with the slow lysis of fibrin deposits and their resorbtion, deblocking the airwaves. Fagocytic cells remove the exsudate and cellular debris from the affected bronchiolae, so the basal membrane is again prepared for repopulation with young undifferentiated cells that will prolifferate and grow into normal and functional bronchiolar cells. (*Fig. 5*, *Fig. 6*)

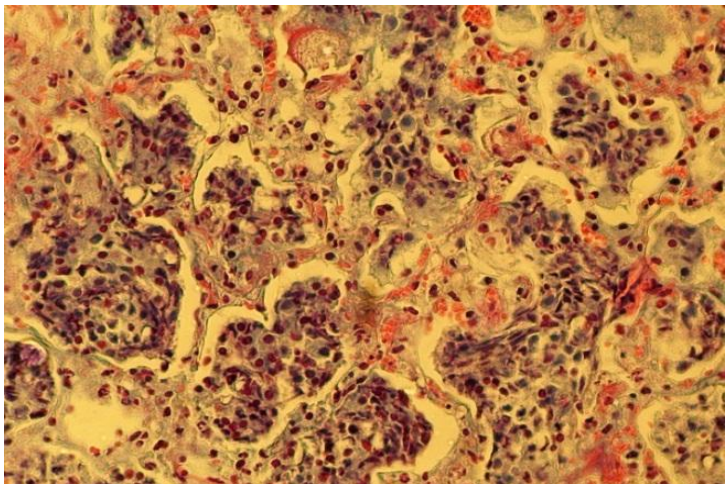

Fig. 5. Roe deer. Lung. Leukocytic masses in the alveolae. HEA, x400

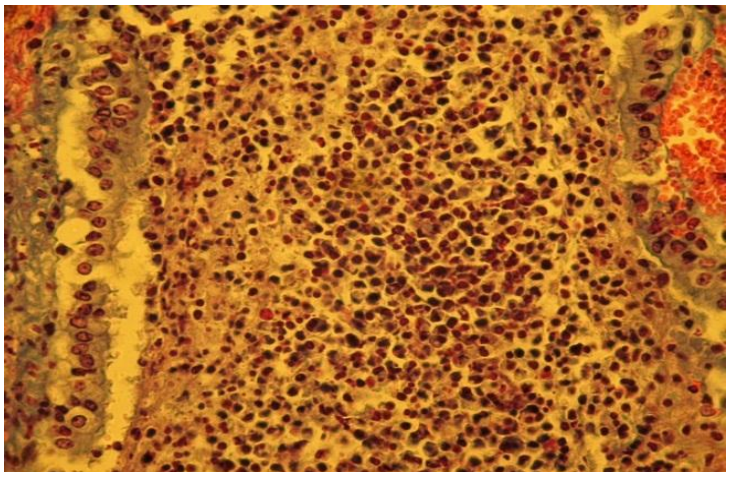

Fig. 6. Roe deer. Lung. Massive leukocytic exsudate in a bronchiole. HEA, x400

As our results show, the evolution of fibrinous bronchopneumonia from the point of view of histological findings is pretty similar to the lesional aspect of the disease in domestic ruminants. The process suffers the same stadialisation, and slight reactive particularities should be seen as individual rather than species characteristics (Williams, 2001).

It would be interesting to determine if the evolution of the process differs as extension in time, and if resolutive processes have a similar tendency to cicatrisation or functional impairement in both domestic species and wild ruminants, and this may be the subject of further research.

Conclusions

1. No important differences could be identified in the evolution of pasteurellosis in roe deers and domestic ruminants.
2. In both species, the characteristic lesional aspect of fibrinous bronchopneumonia was noticed.
3. Stadialisation of the process at sublobular level was obvious in roe deers as well as in domestic ruminants.
4. The main leukocytic populations involved in the process of local detersion are represented by neutrophiles and macrophagues.
5. Further research should clarify if the extent of the inflammatory process, its further evolution (cicatrisation or functional impairement) and its time frame are similar to those identified in domestic ruminants.

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EXFOLIATIVE CYTOLOGICAL ASPECTS OF THE INTERNAL SEROUS CAVITIES IN COMPANION CARNIVORES

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Abstract
Between 2009 - 2010 we made cytological examinations of peritoneal and pleural liquid from 7 dogs and 5 cats. The samples were examined macroscopic, biochemical (protein concentration) and microscopic (quantitative examination - NTC/µl and qualitative – normal and pathological cellular types). The results we got from the examined dogs allowed the framing of the peritoneal effusions in the neoplastic type (2 pleural effusions with carcinoma cells and 1 peritoneal effusion with mesothelium type neoplastic cells) and inflammatory type (2 effusion samples with granulocytes – neutrophil, eosinophil) and 2 samples of modified transsudate. The results we got from the examined cats showed in 4 peritoneal effusion samples the biochemical and cytological features of PIF (proteins 4,5-8g/dl, the presence of nondegenerate neutrophils on a granular proteic background) and in 1 sample of pleural liquid the presence of neoplastic lymphocytes. In conclusion the cytology has a high specificity for the diagnosis of the neoplasms, inflammatory and infectious processes, which can be diagnosed in an early stage, but a certainty diagnosis is obtained by corroboration of the cytologic examination with the anamnesis, the biochemical and other paraclinical examination.

Keywords: cytology, effusion, small animals

The excessive accumulation of organic or pathological fluids in internal serous cavities represents a common lesion which is caused by the modification of hydrological and oncotic pressure, lymphatic drainage, bleeding (1, 2). The cytological examination of puncture fluid presents a high specificity for the cancer diagnostic or inflammatory and infectious processes which can be find in the early stages (2, 3, 4).

With the anamnesis and laboratory tests the cytological examination has a major contribution in setting the nosologic diagnosis.

Matherials and method

The examinations were performed within the Pathological Anatomy Laboratory of the Faculty of Veterinarian Medicine on the samples of pleural and peritoneal effusions, obtained from 7 dogs and 5 cats peritoneal and pleural cavities puncture.

The studied samples has been macroscopic examined-by the color, clarity and viscosity of the puncture liquid, biochemical-the protein concentration detected by refractometry, microscopic examination-quantitative examination (NTG/lµ-using the hemocytometric method, the Burk-Türk counting chamber) and qualitative examination-the pointing of normal and pathological cellular types by microscopy (first with small objective x10, x40 and then with the immersion objective x100) of the smears displayed from the sediment after centrifugation at 1500 Rpm, for 10 minutes and colored by May-Grünwald-Giemsa method.

The samples were accompanied by informations about the presumptive diagnosis for a better management in interpretation the results of the cytological examination.

On the macroscopic examination of PT/dl and NTCN/µl concentration the fluid were classified in modified transsudates and exudates. After microscopic examination and using the specified literature classifications, the puncture liquid was classified as neoplastic or inflammatory fluid.

Results and discutions

The results regarding NTCN/µl and PT/dl concentrations from the examined puncture liquid were shown in Tab. 1. From the quantitative examined samples 2 were modified transsudate and 6 were exudate.

Modified transsudate were characterized by NTCN/µl between 1400-3600/µl and PT between 2,7-4g/dl. The exudate were characterized by a great nucleated cells number (7100-45000/µl) and a high level of proteins (4,5-8g/dl).

Table 1. The classification of effusions
after *Crespeau F.,* Ècole Nationale Vétérinaire d'Alfort, 2007 (* reference values)

Material	Macroscopical aspect	NTC/µl	PTg/dl
Transsudat	colorless, clear	*< 1500	*< 2,5
Modified transsudat	Variable colour (white - yellow - red)	* 1000 - 7000 1400 - 3600	* 2,5-7,5 2,7-4
Exudat	Variable colour, high turbidity, floaters	*> 7000 7100 - 4500	* > 3 4,5 - 8

From the examined samples, 4 were neoplasic: 2 pleural effusions with carcinoma cells at 2 dogs, one peritoneal effusions with neoplasic cells-mesothelium type find in a dog and one pleural liquid sample with lymphoblastic cells-neoplasic type at a cat. Modified transsudate is characterized by a small cells number, from wich exceed activated mononucleate-macrophage cells from sanguine monocytes, mature lymphocytes and a small number of neutrophils (Fig. 1).

The cytological samples from exudates has shown the evolution of inflammatory process with granulocyte type cells (neutrophiles, eozynophiles). On a fade bleeding background was revealed the inflammatory cell type population-major neutrophilia,in different cellular degradation stages even picnosis. Also were observed lymphocytes, a few number of mesoteliale cells and macrophages (Fig 2).

Table 2. Peritoneal and pleural liquid framing

Nr. of cases	Species	Investigated serous cavity	Main lesion	Disease
2	dog	peritoneal	Modiffied transsudat	cirhossis
1	dog	peritoneal	Serous exudat	Canine infectious hepatitis
1	dog	peritoneal	Eosinophil exudat	Post-vaccination allergy
2	dog	pleural	Inflammatory liquid neoplasic tyoe - cancer	Lung cancer
1	dog	peritoneal	Inflammatory liquid neoplasic type - mesothelian	mesotheliomatosis
4	cat	peritoneal	exudat – PIF	FIP
1	cat	pleural	Inflammatory liquid neoplasic type- limphoma	Mediastinal limphoma

After a post-vaccination allergic reaction on a dog, eosinophils with intracytoplasmatic granulations, round or ellipsoidal, with various dimensions were observed (Fig.3).

The results obtained from the investigated cats had revealed in the peritoneal puncture liquid cytological features which suggest a disease called feline infectious peritonitis (FIP) - the wet form. On a granular proteic background (protein concentration 4,5-8 g/dl), it was observed a mixed cells population, formed by normal neutrophiles and small mature lymphocytes (Fig 4, Fig 5, Fig 6).

Although the cytological examination results of the liquid are not specific for feline infectious peritonitis (FIP), they allow the establishment of a presumptive diagnosis of FIP in association with the clinical examination (5).

The pleural fluid associated with a mediastinal lymphoma-diagnosed at a cat, on a fated bleeding background was observed a major lymphoblastic cellular population-young cells, large, with fine dotted chromatine and visible nuclei, a moderate quantity of fade basophilia cytoplasme. Small lymphocites, mature, with large nucleus, well condensed chromatine, small amount of cytoplasma basophilia, in a small number (Fig. 7, Fig. 8).

In a pleural fluid associated with lung carcinoma - diagnosted at a dog, on an inflammatory background and faded bleeding were observed tumor cells-epithelial type, on a posters cells shape (large dimensions) or isolated, showing atypical cells: large cells with adherent cellular membrane, anysocitosis, anizocarioza, polynuclear, large and prominent nucleolus, cytoplasma hyperbasophylia (Fig. 9, Fig 10).

The cytological interpretation of the sample with neoplasic cells-mezotelial type from the peritoneal fluid showed a large number of malignancy criteria: anizocariosis, multiple nuclei, posters arrangement or isolated cells with different dimensions (Fig 11, Fig 12).

Cytological examination together with medical history (anamnesis) - intraabdominal nodular formations, 1-2 cm in diameter, cavity fluid collection, has permitted the framing of the sample in neoplasic type fluid associated with mesothelioma.

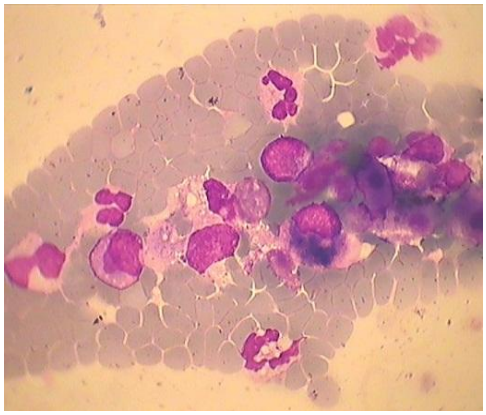

Fig. 1. Dog. Cirhossis. Peritoneal fluid. Modified transsudat. Mixted cells: activated macrophages, mesotheliale activatated cells, neutrophiles. Col. MGG, x 1000

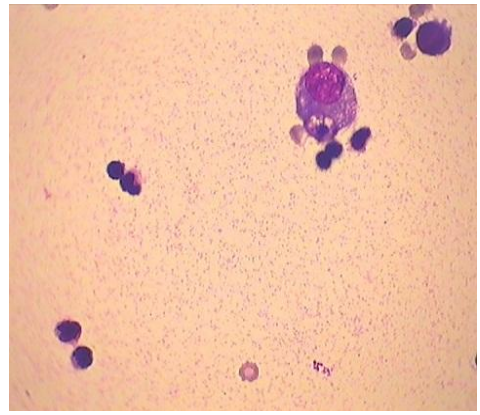

Fig. 2. Dog. Canine infectious hepatites. Peritoneal fluid. Serous exudat: fond granular proteic. Backround, normal neutrophile, mature limphocites, activated macrophage. Col. MGG, x 1000

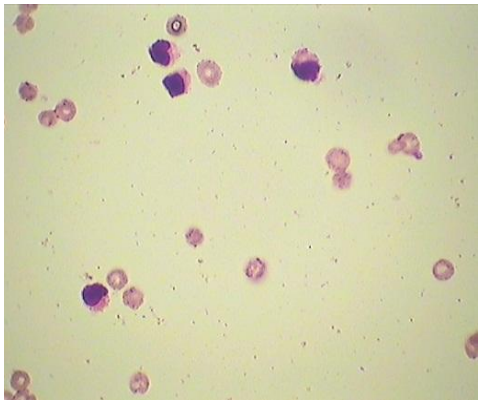

Fig. 3. Dog. Post-vaccination allergy. Peritoneal fluid. Eozinophilic inflammation. Col. MGG, x 1000

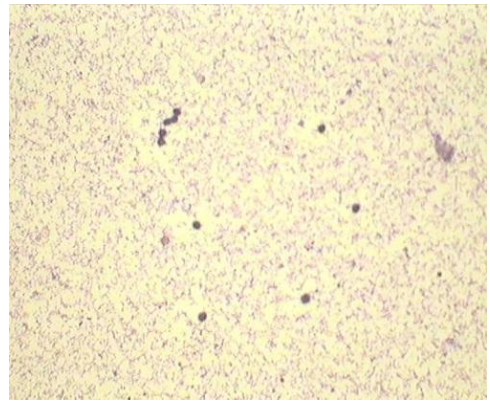

Fig. 4. Cat. FIP. Peritoneal fluid- inflammation. Granular proteic backround (high level of proteins), small limphocites. Col. MGG, x 40

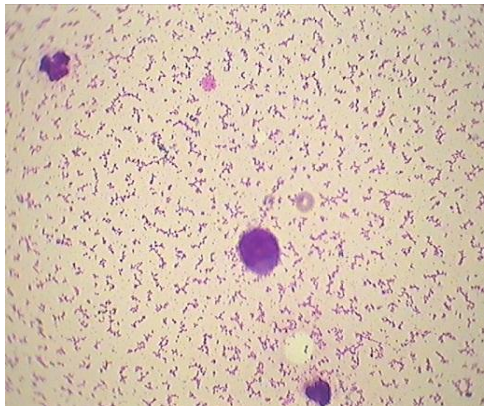

Fig. 5. Cat. FIP. Peritoneal fluid - inflammation. Normal neutrophils. Limphocites. Col. MGG, x 1000

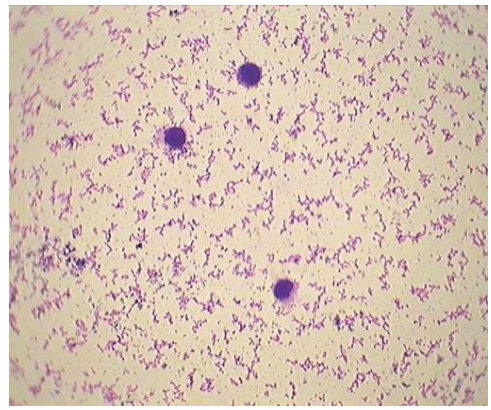

Fig. 6. Cat. FIP. Peritoneal fluid- inflammatory. Col. MGG, x 1000

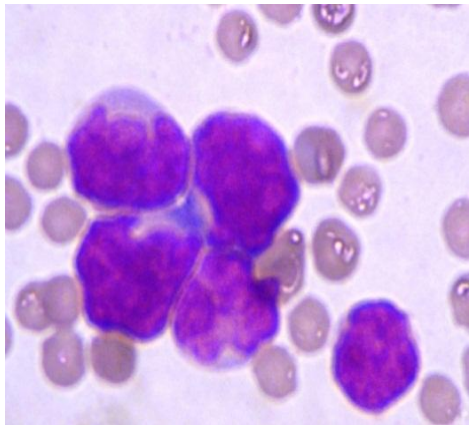

Fig.7. Cat. Mediastinal lymphoma. Pleural fluid-inflammation. Large limfoblastes, citoplasma basophilia faded. Col. MGG, x 1000

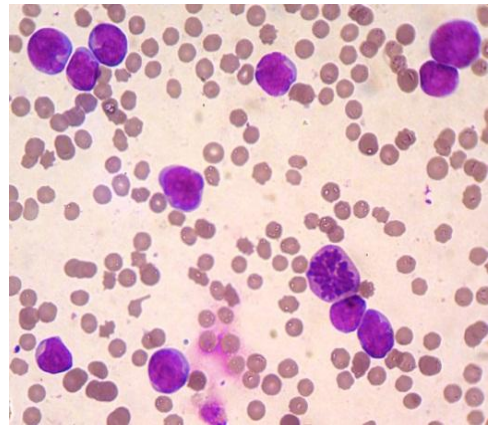

Fig. 8. Cat. Mediastinal lymphoma. Fluid pleural –inflammation. Cellular division. Col. MGG, x 1000

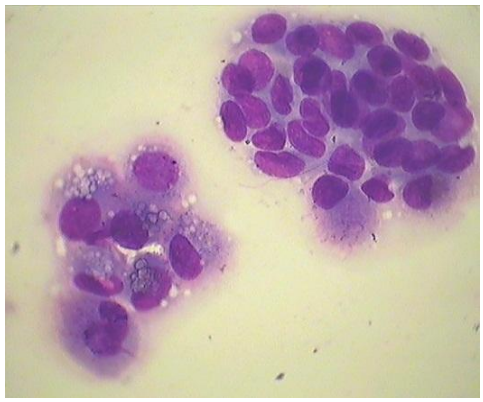

Fig. 9. Dog. Lung cancer. Pleural fluid-inflammation. Tumoral cells with fine vacuolization. Col. MGG, x 1000

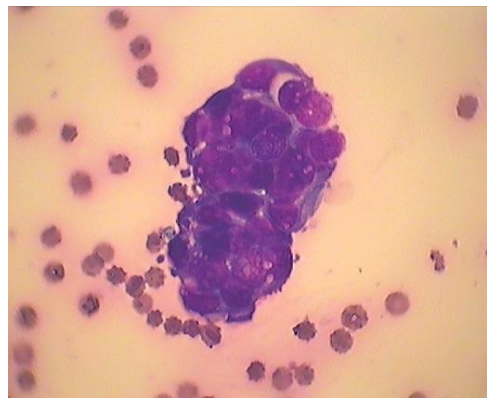

Fig. 10. Dog. Lung cancer. Fluid pleural-inflammation with cell membranes Anizocromisis nuclear. Col. MGG, x 1000

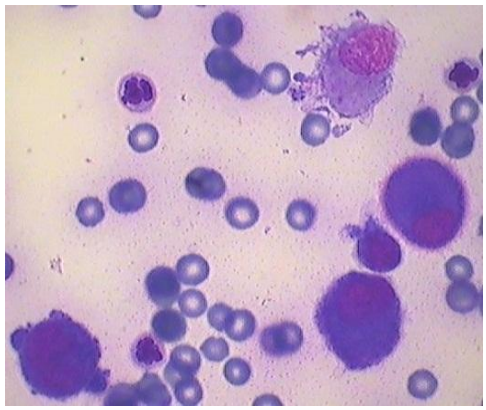

Fig.11. Dog. Mezotelioma. Peritoneal fluid - inflammation. Mezotelium cells basophilia and one macrophagic cells. Vacuolar citoplasma. Col. MGG, x 1000

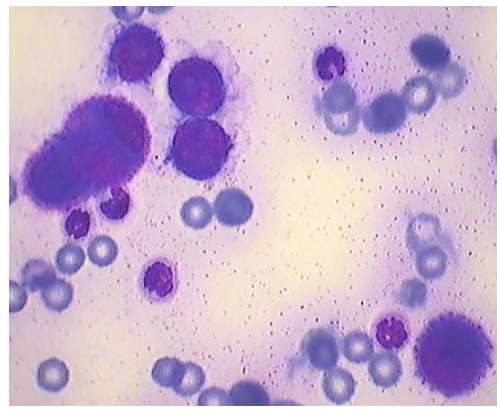

Fig. 12. Dog. Mezotelioma.Fluid peritoneal-inflammation. Bleding background, Anizocitoza, anizocarioza, high ratio N/C. Col. MGG, x 1000

Conclusions

1. The quantitative cytological examination of cavity fluid lead us to classify the examined samples in known classes: modified transsudates and exudates.
2. The qualitative cytological examination of cavity fluid (by microscopic examination) lead us to establish the predominant cellular type and classify the cavity liquid as neoplasic type fluid associated with tumor processes.
3. Modified transudates are liquids containing a moderate number of cells (1400-3600/µl) and proteins between 2,7-4g/dl.
4. Exudates shows a high number of proteins (4,5-8g/dl) and NTCN/µl between 7100-45000/µl. In addition with anamnetic data (age) and clinical examination a presumtive diagnosis was made: feline infectious peritonitis (FIP) - wet form.

5. In this study through cytological examination were diagnosed 4 tumor processes associated with cavity fluid: lung carcinomas at two dogs, one mesothelioma at dog and one mediastinal lymphoma at a cat.
6. The results regarding the framing of internal serous cavities liquid samples in cytological known classes and the guidance of nosologic diagnosis in depending on those classes shows the importance of cytological examination in veterinary medicine.

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CYTOLOGICAL AND IMMUNOCYTOCHEMICAL PARTICULAR ASPECTS OF THE PLEURAL CAVITARY EFFUSIONS IN CATS – CASE STUDIES

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Abstract
In the present work there is presented an immunocytochemical study concerning the structural cytological and immunocytochemical aspects of pleural cavitary effusions in cats. For the completion of this purpose, 3 cats of various ages of common breeding were studied, that had been presented to the clinical examination manifesting diverse pathological estates associated to monocavitary or polycavitary pleural effusions. Thus, the data of the clinical examination was correlated with the obtained results of the cytological pleural effusions from cats, made using the punction with a fine needle of the serous cavities, and the immunocytochemical examination, using specific antibodies for each class of cells, in order to establish the cell origin and a definite diagnosis.
Key words: cat, immunocytochemistry, pleural fluid

Introduction

The immunocytochemical examination has become important not only for establishing a precise diagnosis but also in order to estimate the prognosis and the indicating factors of the cytologic material. Using immunocytochemical techniques is specific for the discovery of mesotheliomas and carcinomas of unknown origin in the case of effusions or aspirations from lymphnodes. Some markers are considered to be helpers to the differentiation between the follicular adenoma and the carcinoma, in the case of aspirations. (*R. Hoinghaus, 2008).*

In some cases, the immunocytochemistry can be used for a characterization of myoepithelial cells, obtained through the punction of mammary tumors, using a fine needle, in an attempt to distinguish benign and malignant lesions.

The punction using a fine needle is a correct alternative if the examinator desires to obtain the cells that are necessary in order to establish their nature, particularly in the cases in which the pre-operatory therapy is the initial option, in inoperable recurrent or metastasing tumors as well as advanced tumors in which the studies concerning the serial hormone could bring new data to the therapeutic response *(Marluce Bibbo, 2007).*

Material and method

The study material consisted of draws coming from pleural effusions, taken from 3 common breed cats of 6, 10 and 2 years of age, that were brought for an initial consultation to the Clinics of the *Veterinary Medicine Faculty of Iasi,* and that presented respiratory and cardiac disorders, associated to the accumulation of pleural liquid.

For the cytologic smears, there was made the fine needle punction of the serous cavities. The punction was made without the use of an anesthetic and the elected place was shaven and disinfected. Needles of 22-23 Ga attached to 5 ml seringes were used. The drawing was made using sterile tubes containing an anticoagulant (EDTA) for the determination of the physical, chemical and microscopic characteristics. The draw was spread on the plate using the blood smear method. The probes were colored using May Grünwald-Giemsa.

For the immunocytochemical examination following the fine-needle punction there was used an immunocytochemical method based on establishing the link between and antigen, that is a cellular component and a specifically marked antibody. In order to emphasize the antigen-antibody complexes, the indirect method using biotin-streptavidin as an ensymatic conjugate was used.

The preparation of the smears for the immunocytochemical study was made in various stages that imply the previous spinning of the effusion liquid, the fixation inside an ethilic alcohol of a 95% concentration and the rehydration.

The protocol of the immunomarking

1. Heating the glass plates up to 37^0C

2. Applying on each plate 2 ml of PBS solution for blocking the endogenous peroxidase and incubating the plates (4 minutes)

3. The automatic distribution of the dilluted primary antibodies inside the PBS (16 minutes):

For the CD79 antibody there was used the dillution……...1/50
For the CD3 antibody there was used the dillution……1/200
For the CKAE1/AE3 antibodies there was used the dillution ……1/500

4. The standard incubation of the antibodies: applying primary antibodies on each plate (for 30 minutes)

5. The After-Fixation using a *0731Fixative solution* – applying 1 drop of solution per plate (4 minutes)

6. Adding an Universal Secondary Antibody, 2 drops per plate, through their automatic distribution (30 minutes)

7. Applying 2 ml of DAB solution on each plate (15 minutes)

8. The counter-coloring that is Standard, with a *II Hematoxylin* solution (coloring the nucleus) (8 minutes)

9. The After-Coloring using a *Bluing Reagent* solution (8 minutes)

10. Washing the probes for 5 minutes using tap water

11. The dehydration using successive baths: alcohol 70% 3 minutes - alcohol 95% 3 minutes - alcohol 100% 3 minutes - toluen 3 minutes.

12. Drying the plates for almost 6 hours.

The result of the immunocytochemical method: the specific antigens are unmasked because of the discovery of a brown coloration at the level of the cell cytoplasm that contain the antigens.

The microscopic analysis was initially made using a small objective (x10, x40) followed by an immersion objective (x100). There were followed the identification, the morphology and the proportion of the main cell types, as well as emphasising an abnormal population and its' characteristics.

Results and discussions

The cytological and immunocytochemical examination of cavitary effusions

From the sterile draw on the EDTA, there was taken a quantity of 1 ml for the cytological examination. The main antibodies that were used in the case of the immunocytochemical examination are CD3 – human policlonal antibodies, CD79- human monoclonal antibodies and CKAE1/AE3- human monoclonal antibodies.

The results of the immunomarking made on the 3 analysed cases are represented in the 1 Table.

Table 1. The results of the cases analysed from an immunocytochemical point of view

Species	**Sex**	**Breed**	**Age**	**Draw**	**ICC**	**ICC result**	**Results**
Cat	F	European	6	Pleural effusion associated to a pleural tumor	CD3 CD79 CKAE1/ AE3	CD3 (-) CD79(-) CKAE1/ AE3(+)	Pleural carcinoma
Cat	F	European	10	Recurring pleural effusion of a neoplastic type	CD3 CD79 CKAE1/ AE3	CD3 (-) CD79(-) CKAE1/ AE3 (-)	Material of intense matter
Cat	M	European	2	Pleural effusion associated to a mediastinal tumor	CKAE1/ AE3	(-)	Decreased cellularity (probe was poor in cells)

F: female, M: male, ICC: immunocytochemistry

The analysed cases in this study are the following:

1. In the case of the 6 year old common bred cat presenting dispnoea for 3 weeks, while using the classical MGG coloration, at the microscopic examination of the smear that was made on a draw taken from an intensely hemorrhagic pleural effusion, there was discovered a neoplastic young cell population, with an intense anisocytosis, severe anisopoikilokaryosis, monstrous nuclei, multiple nuclei baring cells, an abundant and intense basophile cytoplasm and numerous atypical mitosis. (Fig. 1).

In conclusion, the cytopunction is characteristic for an intensely hemorrhagic effusion. The images are mainly compatible with a carcinoma or a mesothelioma.

The immunocytochemical examination made on this draw shows a positive immunomarking of the neoplastic cells with specific antibodies CKAE1/AE3.

The antibodies CKAE1/AE3 present a specificity for cells of an epithelial nature, that was proven correctly marked – positive, (+) for this draw (CKA1/AE3 +), whereas the CD3 antibody (with a specificity for the T lymphocytes) and the CD79 antibody (with a specificity for the B lymphocytes) have shown a negative marking (-). Following the immunocytomarking, the definite diagnosis was made, and consisted of a neoplastic pleural effusion associated to a pleural carcinoma (Fig. 2).

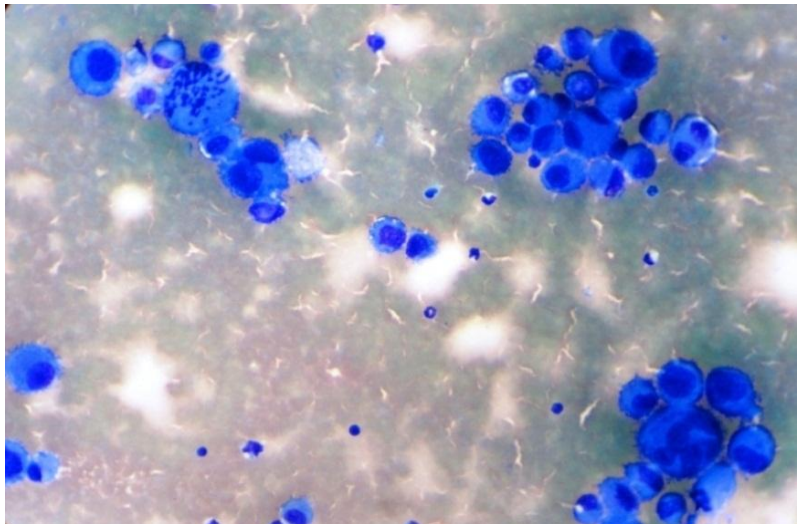

Fig. 1. A tumoral pleural effusion in the case of a cat.
Multiple nuclei-baring tumoral cells. Atypical mitosis. Col.MGG, x400

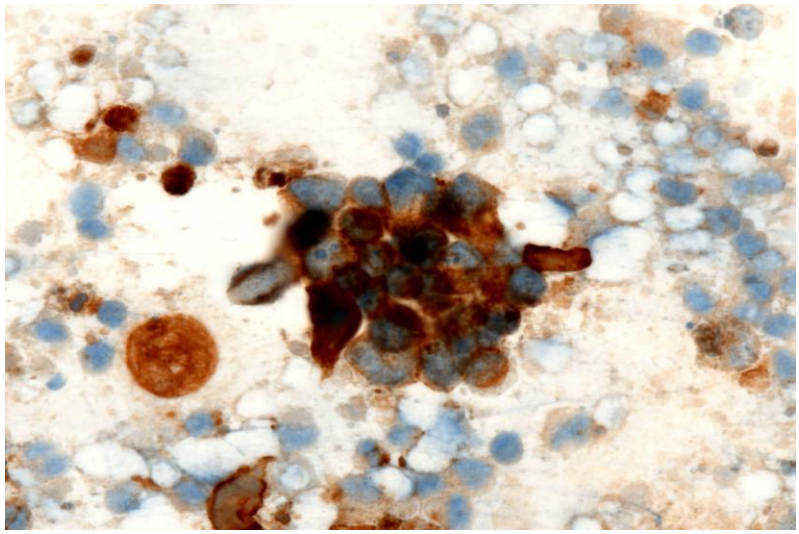

Fig. 2. A pleural effusion in a cat. A positive ICC of carcinomatous cells with the CKAE1/AE3 antibodies.
Pleural carcinoma. ICC, x 200. Positive reaction

2. In the case of the recurring pleural effusion of a neoplastic type, from a 10 year old cat, using the MGG coloration, there was observed a moderate blood contamination and an inflammatory population composed of neutrophile granulocytes and macrophages. The macrophages present numerous cell detritus inside the cytoplasm. There are also present spherical cells of various sizes, with an intracytoplasmatic material (mucus) and numerous atypical mitosis – a cytological aspect in the favor of a malignant neoplastic effusion. The

origin of these cells is compatible with an epithelial one (carcinoma) or with a mesothelial one (Fig. 3).

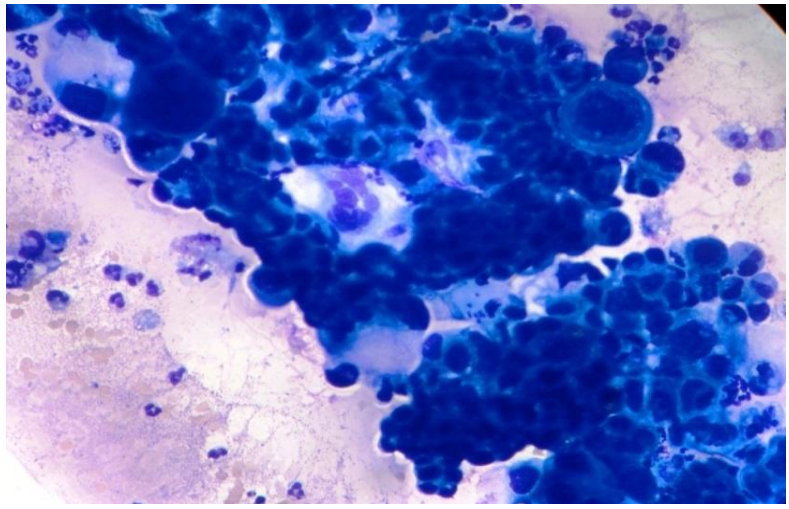

Fig. 3. A neoplastic pleural effusion in a cat.
An eosinophilic matter (abundently proteic material).
Active macrophages. Intracytoplasmatic cell detritus. Col. MGG, x 400

The immunocytomarking was proven to be inconclusive for the final diagnosis (a suspicion of pleural carcinoma or mesothelioma) because the material of the smear presented a dense film of an eosinophilic material (proteins). The marking for the 3 antibodies, CD3, CD79 and CKAE1/AE3 was negative (-) (Fig. 4).

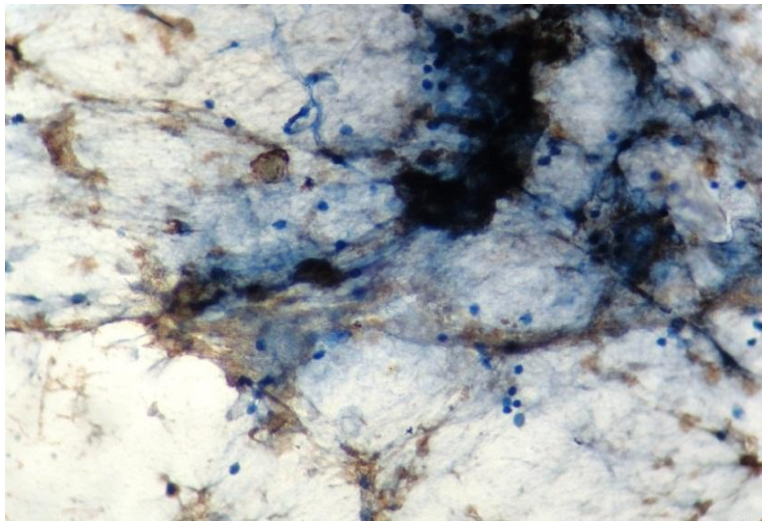

Fig. 4. A neoplastic pleural effusion in a cat. Negative immunomarking of epithelial cells for the CKAE1/AE3 antibodies. The blocking of the antibodies by the proteic matter background (+++) (1). Negative marking, x400

3. A male 2 year old common bred cat presented a cranial mediastinal neoplasm, a cough and an occasional dispnoea. Using the usual MGG coloration, the microscopic examination following the cytopunction of the mediastinal formation is characterized by a slight blood contamination without any erytrophagocytosis or hemosiderosis images.

The main cells present an anaplastic character and are either isolated or disposed in intensely basophilic placard. They have a polyedric shape, a 25 to 40 µm diameter and show a strong anisocytosis with unclear cytoplasmatic limits. The cytoplasm is abundent, the nucleus is oval, the chromatine is fine baring 1 to 2 large nucleoli and with a high power of anisopoikilokariosis. There are also seen remnants of free nuclei (a nuclear fragility) and small lymphocytes in a small number. In conclusion, the draw shows a malignant neoplastic formation, with an intensely anaplastic aspect that is compatible with a malignant epithelial thymoma (Fig. 5).

In the case of the draw that came from the pleural effusion colored using MGG there can be observed a background of the smear that consists of a light granullar proteic material and a slight blood contamination, without any signs of erytrophagocytosis or hemosiderosis. The draw is composed especially of mature lymphocytes and undegenerated neutrophilic granullocytes, as well as several foam-shaped active macrophages. The cytological aspect is compatible to a modified transsudate or a starting chilothorax (Fig. 6).

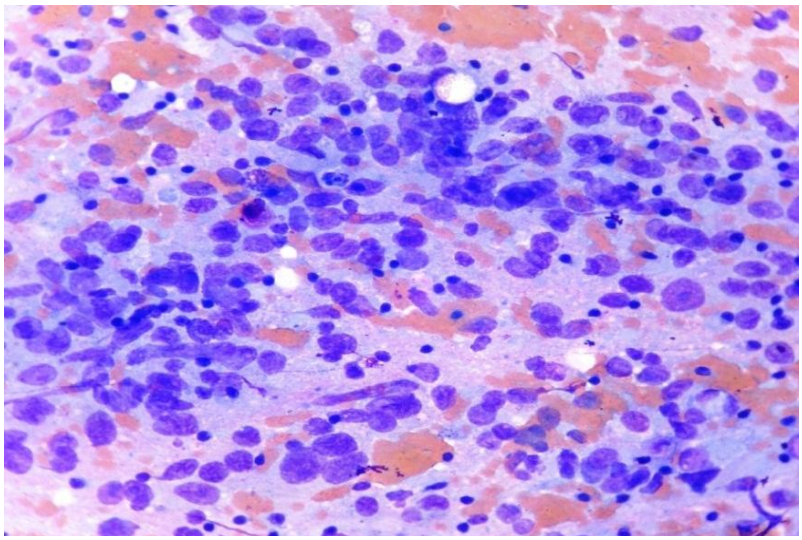

Fig. 5. A mediastinal neoplasm in a cat. Tumoral cells.
Outstanding anisocytosis. Free nuclei. Col. MGG, x400

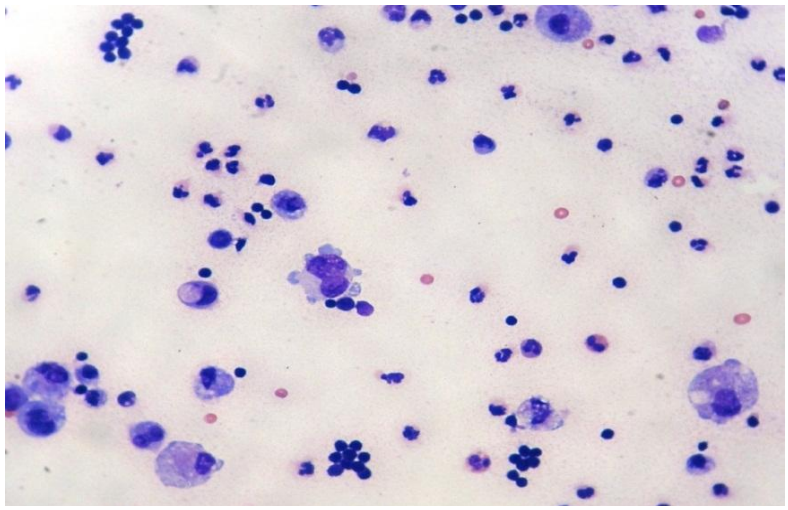

Fig. 6. A pleural neoplastic effusion in a cat. Foam-shaped active macrophages. Mature lymphocytes. Neutrophilic granullocytes. Col., MGG x 400

The material that was analysed for the immunocytochemical examination came from some lliquid inside a pleural effusion. The presumptive diagnosis consisted of a mediastinal thymoma, but following the immunomarking using the CKAE1/AE3 antibodies (made from the effusion) the result has proven to be negative because of the low number of cells for this type of neoplasm (the effusion was poor in cells) (9) (Fig. 7, Fig. 8).

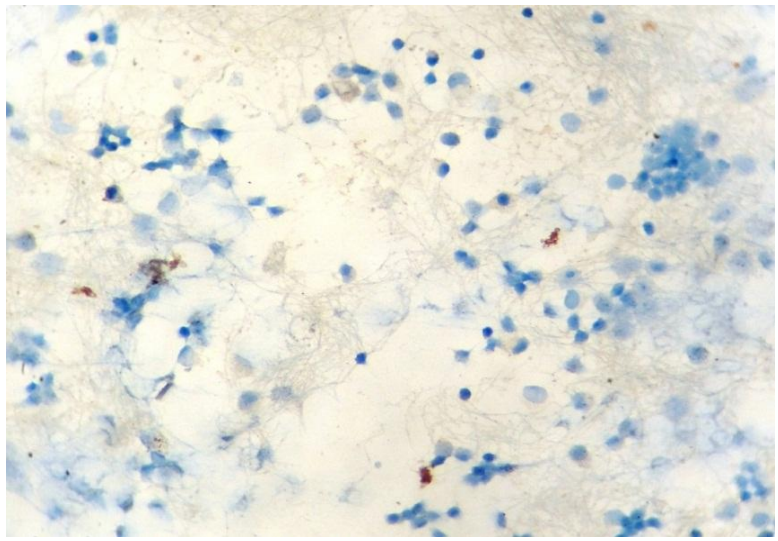

Fig. 7. A neoplastic pleural effusion in a cat. Immunomarking for the epithelial cells without the CKAE1/AE3 antibodies.
A negative control. The probe was poor in cells. Negative marking. (-), x400

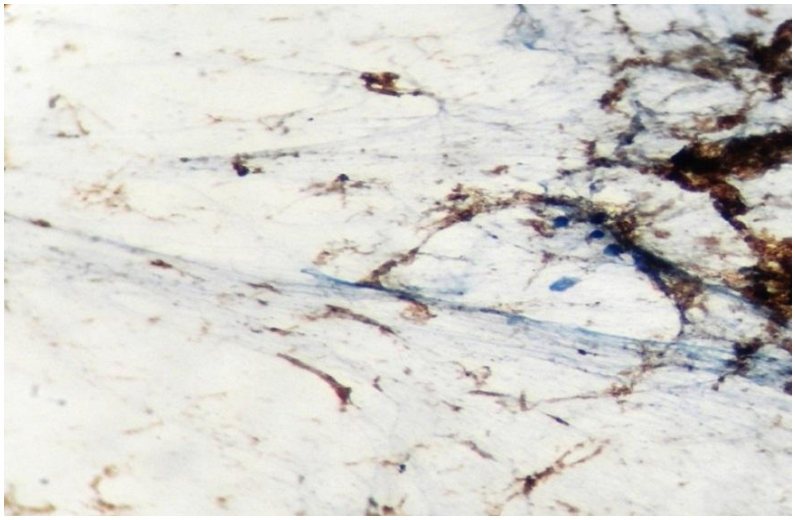

Fig. 8. A neoplastic pleural effusion in a cat. Negative immunomarking of epithelial cells for the CKAE1/AE3 antibodies. Decreased cellularity. Negative marking (-)

Conclusions

The cytological and immunocytochemical examination of the 3 draws from cavitary effusions evaluated in the course of this study have permitted the elaboration of the following main conclusions:

1. In the case of the effusions that were associated to neoplastic processes there were emphasised the malignancy criterias of tumoral cells: anisocytosis, anisocariosis, multiple nuclei-baring cells, large nucleoli and an intense vacuolisation of the cytoplasm.
2. In the case of the studied draws that were cytologically examined, there was not possible to identify the origin of tumoral cells because of the morphologic changes that they had suffered. As follows, the draws were examined using the complementary immunocytochemical examination.
3. Using the immunocytochemical method, the malignant tumoral cells have been identified in a single case from the three that were evaluated (the first case with the pleural carcinoma).
4. In two of the cases, the immunocytomarking was negative because the specific antibodies were blocked by the intensely proteic background and the small number of cells from the draw, therefore the diagnosis consisted of suspicioned malignancy.
5. The immunocytochemical marker with a high sensitivity in noting the differences between effusions associated to a mesothelioma and a carcinoma has proven to be Citokeratin A1/AE3.
6. Combining the routine cytopathologic evaluation with an immunocytochemical examination can lead to a specific correct diagnosis, that can be used for the final treatment (surgical treatment, a neohelping treatment or an aiming therapy) and

establishing a set of specific markers to cavitary effusions can improve the identification and the administration of aimed therapies.

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EFFECTS OF KEFIR ON PROTEIN METABOLISM OF LAYING HENS

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Abstract
Kefir is a mixture of bacteria and yeasts in a matrix of proteins, lipids, and carbohydrates. Kefir is obtained after fermentation of milk at ambient temperatures for overnight and fermentation of the lactose yields a sour, carbonated, slightly alcoholic beverage (1). Kefir contains beneficial bacteria help the digestive system run smoothly by improving digestion and further facilitates the absorption of the nutrients. Moreover, kefir contains +many proteins-amino acids essential for metabolism (2). Hens can not digest nutrients effectively especially proteins, so we aimed in this study to investigate the different levels of kefir in drinking water on the protein metabolism of the laying hens.
Keywords: Kefir, protein metabolism, laying hens

Materials and methods

Analysis of Kefir: For culturing the kefir samples taken, Sabouraud's dextrose agar and enriched culture media were used. Following the incubation period for 18-72h, both gram (-) and lactophenol cotton blue staining methods were employed. For microscopic evaluations, microorganism identifications of the samples were then made using the conventional methods.

Animals: A total of 144 Lohmann Brown layers, aged 24 wks, were allocated randomly into four trial groups, as; Groups C (control, n=36): no treatment, L (low, n=36): 5 cc, M (medium, n=36): 7.5 cc, and H (high, n=36): 10 cc kefir *per* lt of water. Animals were fed for 11 weeks with basal diets complying with the NRC recommendations. Samples were taken for three times: At start, 5th week and end of the experiment.

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of serum, egg-yolk and egg-white proteins were conducted according to Laemmli (5). Proteins were visualized by coomassie brillant blue R-250 and evaluated by Phoretix 1D (TL120) software and expressed as "pixel" for egg-yolk and egg-white.

Statistic analysis

Differences between groups were analysed by one-way analysis of variance (ANOVA), using the statistical package SPSS for MacOsX, version 20.0 with the Duncan multiple comparison test.

Results

1. There were 11, 23 and 24 proteins were detected in egg-white, serum and egg-yolk respectively. The total protein concentration of serum was increased in the H group during the experiment and at the end of the 11st week, significantly higher than the other groups ($p<0,001$).
2. Egg-yolk total protein concentrations were increased in the all kefir groups first 5 weeks, but then decreased during the last period. On the other hand egg-white total protein concentrations were decreased in all kefir groups dose dependent manner.

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HISTOLOGICAL STUDIES ON THE VOMERONASAL ORGAN OF THE EGYPTIAN BUFFALOES *(BOS BUBALIS)*

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Abstract
Vomeronasal tissues from eight mature male buffaloes, aged from two to four years, were prepared for light and scanning electron microscopy,. The vomeronasal organ of the Egyptian buffaloes was a paired tubular structure and had a considerable length, between 15 and 17 cm. The rostral part of the organ was lined by psudostratified epithelium ciliated with glandular area under it. An occasional goblet cell can be seen in this area; the cytoplasm and ciliated border of these cells were strongly PAS-positive. There was loose arrangement of glandular tubules in a rather closely woven fibrous to very dense fibrous tissue, which formed from collagen and elastic fiber in which were embedded numerous glands which serous or mucous either mixed type which gave positive reaction to the PAS, these glands may extend to the cartilage. The glands open in the surface by pores. Several arteries and veins had relatively muscular walls. There was a network of small blood vessels very close to the basal lamina, sometimes so close that the vessels indent the lamina and push well up to the level of the basal cells. Lamina propria submucosa was characterized by blood vessels especially veins which were large and thinner ones also blood capillary which gave erectile appearance these vessels were irregularly distributed around vomeronasal duct.

Key words: Egyptian Buffaloes, Vomeronasal organ, Light and Scan Electron Microscope.

Introduction

In the majority of mammals, odours are detected in two ways: by means of the main and the accessory olfactory systems. The previous of the two types commonly called the vomeronasal system share a common pattern of organization, despite significant morphological and physiological differences, including the size of the structures of which they are formed (Halpern, 1987 and Brennan, 2001). In microsmatic mammals including man, it reaches maximal development during foetal life and little remains in the adult.

In the domestic species, including horses, sheep, cattle, pigs and dogs, it remains an apparently functional organ of some size in the adult (Kratzing, 1971a). Around the vomeronasal duct there is a considerable amount of glands, vessels, nerves and connective tissue, which organize the soft tissue of the vomeronasal organ (VNO).

Additionally, a thick capsule wraps all these structures (Vaccarezza, Sepich, and Tramezzani, 1981 and Keverne, 1999). The vomeronasal organ (VNO) is a chemosensory structure that has morphological indications of functionality and New World primates examined to date. In these species, it is thought to mediate certain socio-sexual behaviors.

The functionality and even existence of the VNO in Old World primates has been debated. Most modern texts state that the VNO is absent in Old World monkeys, apes, and humans. A recent study on the VNO in the chimpanzee (Smith et al., 2001) challenged this notion, demonstrating the need for further comparative studies of primates.

Many researchers described the vomeronasal organ in different species, bovine (Adams, 1986); in pigs, cows and horses. (Salazar, et. Al 1997); in porcine (Ishida et al 2008); in rabbits (Gaafar 1998) and in rat (Garrosa, 1992).

Material and methods

Specimens were taken for light and scan electron microscopy from 8 male (5 for light microscope and 3 for Scan electron microscope) Egyptian buffaloes (Bos bubalis), aged between two to four years. These specimens were obtaining from Benha and Toukh slaughter houses, Egypt. Immediately after slaughter the vomeronasal organ was located by sectioning the head transversely at the level of the first cheek tooth.

For light microscopic study specimen with 2 or 3 mm in thickness were taking and put in 10% buffered neutral formaldehyde solution, then dehydrated and embedded in paraffin. Sections of 5-6 µm in thickness were obtained and stained with Hematoxylin and eosin (H&E) for general histological observations, Crossmon's trichrome (Crossmon, 1937) for collagen fibers and muscle and Verhoffe's stain for elastic fibers (Bancroft and Stevens, 1996).

For scanning electron microscopy, small specimen dissected into smaller blocks, dehydrated, critical point dried, further dissected if necessary, mounted, sputter coated with gold and viewed on a JEOL JSM 5500 LV SEM. The scanning electron microscopy was done at the Faculty of Agriculture, Alazhar University. The length of the vomeronasal organ was measured using SemAfore software.

Result

The vomeronasal organ of the Egyptian buffaloes is a paired tubular structure attached to the vomer bone and the floor of the nasal cavity, and is entirely covered by the corresponding mucosa (Fig. 1). The vomeronasal organ has a considerable length, between 15 and 17 cm, and formed from several layers (Fig. 2). The rostral part of the organ is lined by psudostratified epithelium ciliated with glandular area under it. The epithelium over this glandular area is consists of three types of cells forming two layers: a row of basal cells, then a more superficial zone consisting of ciliated cells (Fig. 3&4) with well-rounded apices which may be supporting or secretory in nature (Fig. 5 and 6).

An occasional goblet cell can be seen in this area; the cytoplasm and ciliated border of these cells are strongly PAS-positive (Fig. 7). The epithelium is usually three to four nuclei in depth over the glandular area (Fig. 8), but some folding and variation in height occur. There was loose arrangement of glandular tubules in a rather closely woven fibrous (Fig. 9) to very dense fibrous tissue, which formed from collagen and elastic fiber in which are embedded numerous glands, (Fig. 10) which serous or mucous either mixed type which gave positive reaction to the PAS (Fig. 11), these glands may extend to the cartilage (Fig 12). The glands open in the surface by pores (Fig. 13).

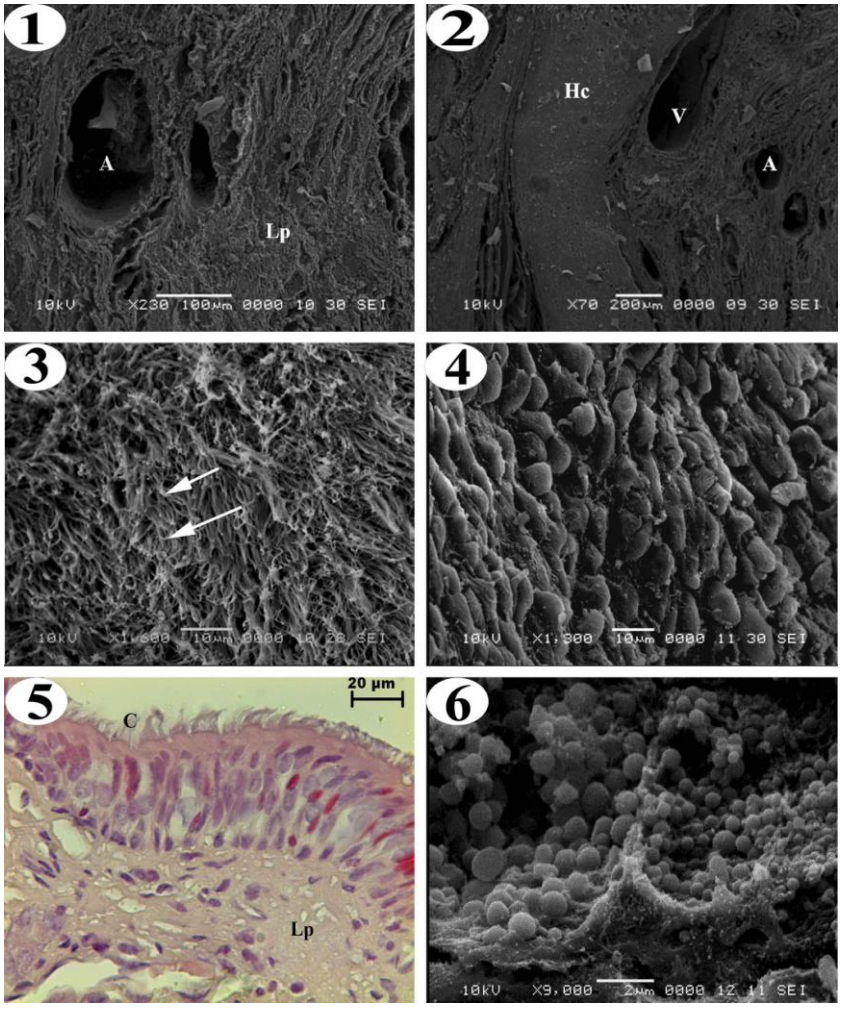
Several arteries and veins have relatively muscular walls (Fig. 14). The epithelium of caudal portion of the organ is a row of basal cells with dark, rather irregularly shaped nuclei, and cytoplasm which is strongly eosinophilic. The next zone consists of larger, rounded cells with large pale nuclei. These cells show great variation in staining in different parts of the section; some are heavily stained, while in other areas both nucleus and cytoplasm are only lightly stained (Fig 15).

Above these, there is a superficial zone of tall, columnar cells with long, ovoid nuclei and apical cytoplasm ending in a well-marked terminal bar and ciliated border. There are some area have no glands, but in several places the epithelium dips down into a crypt-like formation lined with mucus-producing cells which stain a bright magenta with PAS technique (Fig. 16).

In medial wall of vomeronasal organ was lined by olfactory epithelium or the sensory epithelium was showing the sustenticular cells, the receptor cells, their process and the basal cells (Fig. 17). The basal lamina of the epithelium is not very well defined, even with PAS technique. Below it, very fine bundles of nerve fibres belonging to the vomeronasal nerve (Fig. 7).

Larger bundles of these unmyelinated fibers can be seen deeper in the propria, close to the underlying cartilage. There is a network of small blood vessels very close to the basal lamina, sometimes so close that the vessels indent the lamina and push well up to the level of the basal cells. Lamina propria sub mucosa was characterized by blood vessels especially veins which are large and thinner ones also blood capillary which gave erectile appearance (Fig 18) these vessels are irregularly distributed around vomeronasal duct.

Small arteries are usually seen near to the cartilage. The vomeronasal organ is completely surrounded by a vomeronasal cartilage. This cartilage is hyaline type (Fig. 19) and is incomplete dorso-laterally.



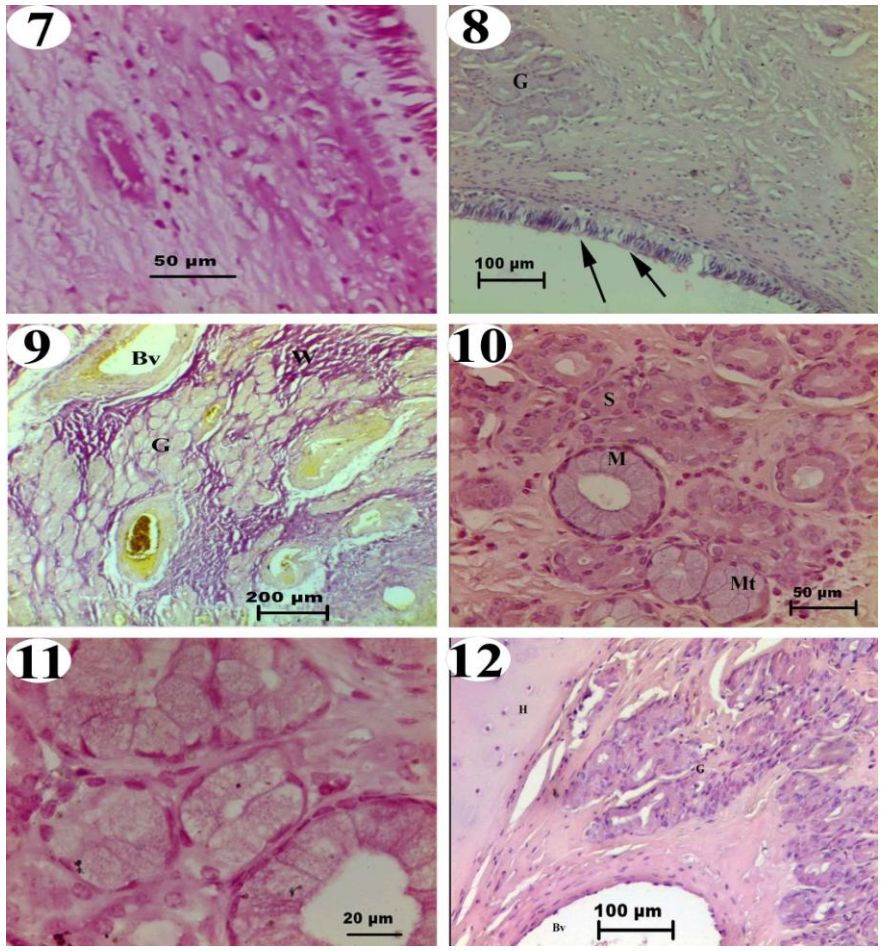


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Fig (3): Scanning electron micrograph showing the cilia of the surface epithelium lining of vomeronasal organ in ox (arrows). Scale bar 10 µm.

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Fig (5): Photomicrograph of the lateral wall of the vomeronasal organ of ox with light microscope stained with H&E stain. Showing the psudostratified epithelium with pigment cell in between, cilia (C), cellular lamina propria (Lp) and ill distinct basal lamina. Scale bar 20 µm.

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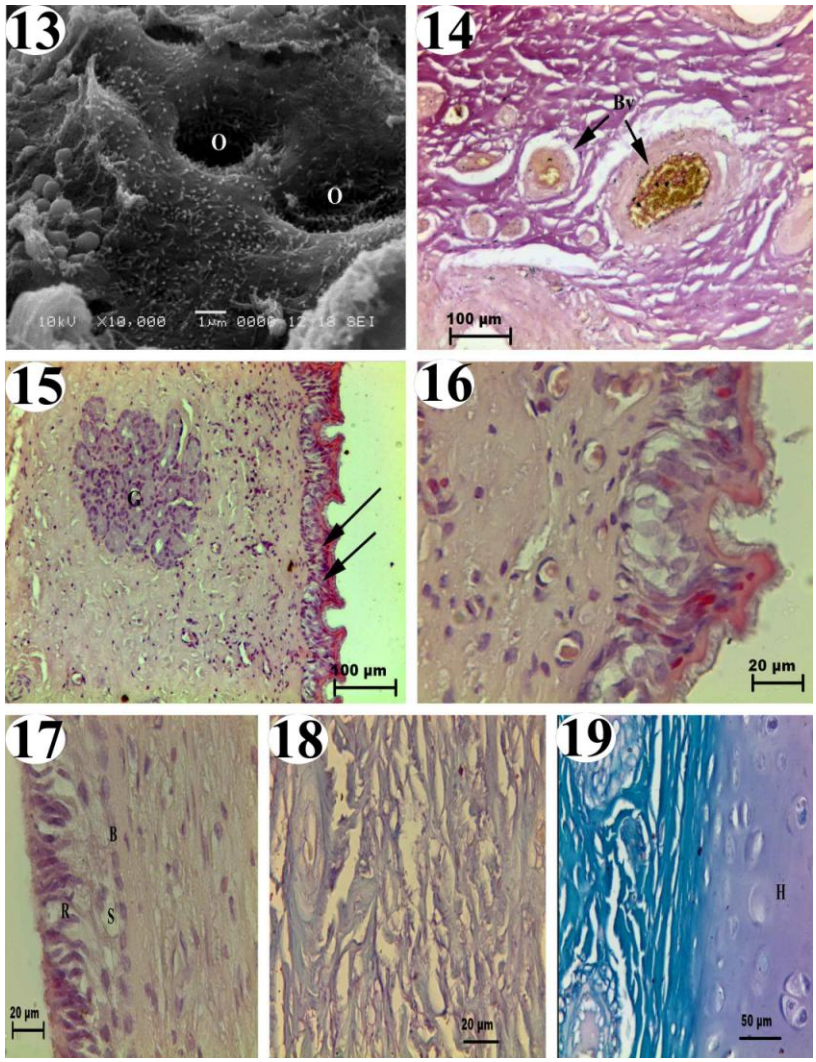

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Discussion

Although there are several interesting publications concerning the anatomy of the vomeronasal organ in cows (Pearlman, 1934; Taniguchi & Mikami, 1985; Adams, 1986; Salazar et al. 1997), the morphological and functional characteristics of the vomeronasal vessels are not considered in depth. Nevertheless, the general idea is that the mentioned vessels seem to organize a sort of erectile tissue by a flow combination of the small but powerful arteries and the numerous veins. This is in contrast with the present study.

The pattern of distribution of the vessels which reach the vomeronasal organ is quite similar in cows to other mammals (Salazar et al. 1997; Jacobs, et al 1981 and Garrosa, et al 1998). Histological descriptions of the vomeronasal organ frequently show a uniform organization all along the tube: vomeronasal epithelium in the medial wall and respiratory-like epithelium in the lateral wall.

However, Schilling (1970) observed in *Microcebus murinus* that the rostral portion of the vomeronasal organ had only one type of epithelium around its lumen. Our study in the ox confirms the observations of Saksena and Chandra (1980) that, although the vomeronasal and psudostratified epithelia are present in most of the length of the tube, only the psudostratified epithelium can be found at its cranial end. The cell types described in the lateral wall of the vomeronasal organ of the ox are similar not only to those of the very Indian buffaloes (Saksena and Chandra 1980), but also to those of sheep (Salazar et al. 1998 and Booth and Katz 2000) and in rabbits (Taniguchi and Mochizuki 1983). In the ox, as in other animals, only a few secretory cells are found in this epithelium. Our observations agree with those of Taniguchi and Mochizuki (1983) regarding the nature of this ciliated epithelium, which seems to be a modification of that of the vomeronasal glands.

The vomeronasal organ in sheep is composed by a pair of tubules closed caudally. The sensory epithelium is located on the medial surface and the non-sensory and the ciliary laterally. Receptor cells possess a big cellular body of a neurogenic type with proximal and

distal ends (Kratzing, 1971a). The vertical diameter of the vomeronasal organ lumen has a width of 1 cm in its central part (May, 1964). In goats, (Besoluk et al., 2001).

Its lumen is lined with the cartilage that builds the organ and, in this species; it is observed ventrally as well (Besoluk et al., 2001). In horses, the rostral end of the incisive canal is closed, unlike swine and cows. There is no communication between vomeronasal organ and the oral cavity. The rostral part of the canal is characterized with layered columnar epithelium (Dellman & Eurell, 1998).

At the same time, the bigger caudal part is built of simple columnar epithelium (Barone, 1997; Salazar et al., 1997). In cattle, the caudal part of vomeronasal organ ends at the level of the first or second premolars (Kumar et al., 1981 and Gulimova, 2002). The lumen of the ventral part of vomeronasal organ is not covered with the cartilage that constitutes the organ (Kjell & Trotier 1998 and Keverne 1999). This finding is much closed with the present study. The cells of the vomeronasal epithelium of the rat, particularly in their apical processes and the way they are arranged to form the epithelial border, show some differences from those described in other animals which may have important functional implications.

The bipolar cells of the rat and of the rabbit show a significant difference: the absence of cilia in the bipolar cells of the rat and their presence in the bipolar cells of the rabbit (Luckhaus, 1969 and Kratzing, 1971 b). The microvilli of the bipolar cells of the rat are slightly different from those of the supporting cells but this is not the case in either sheep or cats (Kratzing, 1971 a; Seiffert, 1971).

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ULTRASTRUCTURAL ASPECTS IN BACTERIAL INFECTIONS OF CYPRINIDS FROM A FISH FARM SITUATED ON JIJIA RIVER

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Abstract

Between 2010 and 2011, in cyprinid farm located in Iasi and on the Jijia River, we diagnosed several infections with bacterial strains, characteristic of mobile species of Aeromonas, developed as a result of the stress induced by biotic and abiotic factors. The samples were collected from 42 fish the following species: common carp (Cyprinus carpio) and bighead carp (Aristichtys nobilis), all of them showing red ulcers, circumscribed by whitish rings, fragmentation of their dorsal fin and hemorrhagic diathesis. Skin samples were taken from the affected areas, especially for the SEM examination with the purpose of evaluating not only aspects of normal morphology but also aspects of some modifications characterising the affected areas together with the presence of etiologically incriminated bacteria: Aeromonas spp. All the strains that were tested showed mobility and oxidase-positivity, but they reacted differently to the other tests. Consequently, they were taxonomically grouped into the following species: Aeromonas hydrophila, Aeromonas caviae and Aeromonas sobria. Scanning electron microscope (SEM) was used for the first time in characterization of the bacterial lesions produced by Aeromonas strains on Cyprinus carpio and Aristichtys nobilis skin. The diagnosis of septicemy with conditionately pathogen species of Aeromonas was correlated with the results of physico-chemical investigations of water and data concerning breeding conditions of the investigated livestock.

Key words: cyprinids, *Aeromonas,* SEM (Scanning Electron Microscopy), skin

Introduction

In the fish farms and in the water, especially, bacteria are important pathogens for wild and cultivated fish and are responsible for serious economic losses. Some bacteria cause only surface diseases as skin infections, but some inflict systemic disease. The prevalent fish diseases in fish farms are usually initiated by bacteria (Inglis et al. 2001).

There are primarily two different kinds of bacteria causing disease, namely obligate pathogens and facultative pathogens. The latter possess the ability to survive in water for an indefinite period of time and whenever environmental circumstances are conductive, infectious fish diseases are very likely to disperse. A significant number of potentially pathogenic bacteria of fish usually live commensally with the host or develop individually in the environment. Both types of bacteria become pathogenic as soon as any kind of stressor affects the immune system of fish (Kirjusina et al. 2007).

Fish bacterial infections can develop into bacteremia, which presupposes the occurrence of bacterial organisms in the bloodstream, with no clinical signs. Others may as well develop into septicemia, which indicates the presence of bacteria and toxins in the circulatory system, which can accelerate disease as well as the clinical signs. Gram-negative bacteria are responsible for the production of either exotoxins or endotoxins, which are made up of proteolytic enzymes that destroy host cells and can produce necrosis or can cause blood vessels porosity or hemorrhage (Kirjusina et al. 2007).

Aeromonas species are uniformly distributed and can be isolated from a large variety of environmental and water samples. *Aeromonas hydrophila* is considered the most plenteous pathogenic bacteria isolated from the environment (Briede, Medne 2004).

One of the main reasons why fish skin is considered an important defense system against pathogens is that the latter ones have regular contact with a wide range of microorganisms living in the aquatic environment. Fish skin is responsible for a series of highly developed antimicrobial defense mechanisms (Ellis, 1981). The part played by fish skin mucus in antibacterial defense has been known for a long period of time (Jones, 2001). Some of the most important components are, in this respect, the antimicrobial, bioactive substances which consist of lysozyme, complement, C-reactive protein, hemolysins and lectins and the epidermal migration of inflammatory cells and their secretion, which is utterly important in relation to fish immunity (Depountis et al. 2006, Lebedeva 1999). The mucus is responsible for preventing colonization by parasites, bacteria, and fungi.

A wide range of authors have investigated fish bacteriosis from a histological, microbiological, and taxonomical standpoint (Lemaitre 1996). The specialised literature focusing on the lesions determined by bacteria, in general, and *Aeromonas*, in particular, comprises only a few scanning electron microscopy investigations (Gostin et al., 2011). Martínez and collaborators (Martínez et al. 2004) used scanning electron microscopy to analyse the lesions determined by means of an experimental infection of *Flavobacterium psychrophilum* in fins of Atlantic salmon *Salmo salar*.

The purpose of the present paper is to provide the electronomicroscopic details of the lesions determined by bacteria and to emphasise the revelance of these investigations for the study of fish bacteriosis. Scanning electron microscopy was used for observing the lesions, with the purpose of investigating the invasion of bacteria at the level of the skin tissue.

Due to the considerable impact that aeromonosis can have on cultivated fish and sometimes on wild fish populations, one can conclude that more attention should be paid to studying the various aspects of the disease. The examination of wild fish populations will therefore be an integral part of future investigations.

Materials and methods

The investigations were carried out on 42 common carp *(Cyprinus carpio)* and bighead carp *(Aristichtys nobilis)* patients from Piscicola – Larga Jijia fish farm and free from the watercourse of the Jijia River, ranging in size from 25 to 65 cm. All the investigations included bacteriological and scanning electron microscopy (SEM) analyses.

After compiling a list of the external lesions of fish, several tests were performed using the Gram staining method in order to identify the bacteria at the level of the cutaneous ulcers.

Bacteriological examination was made on samples of skin lesions, kidney and blood.

The used media were TSA (trypticase soy agar) or BHIA (heart brain infusion agar) – a medium which favours the occurrence of the majority of pathogenic bacteria in fish.

The inoculated media were incubated at 25°C, for 24-48 hours, and the identification of the resulting cultures was based on testing the metabolic characteristics. Since, based on the lesional aspects of the direct microscopic exam and of the cultures, we suspected the etiologic involvement of *Aeromonas* bacteria, colonies from each plate were replicated for biochemical confirmation. Initially, the oxidase test was carried out, a test which differentiates the *Aeromonas sp.* from enterobacteriaceae, and then a series of tests aimed at

differentiating the species within their kind: the glucose fermentation test and the hydrogen sulphide test using the TSI (Triple Sugar Iron) medium, the aesculin hydrolysis, the arabinose and salicilin fermentation, the Voges-Proskauer reaction, the examination of mobility in a MIU (Mobility-Indole-Urea). The bacterial strains which had an atypical reaction to certain tests were tested again by means of the API 20 NE galleries. The antibiograms were carried out using the diffusimetric method.

Scanning electron microscopy (SEM) investigations: the investigated material consists of small skin pieces. The material was fixed in glutaraldehyde (2%) for 2 hours, osmium teraoxide (1%) for 4 hours and washed with phosphate buffer. After dehydration in a graded ethanol series (40%, 70%, 80%, 90%, and 100%) and acetone, the material was critical-point dried with CO_2 (using an EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (using an EMS 550X Sputter Coater) and, finally, examined by scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 27.88 V.

Simultaneously with investigations performed on fish, water parameters determinations were made on breeding basins: temperature, pH, nitrites, nitrates, ammonia, clorures, organic matter.

Results and discussions

Between 2010 and 2011, in summer and autumn, several cases of fish showing red ulcers circumscribed by whitish rings, fragmentation of their dorsal (Figure 1, Figure 2) and hemorrhagic diathesis, were registered (Figure 3). Affected species included the common carp and the bighead carp, one or two summers old, from the cyprinid fish farm located along the Jijia river.

Fig. 1. Carp erythrodermatitis. Red cutaneous ulcers surrounded by a whitish ring

Fig. 2. Carp erythrodermatitis. Carp with ulcers in their ventral and caudal areas

The direct bacterioscopic examination of cutaneous lesions highlighted the presence of gram-negative bacilli and coccobacilli (Figure 4). The bacteriologic examination was carried out on blood samples taken from the heart and the anterior kidney. After storing the samples in a thermostat, on both media, round, no pigment or cream colonies of 2-3 mm diameter developed within 24 h.

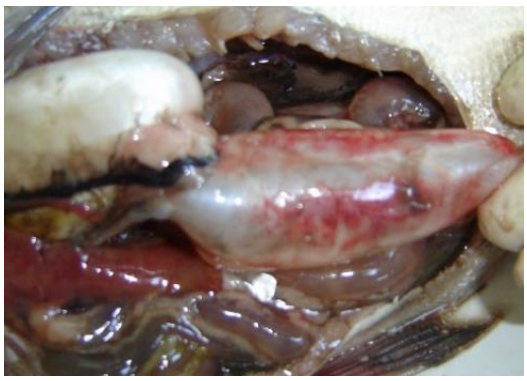

Fig. 3. Bighead carp - hemorrhagic diathesis

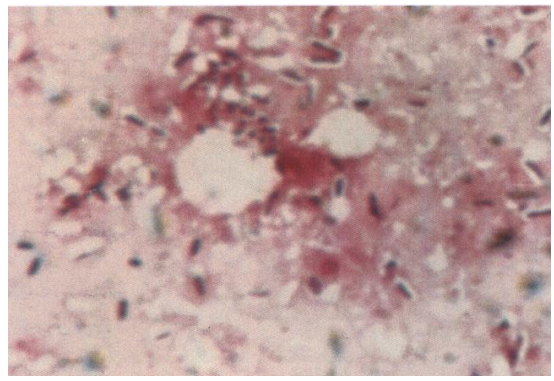

Fig. 4. Carp erythrodermatitis. Smear resulted from the cutaneous ulcers. Gram negative bacilli and coccobacilli

All the strains that were tested showed mobility and oxidase-positivity, but they reacted differently to the other tests. Consequently, using API 20 NE strips, they were taxonomically grouped into the following species: *Aeromonas hydrophila, Aeromonas caviae,* and *Aeromonas sobria* (Table 1).

As far as the examined carps were concerned, several mixed cultures of *Aeromonas species* were isolated from the cutaneous ulcers and from the kidneys and blood; *A. hydrophila* was isolated in pure cultures and in association with one of the two species or with both species.

Table 1. Biochemical characteristics of the *Aeromonas* isolates using API 20 NE

Test	***A. hydrophila*** **N=42**	***A. sobria*** **N=12**	***A. caviae*** **N=22**
Nitrat reduction	+	+	+
Indol production	+	+	+
Glucose acidification	+	+	+
Arginine dihydrolase	+	+	+
Urea hydrolysis	-	-	-
Esculin hydrolysis	+	-	+
Gelatin hydrolysis	+	+	+
Para-Nitro-Phenyl-(beta)D Galactopyranoside	+	+	+
Glucose assimilation	+	+	+
Arabinose - " -	-	-	+
Mannose	+	+	-
Mannitol	+	+	+
N.Acetyl-Glucosamine	+	+	+
Maltose	+	+	+

Gluconate	+	+	+
Caprate	+	+	+
Adipate	-	-	-
Malate	+	-	+
Citrate	+	D(+ 85%)	D(+ 70%)
Phenyl acetate	-	-	-
Cytochrome oxidase	+	+	+

N=Number of strains tested; **+** = 100% positive reactions; **-** = 100% negative reactions; D=different reactions

In the case of a few specimens of common carp and bighead carp that had cutaneous lesions and were diagnosed with erythrodermatitis, we also carried out an electronomicroscopic exam of the skin, by using a scanning electron microscope (SEM), in order to observe the processes occurring at this level.

The epithelial cells are endowed with a singular microridge pattern (Figure 5). These ornamentations are responsible for creating a pattern which is specific to each species (Bergsson et al. 2005).

Due to the essential part they play in retaining the mucous substances secreted at the surface of the skin (Bergsson et al. 2005, Hirvelä-Koski 2005), they are responsible for protecting the fish against bacterial, fungal, and parasite attacks. The upper cell layers are not constantly renewed. Nevertheless, they are individually replaced as soon as they die.

The pore enlargement of the mucus secreting cells can be identified the common carp and bighead carp, too (Figure 6).

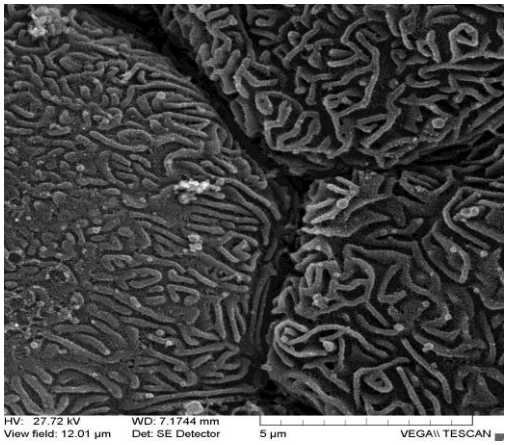

Fig. 5. Detail from epithelial cells of the normal skin in *Cyprinus carpio*

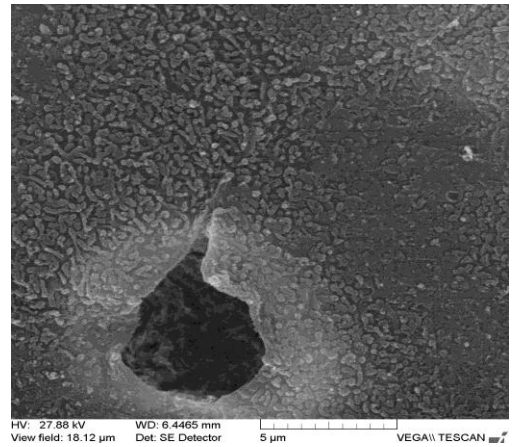

Fig. 6. SEM. Bighead carp normal skin, presenting the opening of mucus secreting cell

In the case of the samples that were taken from the areas affected by ulcerous lesions, we were able to identify the presence of erythrocytes, with a globular aspect. We were also able to identify the presence of certain bacteria whose shape was very similar to that of a

„rod" and which were confirmed to be representative of the *Aeromonas species,* following the microbiologic examination.

Round bodies, with irregular surface, with may have been lymphocytes were also seen near the lesion. In the same time, numerousness red blood cells included in a network of thin fibers fibrin (which is a type of protein) are visible in the lesion vicinity (Figure 7, Figure 8, Figure 9, Figure 10).

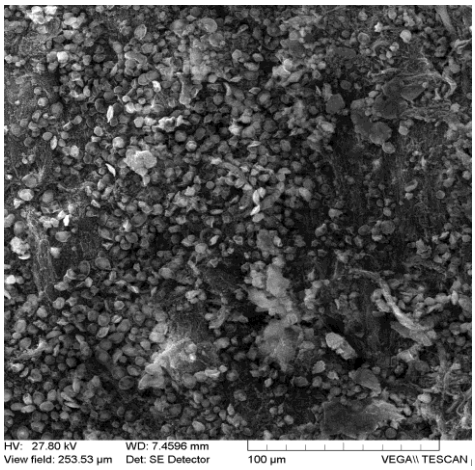

Fig. 7. SEM. Red blood cells congestion within the injured area

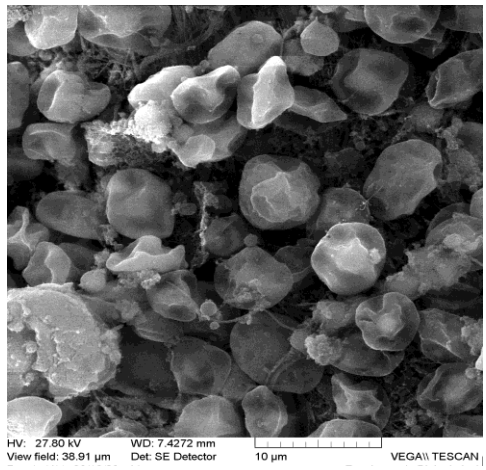

Fig. 8. SEM. Globular aspect of red blood cells

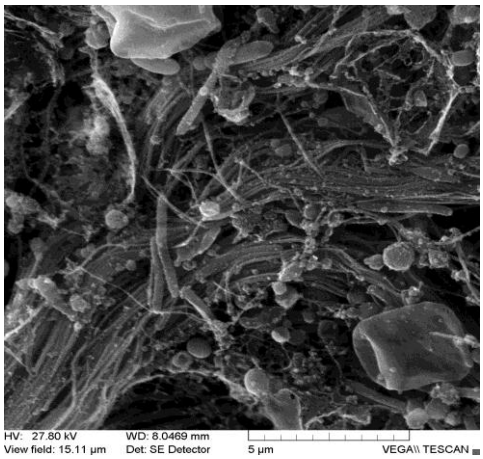

Fig. 9. SEM. Red blood cells and *Aeromonas sp.* present among the muscular fibres.

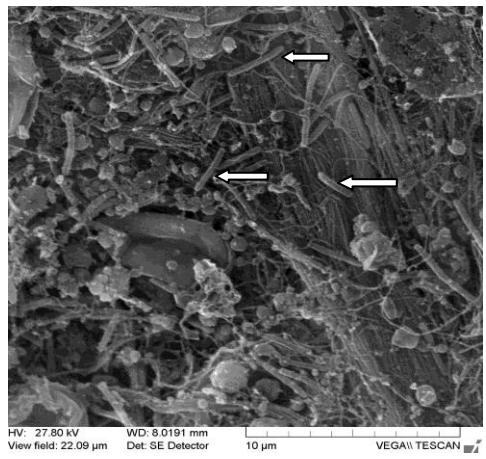

Fig. 10. SEM. Red blood cells and groups of cells similar to rod-shaped species of *Aeromonas* (arrow).

Aeromonas preferentially invades previously damaged tissue, typically an area of erosion. The integrity of the skin plays an important role in protection against bacterial

infections. Bacteria may be destroyed by antimicrobial products (lysozymes, agglutinins) present in the mucus (Fishelson 1998).

In the areas where the skin was injured, we were able to identify the presence of the *Aeromonas species* of bacteria, along the lines of fine fibrillar strands, possibly fibrin, as well as the presence of red blood cells in the injured areas. (Figure 11, Figure 12)

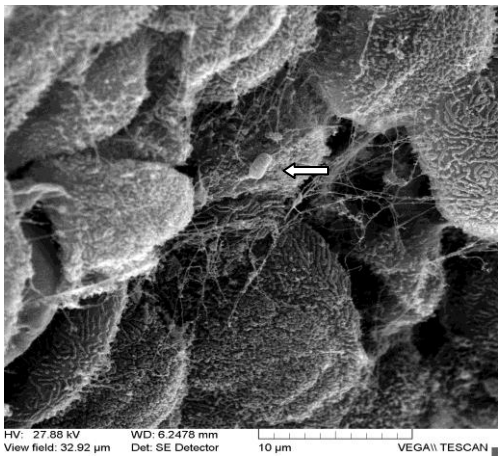

Fig. 11. SEM. Fine fibrillar strands. Aeromonas species of bacteria (arrow)

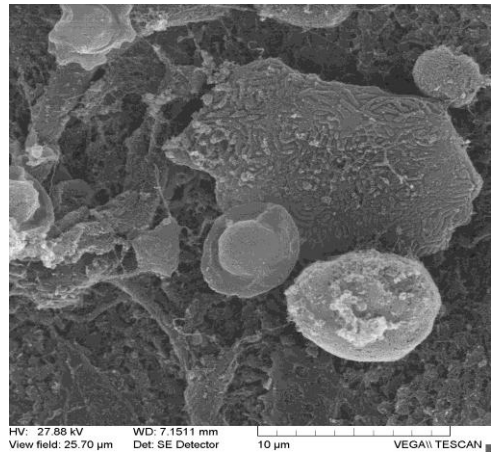

Fig. 12. SEM. Detached epithelial cells in the vicinity of one lesion, notice presence of blood cells

By corroborating the anatomoclinical aspects and the bacteriologic test results, we were able to diagnose the examined specimens of fish with erythrodermatitis and infectious septicaemia with *Aeromonas sp*.

Antibiograms revealed an antibioresistance of the "multi-step" type, 20% of the examined strains being resistant to more than 4 antibiotics.

All the strains were sensitive to neomycin, gentamycin, chloramphenicol, and ciprofloxacyn and showed resistance to ampicillin. The majority of the strains (75%) showed sensitivity to the amoxicillin-clavulanic acid association.

Results of determinations performed on water samples lead to the conclusion that diagnosed aeromonosis appeared due to the imunosupression induced by water temperature (10-15^0 C), below ciprinids thermic confort (22 -25^0 C). Chemical parameters were within normal values (Table 2).

Table 2. Chemical parameters of river water fish growth

Parameters	**Recorded value**
pH	7,02
Nitrites (mg/l)	Absent
Nitrates (mg/l)	Absent
Ammonia (mg/l)	Absent
Chlorures (mg/l)	215
Organic matter (mg/l)	5,6

Conclusions

1. The main purpose of the present paper is that of analysing the contribution of scanning electron microscopy investigations to gaining a better insight into fish bacteriosis, with a special focus on *Aeromonas* infections on *Cyprinus carpio* and *Aristichtys nobilis* skin.
2. The bacteriologic examinations used the API tests and diagnosed the following species: *A. hydrophila, A. caviae, and A. sobria.* The strains of the *Aeromonas species* showed resistance to ampicillin and were sensitive to the amoxicillin-clavulanic acid association.
3. In the areas where the skin was injured, the electronomicroscopic examinations indicated the presence of the *Aeromonas species* of bacteria, as a result of some fibrin deposits, as well as the presence of red blood cells in the injured areas.
4. Fish skin plays an essential part in the defense against bacterial infections. The proliferation of bacteria occurs mainly after the exfoliation of the epithelial cells. On intact skin or in the areas surrounding the lesions only rare bacterial cells were observed. The essential part played by the mucus in antibacterial defense was herein emphasised. The loss of integrity of the epithelium, specific to this disease, is also a gateway for other bacterial or parasitic pathogens.

Acknowledgements

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HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES OF THE SPERMATOGENESIS IN SEXUALLY MATURE NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

The morphology fish gonads at the gross anatomical or histological levels has long been studied by biologists to identify annual reproductive cycles, length of the breeding season, onset of reproductive maturity, spawning rhythms, fecundity, and the various other aspects of reproductive biology that can be applied to fish gonadal development and function. The study summarized here was designed to elucidate the histology and the fine structures of the testes in the mature Nile tilapia. Tilapia is the common name applied to three genera of the family Cichlidae: (Sarotherodon, Oreochromis and Tilapia) including about 70 species. Nile tilapia, Oreochromis niloticus, is the main fish of the genus Oreochromis with high potential for aquaculture in Egypt and around the world. This study focused on Oreochromis niloticus. A total number of 40 sexually mature male fish were used in this study. Fish were reared normally under standard feeding and management regimes at the department of Fish Management and Diseases of the Faculty of Veterinary Medicine, Benha University, Egypt, during the period from May to December, 2011. The testes are described as cylindrical organs which extend longitudinally along the dorsal region of the abdominal cavity, just below the swim bladder, and are attached to the dorsal wall through the mesorchium. They are wrapped by the squamous epithelium of the visceral peritoneum followed by a thin fibrous capsule, the tunica albuginea. Fish were sacrificed and the middle portions of the gonads were removed and processed for light and electron microscopy investigations. The spermatogenic cells include primary spermatogonium, which was singly distributed along the seminiferous lobule and surrounded by the processes of Sertoli cells. The secondary spermatogonia were enclosed in a cyst formed by the processes of Sertoli cells. The primary spermatocytes were a clone of isogenic cells interconnected through cytoplasmic bridges, while the secondary spermatocytes were rarely observed. On the other hand, spermatids were characterized by small condensed nuclei, and were transformed finally into spermatozoa. The fine structure of each of the cell types mentioned above was described and discussed. The interstitial compartment which was made up of the interstitial Leydig cells, myoid cells, granulocytes, blood vessels and nerves, had been histologically and ultrastructurally processed and studied using standard techniques.

Key words: Nile tilapia, mature male fish, testes, spermatogenic cells, spermatocyst, Sertoli, interstitial cells.

Introduction

According to the location of spermatogonia, the testes of the fish have been classified, into restricted and non-restricted types (Grier *et al.*, 1980). Another classification has also been adopted: lobular, tubular, or anastomosing tubular (Takashima, 1995). Grier and Lo Nostro (2000) concluded that as in all other vertebrates, a basement membrane separates the germinal compartment from the interstitial compartment of the testes. The germinal compartment contains sparmatogenic germ cells and Sertoli cells. The processes of Sertoli cells form the borders of spermatocyt-containing isogenic germ cells in Nile tilapia (Lo Nostro *et al*., 2003; and Abd El-Aziz, 1992). Schulz *et al.* (2005) concluded that the germ cells within the spermatocyt are linked cytoplasmically by intercellular bridges indicating synchronous differentiation in other teleost fish.

The spermatogenic cells include primary spermatogonia type A and B, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa, as had been identified by Dougbag, et al., (1988) in Nile tilapia, and by Nakaghi *et al.,* (2003) in *Colossoma macropomum*. Lo Nostro *et al.,* (2003) described type A and type B spermatogonia of *Synbranchus marmoratus* as having a single nucleolus and grouped mitochondria associated with dense bodies or nuage. (Billard, 1984) reported in *Poecilia reticulate* a reduction in the nuclear size and an increase in nuclear chromatin density of type B spermatogonia compared to that of type A, which are individually surrounded by Sertoli cells. This is contrast to the to the observations extended by Van den Hurk *et al.,* (1982) in *S. gairdnieri*, and Billard (1984), who reported the presence in adult *Poecilia reticulate* of annulated lamellae only in spermatogonia. On the other hand, Meijide, *et al.,* (2005) have detected the above mentioned organelles in both spermatogonia and pachytene spermatocytes of *C. dimerus*. Interestingly, it was observed that when spermatogonia turned into primary spermatocytes in rosy barb fish (Puntius conchonius), they decreased in size and lost their nucleoli (Çek (2006). Though somewhat smaller and more basophilic, secondary spermatocytes were generally similar to the primary spermatocytes cytological appearance. On the other hand, secondary spermatocytes undergo rapid development to become spermatids (Lo Nostro *et al*., 2003); these spermatids were smaller in size, irregular in shape, and stained strongly basophilic than the secondary spermatocytes. It is generally agreed that most externally fertilizing teleosts have a simple type of spermatozoon, called aquasperm (Jamieson, 1991). The aquasperm has a round or ovoid head and a short neck region (middle piece) containing a few mitochondria. Jamieson, (1991) and Mattei (1991) have indicated that biflagellated sperms were found only in a few species.

In addition to their supportive and protective functions, Sertoli cells in the seminiferous lobules, phagocytize residual bodies of spermatids, and degenerating cells (Chung, 2008). Interstitial endocrine cells (Leydig cells) presence in the testis appears to be typical of teleosts, as in the vertebrate lineage (Nagahama, 1983). Theses steroidogenic interstitial endocrine cells of the fish have three morphological characteristics that include a round nucleus, numerous mitochondria with tubular cristae, and extensive smooth endoplasmic reticulum (Pudney, 1996; and Lo Nostro e*t al*., 2004). On the other hand, a vesicular nucleus has been observed in the steroid-secreting cells of teleosts (Jun, *et al.,* 2006; Chung, 2008). The interstitial tissue contains fibroblasts, collagen fibers, myoid cells, and blood cells, together with Leydig cells (Reinboth and Bruslé-Sicard, 1997; Cinquetti and Dramis, 2003). Myoid cells have the same ultrastructural features, including irregularly-shaped, elongated nucleus with numerous indentations, and clumps of heterochromatin, microfilaments that run parallel to the long axis of the cell as the peritubular or lobule boundary cells, have already been characterized in a pike fish (Grier, *et al.,* 1989), carp (Timmermans, *et al.,* 1993) and in *O. mykiss* (Cauty and Loir, 1995). Regarding nerves and nerve bundles, Nakamura and Nagahama (1995), have documented, in tilapia testes, the presence of thick bundles of nerves penetrating into the testis via the mesorchium, and thin nerve bundles branch from these bundles and extend into the small spaces between the lobules.

Materials and methods

A total number of 40 Nile tilapia fish were used in this study. Below are the procedures used for light and electron microscopy.

I. Light microscopy

Fish were sacrificed and the middle portions of the gonads were removed and fixed in Bouin's fluid. Dehydration was carried out through ascending grades of ethanol. The samples were then cleared in xylene, embedded, and blocked in paraffin wax. Sections about 5-7 µ thick were cut using a rotary microtome, picked up and put on clean slides and stained with Hematoxylin and eosin (H&E) stain for general histological observations; Crossmon's trichrome, Van Gieson's stains were used for observations of collagen fibers and smooth muscles; and the Verhoffe's stain was used for identification of elastic fibers (Bancroft and Stevens, 1996).

II. Electron microscopy

Small pieces of tissue (1x1x1mm) were cut out from the gonads and immediately immersed in the 2.3% glutaraldehyde liquid in 2.14% sodium cacodylate buffer. After washing in sodium cacodylate buffer, the specimens were treated with osmium tetroxide. Dehydration was carried out in ascending grades of ethanol, and the samples were then treated with 2% uranyl acetate and 2% phospho-tungstic acid. The pieces of tissue samples were then embedded in (TABE) resin. Semi-thin sections (0.5-1 µ) were cut on Reichert ultramicrotome using glass knife, stained with toluidine blue, and examined using the light microscope. The desired regions for electron microscopy examination were then selected and ultra-thin sections (70-90nm) were cut. The sections were mounted on uncoated copper grids, double stained with uranyl acetate, and then immersed in lead citrate, washed, dried, and examined in Zeiss EM electron microscope (Bozzola and Russell, 1999).

Results

The Nile Tilapia testes were found to be cylindrical organs which extend longitudinally along the dorsal region of the abdominal cavity just below the swim bladder. The testes were wrapped by the squamous epithelium of the visceral peritoneum followed by a thin fibrous capsule, the tunica albuginea, which was formed of firm collagenous fibers (Fig. 1), in addition to a few elastic fibers. The testes were attached to the body cavity through the mesorchium.

The testicular parenchyma consisted of germinal compartment (the seminiferous lobules), which contained cysts exhibiting all the developmental phases of the spermatozoa, in addition to the presence of free spermatozoa in their lumina, especially in the region that lies towards the efferent ducts (Fig. 2). All stages of cyst development were not simultaneously present along one given tubule. As an example, as a cyst containing spermatogonia could be observed adherent to one cyst containing spermatids. On the other hand, the interstitial compartment was separated from the germinal compartment by a basement membrane, and filled all the intertubular spaces. During the reproductive cycle, the interstitial compartment occupied relatively a small volume compared to the germinal one (Fig. 1)

The seminiferous lobules in Tilapia were of the unrestricted type in which spermatogonia were found throughout the seminiferous lobules that oriented at right angles to the long axis of the testis. The spermatogonia were divided into primary spermatogonia and secondary spermatogonia.

The primary spermatogonia were the largest cells among the spermatogenic cells, and were characterized by their lightly staining cytoplasm, the oval or round shaped euchromatic nucleus with distinct or indistinct nucleolus, and the abundance of round to oval perinuclear

mitochondria, commonly associated with nuage. Free ribosomes, annulated lamellae formed by one cistern were also seen closer to the nuage. However, Golgi complex was rarely observed in this study. The primary spermatogonia were rested directly upon the basal lamina and located between the spermatocysts. The cytoplasmic processes of Sertoli cells surround the adjacent spermatogenic cells (Fig. 3).

Primary spermatogonia underwent several mitotic divisions with incomplete cytokinesis giving rise to secondary spermatogonia, which were interconnected through cytoplasmic bridges and resembled primary spermatogonia, although smaller in size and grouped in spermatocyst containing 2-4 cells (Fig. 4). The cytoplasmic bridges often presented a thickened plasma membrane. The secondary spermatogonium also possessed round to oval euchromatic central nucleus and relatively fewer mitochondria, and sparse nuage and SER.

When meiotic division occurred in primary spermatocytes, they gave rise to daughter cells called secondary spermatocytes with haploid number of chromosomes (1n). The secondary spermatocytes were smaller in size and possessing spherical, decondensed nuclei frequently seen with cell division's morphological changes, and had relatively high nuclei-cytoplasmic ratio (Fig. 5). The cell outlines were not clear in histological preparations; however, they were very obvious at the E.M. level (Fig. 6). The cells developed intercellular bridges which showed thickening in the plasma membrane. Scanty, smaller, oval to rounded mitochondria with electron dense matrix were commonly aggregated at one side of the cell. They were occasionally associated with nuage. Synaptoneamal complexes are evident in the nucleus and anchored to the nuclear envelope during the zygotene stage of the first meiotic prophase (Fig. 7).

Secondary spermatocytes were observed in large nests extending to the lobular lumen and surrounded by the cytoplasmic extensions of Sertoli cell. They resembled primary spermatocytes, although they were smaller and more basophilic (Fig. 5). They were characterized by spherical voluminous nucleus with dense chromatin, fewer organelles and indistinct cell limits. R.E.R was observed around the nucleus, centrioles were clearly evident (Fig. 8), and mitochondria that started to multiply and became larger.

The smallest spermatogenic cell types were spermatids which showed marked reduction in the cellular and nuclear size and became strongly basophilic (Fig. 5). As a result of the decreasing cells size, the cyst presented large intercellular spaces. Spermatids possessed round nucleus with condense irregular chromatin granules and organelles similar to secondary spermatocytes (Fig. 9). With progressing development of spermatids, the dense chromatin granules increased in number and nearly filled all the nucleus and the centrioles formed the basal body from which the flagellum arise.

With the progress of their development, spermatids undergo spermiogenesis, a process during which important morphological changes take place transforming the spermatid into testicular spermatozoa. The process of spermiogenesis, include increased condensation of the nuclear chromatin, formation of the nuclear fossa in front of the centriolar complex, the formation of the cytoplasmic canal around the developing flagellum and the organization of the mitochondria in the middle piece (Fig. 10). The sperm tail or the flagellum was involved in the cytoplasmic canal and consisted of nine peripheral and two central microtubules. It was surrounded by mitochondria in its upper piece and develops two lateral fins at its distal end (Fig. 11). Spermatozoa cysts were also surrounded by the cytoplasmic processes of Sertoli cells. As the spermiogenesis, is completed, the cyst wall will be broken down leading to the

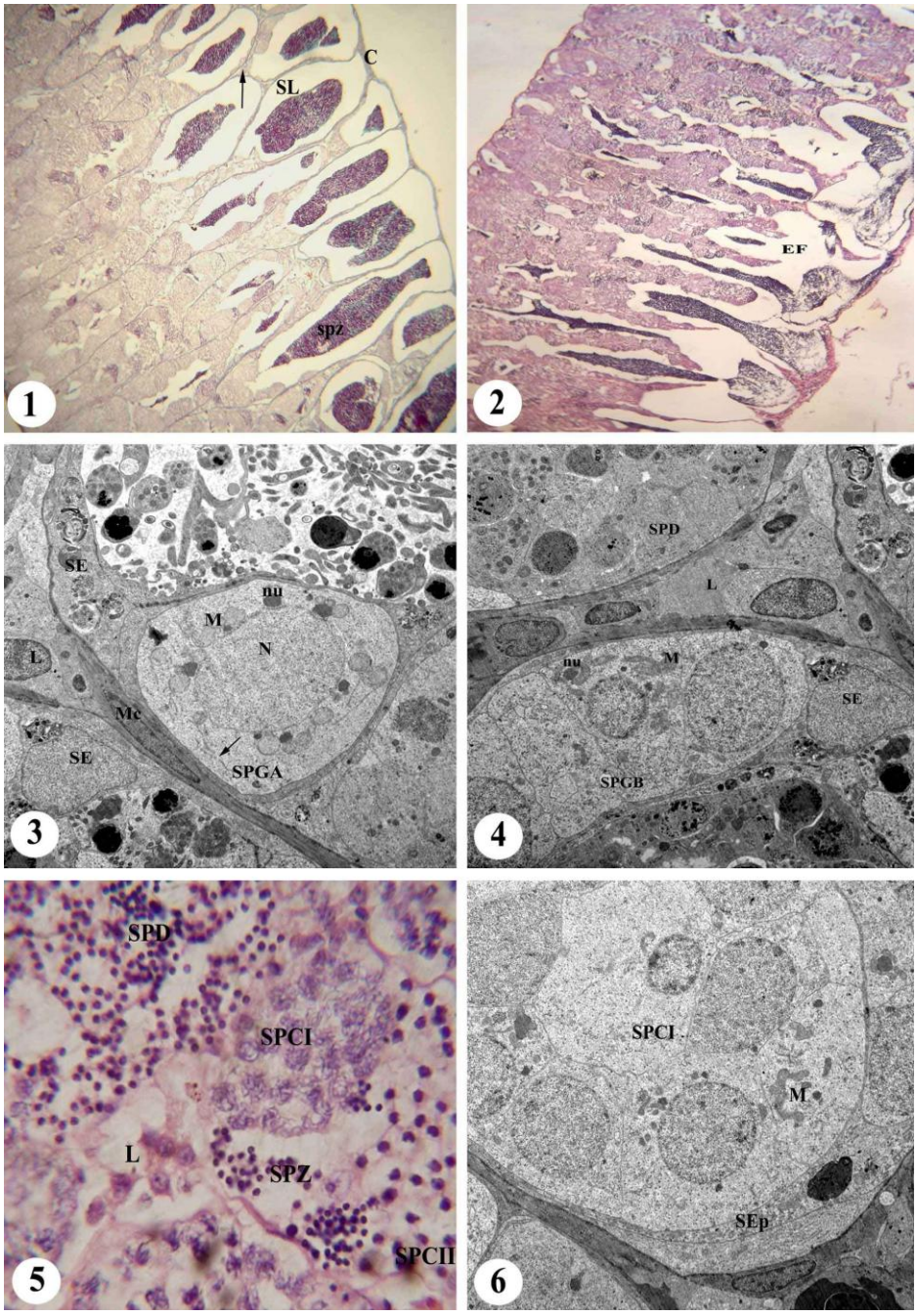
release of SPZ into the lobular lumen during spermiation. With increasing number of free spermatozoa in the lumen, they were transferred into the efferent ductules.

In sexually mature testes, the number of primary spermatogonia and secondary spermatogonia were very few, and the spermatocysts predominated. The lobular walls were distended, most of the spermatocysts were disappeared and only Sertoli cells remained lining the lobular wall and the lumina was engorged with spermatozoa (Fig. 12).

Sertoli cells were the second cell type that was present in the seminiferous lobules. Their broad base rested on the basal lamina giving rises to long processes that surrounded the spermatogonia and the different types of the spermatocysts, thus separating them from the basement membrane. These processes were joined by tight junctions and desmosomes (Fig. 13). The shape of the Sertoli cells nuclei varied from triangular, round, oval or polymorphic shape. Their cytoplasm possessed fewer organelles mainly mitochondria and was rich in intermediate filaments, polyribosomes, lysosomes, glycogen and few lipid droplets. The most characteristic feature of Sertoli cells was the presence of phagocytized material in their cytoplasm that included spermatids and their cytoplasmic material (Fig. 13).

The interstitial compartment of the testes was made up of fine collagenous connective tissue, and few reticular and elastic fibers concentrated mainly near the efferent ducts (Fig. 14). Blood vessels, nerves, myoid and Leydig cells also constituted part of the interstitium. Myoid cells bordered the seminiferous lobule and lied just beneath the lobular basement membrane. They were spindle shaped cells with flat elongated heterochromatic nucleus and scarce organelles (Figs. 3). Their cytoplasm was rich in myofilaments and was surrounded by collagen fibrils. Leydig cells were the second predominant cell type of the testicular interstitial cells compartment, the interstitium. They were frequently localized near blood vessels, and in the angular interstices between the seminiferous lobules (Fig. 5). These cells were also found in the interstitium between the efferent ducts. They were present either singly or in the form of small group aggregates. Their number and cellular organization exhibited cyclical changes according to the maturational stage of the male. They possessed polygonal shape with ill-defined cell boundaries, large ovoid to round nucleus with a well-developed eccentric nucleolus (Fig. 15), as well as numerous spherical or oval mitochondria, lipids and vesicular SER. Granulocytes (Fig. 16), and macrophages with their characteristic irregular shape, due to their pseudopodia, were frequently observed in the interstices near blood vessels.

The seminiferous lobules converged towards the central longitudinal axis of the testes forming the efferent ducts, which were dilated channels filled with spermatozoa and lined by squamous to low cuboidal cells (Fig. 2). Bundles of unmyelinated nerve fibers and strands of smooth muscle fibers in addition to numerous aggregates of Leydig cells were observed at the vicinity of the efferent ducts (Figs. 17&18).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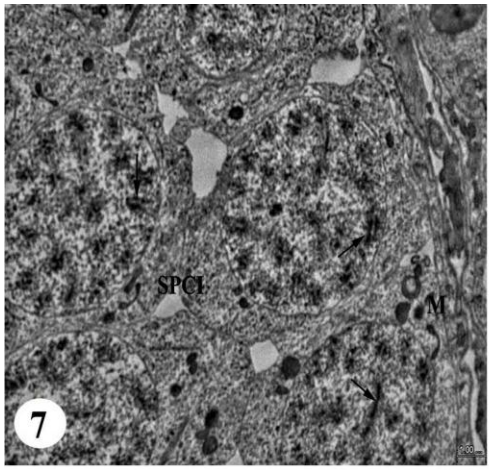
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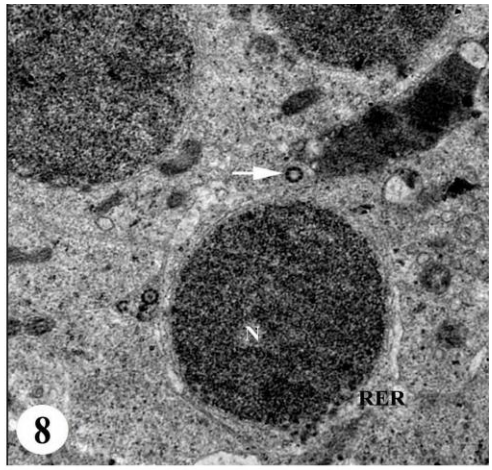
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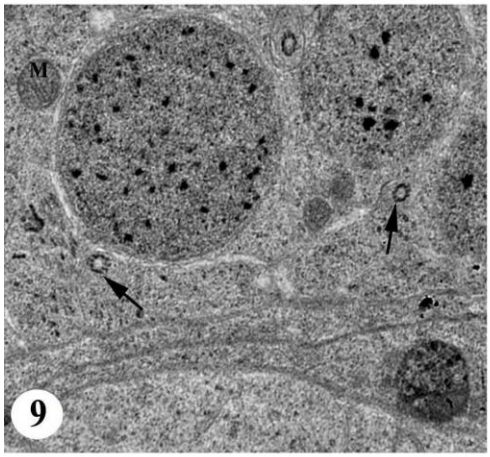
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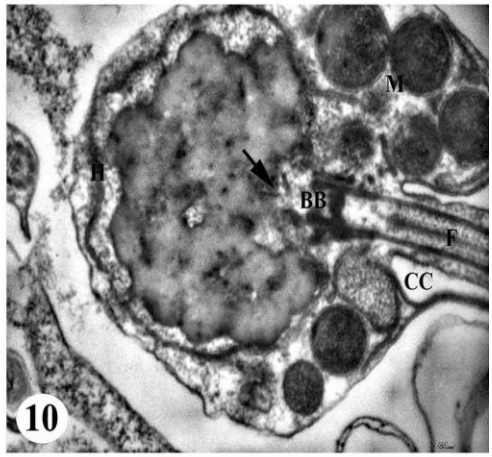
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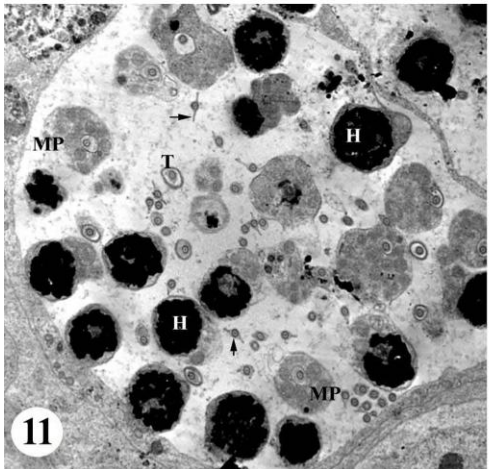
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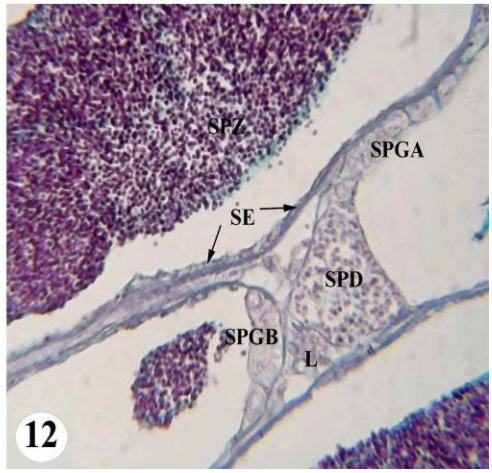
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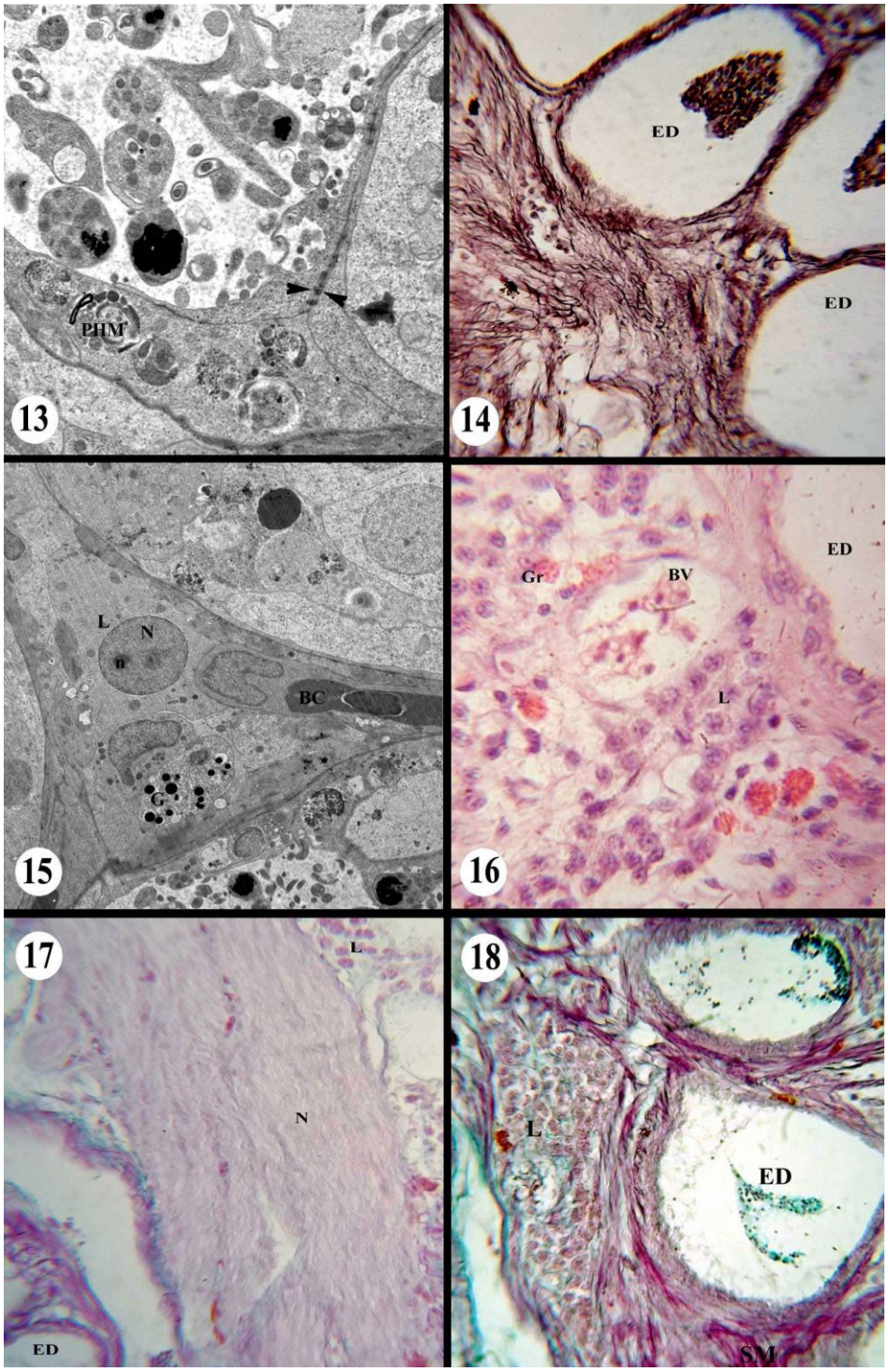
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Fig (1): A photomicrograph of the testes at the fifth month of age representing a thin collagenous capsule (C) and the predominance of the seminiferous lobules (SL) at the expense of the interstitium (I) which is difficult to distinguish. Note that the lumina of the seminiferous lobules are engorged with spermatozoa (SPZ). Crossmon's trichrome stain. X 40.

Fig (2): A photomicrograph of sexually mature testes reveals the lobular organization of the seminiferous lobule which contains different spermatogenic cells. EF: efferent ducts engorged with spermatozoa. H&E stain. X 40.

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Fig (15): Electron micrograph showing triangular interstitial area that contains Leydig cell (L) with unclear cell boundaries, round nucleus (N) with eccentric nucleolus (n). Note that one cell possessing secretory granules (g). Blood capillary (BC) with RBC is also seen in the interstitium. X 1000.

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Fig (18): A photomicrograph of the testes demonstrating the presence of smooth muscle fibers (SM) around the efferent ducts (ED) of the testis. L: Leydig cells. Crossmon's trichrome stain. X 400.

Discussion

In this study, we have described and compared the different spermatogenic cell types and the cells that compose the interstitial testicular compartment in mature males of the Nile Tilapia, *Oreochromis niloticus.* The organization of the teleost testes is variable. Two basic types of structural arrangement have been classified according to the anatomical disposition of the germinal tissue. Firstly, a tubular type with no lumen; which is typical for Atheriniformes, an order of ray-finned fish (Grier, 1984). Secondly, a lobular type with a structure that has a central lumen where cysts develop along the length of the lobules; this type of organization is found in most teleost species as documented by Grier, *et al.* (1980). According to the results of the present study, the testes of Nile tilapia are classified as a lobular type.

The cytoplasmic interconnection of the secondary spermatogonia, primary spermatocytes, and secondary spermatocytes observed in the current study was in agreement with that documented by Abd El-Aziz (1992) in Nile tilapia, and Schulz et al. (2005) in other teleost fish. It is believed that the exchange of molecules between germ cells via their intercellular bridges is responsible for synchronous development (Gilbert, 2000).

The electron-dense material referred to as nuage may be the most definitive marker of early germ cells in a wide range of organisms (Eddy, 1975). The nuage appears as discrete, electron-dense cytoplasmic inclusions that tend to be associated with mitochondria (Eddy, 1975) or with annulated lamellae (Kessel, 1983). This material, the nuage, also referred to as "germinal dense bodies," and "nucleolus like bodies," and "intermitochondrial cement," has been observed consistently, not only in primordial germ cells but also in oogonia, oocytes, spermatogonia, spermatocytes, and even spermatids of fishes (Billard, 1984; Hamaguchi, 1992; Grandi and Colombo, 1997; Quagio-Grassiotto and Carvalho, 1999; Grier and Lo Nostro, 2000; Lo Nostro *et al.*, 2003; Ravaglia and Maggese, 2003). According to Azevedo (1984) the nuage appears to be synthesized in the nucleus; and it contains ribonucleoproteins (Toury, Cle´rot and Andre´, 1977). It may represent ribosomal components that are subsequently assembled in the cytoplasm (Flores and Burns, 1993). In *O. niloticus*, the nuage was observed in association with mitochondria up to the level of primary spermatocytes only. The precise role of the nuage is not known (Hamaguchi, 1993), but its presence is presumably associated with synthetic activities of these cell types.

Contrarily to *M. barberi* in which secondary spermatocytes are numerous (Pecio and Rafinski, 1999), the secondary spermatocytes of Nile Tilapia in the present study, as in most fish species, are scarce and difficult to observe, probably due to their short lifetime. In regards to their transformation into spermatozoa, spermatids of Nile tilapia undergo various degrees of chromatin condensation that starts with the appearance of a few small dense chromatin granules, which increase in number with the progressing development of the spermatid till it covers the whole nucleus of the spermatozoa. Saperas, *et al*., (1993) argued that in the nuclei of Teleostei fish, the chromatin condensation pattern in spermatogenic cells depends on the type of protein associated with the DNA.

In his classification of teleostean sperm, Mattei (1991) did not include the biflagellated sperm within the type I or II. Briefly, type I aquasperm typically has a small rounded or ovoid nucleus, and it does not have an acrosome. However, there are two centrioles present distal to the nucleus. The proximal centriole is often at right angle to the distal one, which forms the basal body of the flagellum; one or both of the centrioles may or may not be located in a basal fossa of the nucleus if, as frequently occurs, a fossa is present. On the other hand, in the type II aquasperm, no rotation of the flagellar axis in relation to the nucleus exists, and the flagellum is positioned parallel to the base of the nucleus. The ultrastructure of *O. niloticus* spermatozoon in the present investigation followed the general description of type I aquasperm's pattern of those of external fertilization species, in that it was uniflagelated and it did not have an acrosome.

Jamieson (1989) indicated that the flagellum's lateral fins are present in most externally fertilizing sperm, except for Ostariophysi (Characiform, Cypriniform, Siluriform). However, in most cases of internally fertilizing spermatozoa, the flagellum's lateral fins are absent. In the present study, *O. niloticus*, which is an external fertilization species; the spermatozoon had two flagellar lateral fins. Therefore, these results are in agreement with those reported by Jamieson (1989).

Lo Nostro *et al*. (2003) reported that Sertoli cells, primary spermatogonia, and the subsequent stages of germ cell development reside in spermatocysts. Sertoli cells are always sequestered from interstitial tissues by a basement membrane. Sertoli cells rest upon the basement membrane and their processes form the borders of spermatocysts. The results of the present investigation revealed that, in Nile tilapia, Sertoli cell processes completely envelop the primary spermatogonia thus isolating them from contacting both the basement membrane and the lobule lumen. This isolation persists throughout the process of spermatogenesis, and until the release of sperm into the lobule lumen (spermiation), even when isogenic clones of germ cells develop synchronously within spermatocysts. These findings are supported by the results reportedby Lo Nostro *et al*. (2003) and Fishelson (2003). It worth mention here that Jun *et al.* (2006) observed in *Kareius bicoloratus* that endoplasmic reticulum, the mitochondria, large number of glycogen particles, and a few lipid droplets appear in the cytoplasm of the he mature Sertoli cell. It seems that the glycogen particles may be involved in the nutrition of spermatids during spermiogenesis. In the present study, the ultrastructural of *O. niloticus* Sertoli cell was similar to those reported by Jun *et al.* (2006), however, the marked structural variation in the cell organization accompanied the germ cell developmental stages reported by them was not observed in this study.

In the present study, the cytoplasmic processes of Sertoli cells were joined by desmosomes and tight junctions, whereas in mammalian testes, Sertoli–Sertoli tight junctions have to be intermittently dismantled to allow migration of maturing germ cells from the basal

compartment to adluminal compartment. This does not occur in the teleost testes since all germ cells develop as an isogenic clone within the spermatocyst lumen (Pudney, 1993). Sertoli cells function as well as phagocytes, including phagocytizing residual bodies and degenerating germ cells, and residual sperms (Grier, 1993). In the present study, phagocytosis of residual bodies was found to have had occurred, and numerous large autophagic or digestive vacuoles were observed in the Sertoli cells in the testes of *O. niloticus* after spermiation. Thus, Sertoli cells in the testes of this species showed similar patterns to those seen in several other species.

The dual function of the testes is to produce spermatozoa and hormones, of which the sex steroids form a dominant group. Sex steroids are required in the gonads for germ cell development (spermatogenesis). The presence of Leydig cells within the interstitial tissue was clearly demonstrated in this study, and it was confirmed, in other studies, that they are the main source of gonadal steroids, mainly androgens. Leydig cells in *O. niloticus*, are the unique cells that display ultrastructure features of steroidogenic cells; mitochondria with tubular cristae, smooth endoplasmic reticulum, and round nucleus as that performed by (Pudney, 1996; Lo Nostro, *et al.*, 2004).

The structural features of myoid cells in this study confirms the suggestion extended by Grier *et al.* (1989) in that these cells could form a contractile network, which would facilitate the expulsion of sperm from the lobules during spawning. The granulocytes detected beside the blood vessels of the interstitium near the efferent ducts are probably involved in the phagocytizing of damaged gonadal cells and residual sperms.

In this study, unmyelinated nerves within the interstitial compartment at the vicinity of the efferent ducts were described, but it was not possible to say for sure whether they belong to the sympathetic or parasympathetic systems.

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INSULIN-MIMETIC EFFECTS OF CINNAMON EXTRACT IN WISTAR RATS

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Abstract

Cinnamon extracts are widely used Arabian and Asian countries as herbal and medical plant. The cinnamon has active substances which have in vivo hypoglycemic activity and probably improve insulin sensitivity. Up till now the molecular mechanism of CE on genes expression is not yet determined. In this study, we examined the effect of CE (200 mg/kg body weight) in diabetic Wistar rats by testing its effect on genes related to lipids and glucose metabolism. In induced diabetic experiments, CE treatment normalized significantly ($p< 0.05$) the increase in lipid profiles and glucose levels occurred in diabetic rats. In parallel, CE lowered glucose levels through the increase in insulin secretion from still working □ cells. The antidiabetic action of CE was confirmed as CE treated diabetic rats showed an increase in the mRNA expression of leptin, PPAR-□ and adiponectin. In conclusion, CE has antidiabetic and insulin-mimetic actions through the regulation of genes related to lipid and glucose metabolism.

Key words: *Cinnamon, Biological actions, Diabetes, Obesity*

Running head: *Cinnamon induces insulin like acyivity in diabetic rats*

Introduction

The bark of the Cinnamomi cassiae (Lauraceae) contains cinnamic aldehyde, cinnamic acid, tannin and methyl-hydroxychalcone polymer (MHCP) as main components. Cinnamon extract (CE) contains biologically active substances with insulin-mimetic properties (Kim et al., 2006). Qin *et al.*, 2003 have reported that cinnamon extract decreases blood glucose in rats and increases insulin sensitivity and glucose uptake in adipocytes (Jarvull-Taylor et al., 2001). In vitro and in vivo studies have shown that cinnamon enhances glucose uptake by activating insulin receptor kinase activity, autophosphorylation of insulin receptor and glycogen synthase activity (Kannappan *et al.*, 2006). Moreover, it posses the ability to reduce lipid levels in fructose-fed rats and affects immune responses by regulating anti-, proinflammatory and glucose transporter gene expressions in mouse macrophages (Cao et al., 2008). Also, cinnamon has hepatoprotective effect against carbon tetrachloride induced oxidative stress and liver injury in rats [Moselhy & Ali 2009]. Diabetes is chronic metabolic diseases associated with an increased risk of coronary heart disease, stroke, hypertension, renal failure, type 2 diabetes, dyslipidemia and all cause mortality (Hall 2003; Havel 2004; Trayhurn and Beattie 2001). Clinically diabetic patients characterized by marked increase in blood glucose levels followed by mild hyperlipidemia (Reddy *et al.*, 2009). Traditional herbal medicine has been widely used for diabetes treatment and is recognized as an interesting alternative to conventional medicine (Kameswara Rao et al., 1997) especially in the third world countries and therefore represent new avenues in the search for alternative hypoglycemic drugs (Day 1998). However, most of them have been shown to exert little or no effect on glycemic control in experimental studies, although some herbs possess hypoglycemic properties. Treatment of diabetes depends on genes related to glucose

metabolism. For example, glucose transport is the rate-limiting step in carbohydrate metabolism (Maughana 2009) which is facilitated by glucose transporters (GLUT) across the cell membrane (Anand et al, 2010). So, compounds facilitating GLUT4 translocation and improve insulin sensitivity can be beneficial for the treatment of diabetes (Kipmen-Korgun *et al.*, 2009; Shepherd & Kahn 1999). Usage of natural products as cinnamon and other dietary modulators with anti-diabetic activity are the first choice of diabetic patients. This tendency is because insulin, to date, cannot be used orally and its repeated injections had many undesirable adverse effects. In addition, most of hypoglycemic agents or drugs are not effective to decrease blood glucose levels in chronic diabetic patients (Cheng & Fantus 2005).

There are strong relationship between obesity and hypertension with diabetes (Mohamed-Ali et al., 1998). Therefore, we can speculate that there is an interaction between cinnamon and diabetes through its effect on insulin and genes related to lipid and glucose metabolism and that is the purpose of this study.

Materials and methods

Streptozotocin (STZ) was from sigma Aldrich, USA. The Wistar albino rats were from Egyptian Co for experimental animals import, Helwan, Egypt. Vehicles and related materials were from ADWIA pharmaceutical company, Egypt, Heparinzed vacuteiner tubes, TriZol reagents, Poly dT, chloroform, ethanol and cytokines primers were from Wako pure chemicals, Osaka, Japan. Biochemical kits for lipids, insulin, leptin and haptoglobin were from *Clini Lab*, Cairo, Egypt.

Cinnamon extracts preparation

Cinnamon extract was extracted based on method of Sheng et al., 2008. Briefly, cinnamon powder (100 g) was dissolved in 1000 ml double distilled water then subjected for revolving evaporator in vacuum state using vacuum pump till the volume of water reduced to 50%. The supernatant was filtered through Whatman paper no. 1 to obtain cinnamon water extract. The final concentration was measured by Lowry method for protein concentration.

Induction of diabetes in Wistar rats

Adult Wistar rats weighting 200-250 grams (90 days old) were used for induction of diabetes. The animals were injected STZ at the dose of 60 mg/kg of the body weight intraperitoneally. STZ induced diabetes within 3 days of injection as confirmed by the increase in blood glucose samples collected 3 days after STZ injection. The blood glucose levels over 200 mg/dl considered diabetic rats. Diabetic and non-diabetic control groups were kept in metabolic cages individually and separately. Next, rats were divided as follows:

(i) Control receiving water as vehicle
(ii) Control diabetic received STZ single dose then water as vehicle.
(iii) Diabetic plus cinnamon extract (CE) in a dose of 200mg/kg body weight (Kim et al., 2006; Kim and Choung 2010) orally and daily for 2 months.

Rats in all experiments were killed by decapicitation after 2 months of CE treatments. Blood was collected to get plasma and tissues were kept in TriZol reagent at -70 ^{o}C until RNA extraction and mRNA expression.

Plasma chemistry analysis

Serum triglycerides (TG), total cholesterol (TC), VLDL and HDL were measured using commercial kits that based on spectrophotometric analysis. Insulin and glucose were measured by commercial kits supported by Wako pure chemicals, Japan.

RT-PCR Analysis and Gene Expression

Livers and adipose tissue were collected from rats, flash frozen in liquid nitrogen and subsequently stored at -70°C. Frozen samples (approximately 100 mg of tissue per sample) were immediately added to 1 ml of TriZol reagent (Invitrogen, Carlsbad, CA) and homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). One milliliter of the tissue homogenate was transferred to a microfuge tube, and total RNA was extracted via chloroform extraction followed by nucleic acid precipitation with isopropanol. The pellet was washed with 75% ethanol and resuspended in molecular biology grade water. Nucleic acid concentration was determined by o.d. 260 nm (Smart-Spec; Bio-Rad Laboratories, Hercules, CA), and RNA integrity was evaluated using an Agilent bioanalyzer (model 2100; Agilent Technologies, Foster City, CA).

RNA (1µg) was treated at 72 °C for 5 min and reverse transcribed using 100 units of Moloney murine leukemia virus reverse transcriptase (Gibco), 50 pmol of poly (dT) primer and 20 nmol of dNTPs in a total volume of 10 µl at 37 °C for 1 h. After heating at 94 °C for 5 min, PCR amplification was performed with 2.5 units Taq polymerase (Perkin-Elmer, Foster City, CA, USA), 3 mM MgCl2 and 50 pmol of forward and reverse primers specific for respective genes in a total volume of 50 µl. The PCR conditions for different tested genes as adiponectin, PPAR-□, leptin and GLUT4 carried out as in table 1. Electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualization under UV lamp will be carried out. Intensities of PCR bands will be analyzed densitometrically using NIH Image program (http://rsb.info.nih.gov/nih-image/).

Statistical analysis

Results are expressed as means ± S.E. of independent experiments. Statistical analysis was done using ANOVA and Fischer's post hoc test, with $p < 0.05$ being considered as statistically significant.

Results

Effect of cinnamon extract on changes of lipids profiles, glucose and insulin in normal, diabetic control and CE-treated diabetic Wistar rats.

CE treatment induced alteration in plasma levels of lipid parameters. As seen in Fig.1.a-d, diabetic rats showed significant increase in cholesterol, TG, VLDL and decrease in HDL compared to normal non diabetic rats. CE treatment normalized the changes induced in diabetic rats. Moreover the CE decreased the significant increase in glucose concentration in diabetic rats (Fig.1-e). Plasma insulin levels was decrease in diabetic rats as results of pancreatic □ cells destruction and CE treatment stimulated the remaining cells to increase insulin secretion (figure 1-f).

RT-PCR analysis and mRNA expression in epididymal fat tissues

Finally, we tested the effect of CE on modulation of leptin, PPAR-□ and adiponectin expression in rat epididymal adipose tissue using RT-PCR analysis. STZ diabetic rats have no alteration in leptin expression but treatment of diabetic rats by CE significantly increased leptin mRNA expression relative to control and diabetic control rats (Fig.2a). Moreover, the expression of PPAR-□, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism was examined. As seen in Fig.2b, STZ induced less significant increase in PPAR-□ expression ($P< 0.05$) but treatment of STZ diabetic rats by CE induced 4 folds increase in PPAR-□ expression to increase peripheral glucose utilization

and lipid metabolism. At the end, we examined the expression of insulin sensitizing protein, adiponectin. Adiponectin expression was double fold increased ($P < 0.05$) in CE treated rats compared to control and STZ diabetic rats as seen in Fig.2c.

Discussion

Diabetes is a chronic metabolic disorder that affects approximately 3% of population worldwide. Gaster and Hirsch, 1998 reported that Sustained reductions in hyperglycemia will decrease the risk of developing microvascular diseases and reduce diabetes complications. Usage of oral hypoglycemic drugs to treat diabetes has several limitations, such as adverse effects and high rates of secondary failure (Kim *et al.*, 2006). Those adverse effects forced the diabetic patients to use herbal medication that have a similar degree of efficiency without side effects and that was the purpose of this study. CE treatment decreased glucose levels in STZ diabetic rats and that confirmed the insulin like effects of CE extract and that is seen by increase in insulin levels in plasma. The possible mechanism by which CE has its hypoglycemic action in diabetic Wistar rats may be by potentiating the effect of insulin in plasma or by increasing either the pancreatic secretion of insulin from the existing beta cells or its release from the bound form (Kim et al., 2006). CE might improve diabetes by normalizing the postprandial plasma glucose level as well as fasting blood glucose level (Kim et al., 2009). In turn, in hyperinsulinemia, cinnamon increases insulin sensitivity for effective glucose disposal in rats although in humans cinnamon does not appear to improve fasting blood glucose levels and lipid parameters in patients with type1 or type 2 diabetes (Baker et al., 2008). Here, CE improved and normalized the the changes in lipid parameters as TG, cholesterol and HDL and that is parallel with results of Kham et al., 2003; Kim and Choung 2010; Qin et al., 2003, and improved insulin resistance induced by feeding high fat diet (Thorens 1996).

The molecular mechanism of CE, we examined the mRNA expression of proteins involved in lipid and glucose metabolism in diabetic epididymal adipose tissue. Treatment of STZ diabetic rats with CE induced significant increase leptin, PPAR-□ □and adiponectin expression. The correlation between glucose, insulin, leptin, PPAR-□ and adiponectin is the mechanism by which CE induced its antidiabetic effects. As the decrease in glucose levels in diabetic rats was correlated with the increase insulin secretion from working □ cells as suggested by our findings and that of Kim and Choung 2010. Also, the decrease in lipid parameters may be associated with the increase of leptin mRNA expression in diabetic rats, because leptin is known to be the potent lipolytic protein and increased peripheral glucose utilization (Ahima and Flier 2000). Moreover, PPARγ mRNA expression was up-regulated in adipose tissue by the administration of the cinnamon extract. As known, PPARγ is highly expressed in adipose tissue and plays an important role in insulin sensitivity and the secretion of adipocytokines such as adiponectin. PPARγ activation through the binding of the synthetic thiazolidinediones, PPAR-□ agonists, results in a marked improvement in insulin and glucose in type 2 diabetic patients, which results in improvement of the whole body insulin sensitivity (Frias et al., 2000; Miyazaki et al., 2001; Raskin et al., 2000). It has been shown that PPARγ increases the synthesis and production of adiponectin in animals and humans (Yamauchi et al., 2001; Fruebis et al., 2001; Maeda et al., 2001). The increase in expression of adiponectin constitutes the mechanism by which PPAR-□ acts in adipose tissue to increase whole-body insulin sensitivity (Yamauchi et al., 2001). So the increase in insulin secretion

and decrease in glucose levels and lipid parameters may be mediated by the increase in PPAR- (Yamauchi et al., 2001) and possibly by adiponectin expression.

This study demonstrated that CE is more anti-diabetic herbal medication through the improvement of insulin sensitivity, decrease in blood glucose levels and increases the expression of proteins related lipid metabolism. Moreover, cinnamon extract is not pure anti-obesity herbal therapy.

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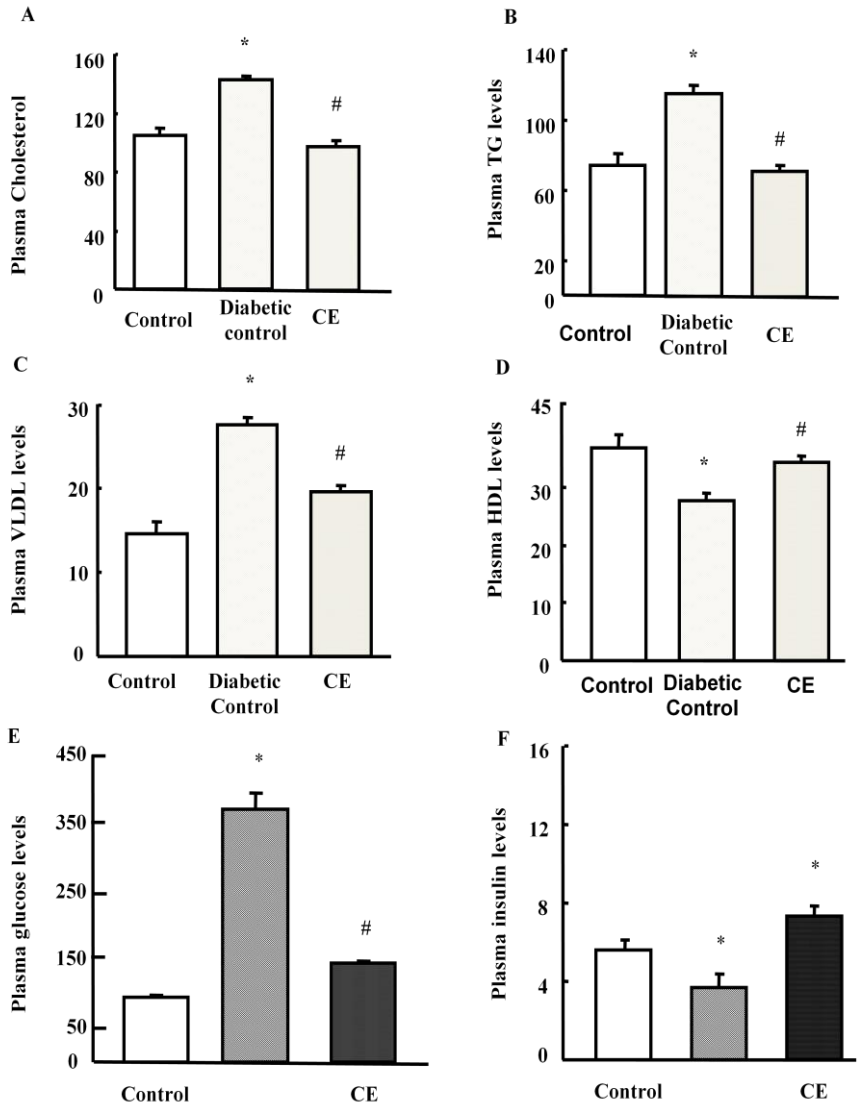

Fig. 1. Effect of cinnamon extract on changes of lipids profiles (a-d) glucose (e) and insulin (f) in normal, diabetic and diabetic plus CE after 2 month in wister rats. Rats were given cinnamon extract in water and food for CE and diabetic rats, vehicle as control for normal rats for 2 months. Plasma levels of cholesterol. TG, VLDL, HDL, glucose and insulin were measured for the 3 tested groups. Values are means ± S.E.M of 6 different rats for each treatmenr. *$p < 0.05$ vs. control and # $p < 0.05$ vs. diabetic rats.

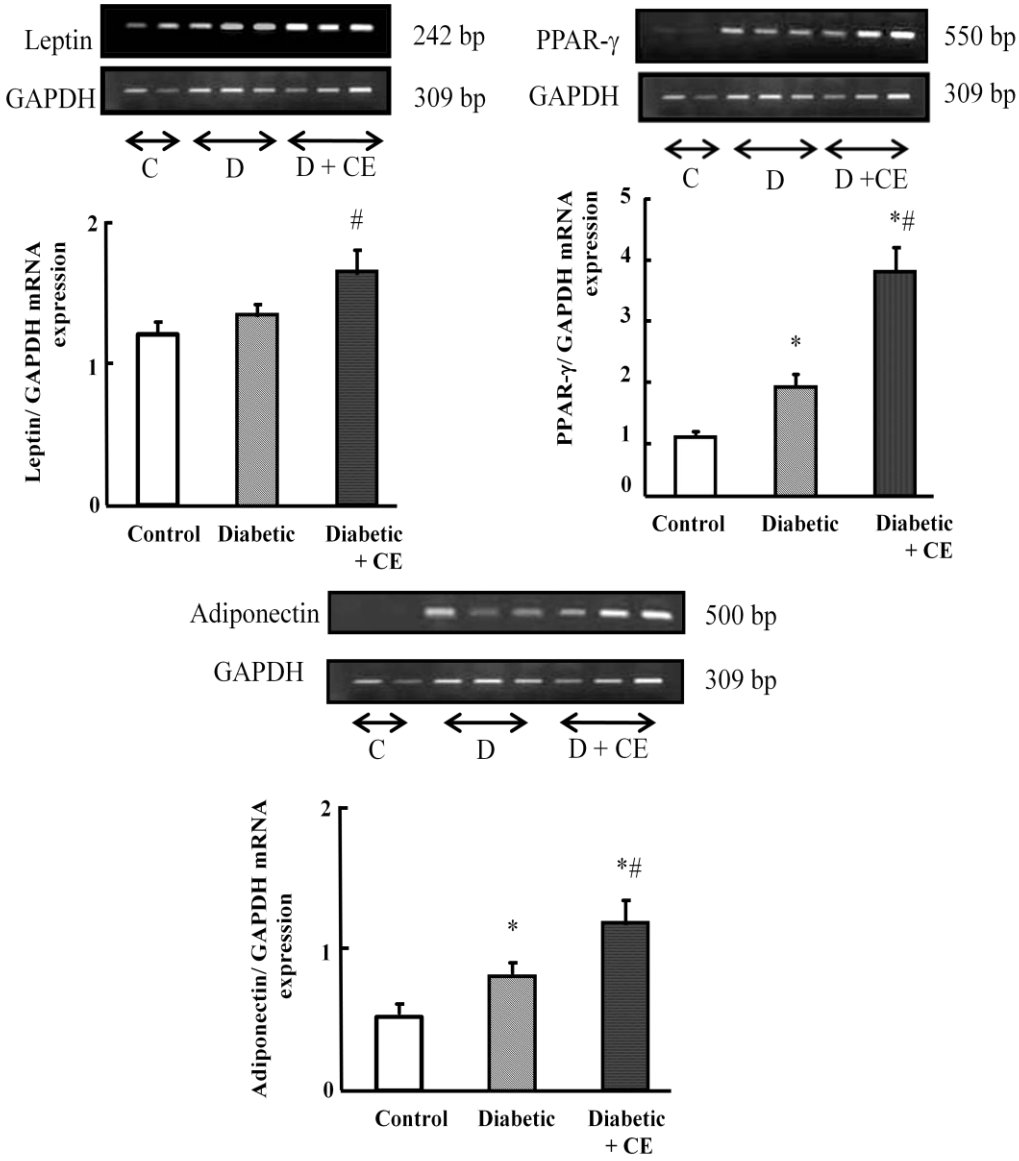

Fig.5. RT-PCR analysis of leptin, PPAR-γ and adiponectin in epididymal fat tissue of wister rats. Rats were treated by either vehicle or CE for 2 months. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out as seen in upper bands for TNF-a and b-actin. The densitometric analysis of expressed bands (lower columns) were normalized with that of GAPDH and then relative to control. Values are means±SEM obtained from 5 different rats. *$p < 0.05$ vs. compared to control and .# $p<0.05$ vs. diabetic groupe alone.

ANTIOXIDANT, ANTIMETASTATIC AND APOPTOTIC PROPERTIES OF IRRADIATED CITRUS PECTIN

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Abstract

Pectin, a natural plant polysaccharide, which presents in all higher plant cell walls, fruits and vegetables, is a substance that appears to be able to inhibit cancer metastasis and it can be degraded by Irradiation to preparing low molecular weight pectin The aim of this study was to evaluate in vitro and in vivo antitumor activity of citrus pectin and irradiated citrus pectin. Cytotoicity assay of citrus pectin and irradiated citrus pectin (ranged from 0.1-40 mg/ml)on EAC cells was evaluated by trypan blue exclusion method. In vivo studies were done by treating Erlich tumor bearing mice with citrus pectin and irradiated citrus pectin for 6 weeks. Tumor volume was determined. Also, NO, MDA,GSH, urea, and creatinine concentrations and CAT,SOD, Gpx. ALT, AST,and GGT activities were evaluated by colorimetric assays, histopathological examination and Characterization of cell death(apoptosis) within tumor tissue was evaluated. In vitro and in vivo studies results revealed that treatment with irradiated citrus pectin is more effective in cancer treatment than treatment with citrus pectin. From our results, it can be concluded that irradiated citrus pectin might be a potential alternative agent for cancer therapy.

Keywords: *citrus pectin, irrdiation, cytotoxicity, Ehrlich Ascites Carcinoma Cells and tumor*

Introduction

Pectin is a naturally occurring biopolymer found in most plants, but is most concentrated in citrus fruits (oranges, lemons, grapefruits) and apples (Sharma *et al.*,2006). Ordinary pectin isolated from citrus fruits has a molecular weight of 100.000–500.000 daltons and can be modified resulting in shorter, less complex molecules. These shorter carbohydrate chains, called modified citrus pectin (MCP), dissolve more readily in water and are better absorbed and utilized by the body than ordinary, long-chain pectin (Azemar *et al.*,2007). The term "modified pectin" refers to any changes to the structure of pectin that are brought about by chemical, physical or biological(including enzymatic) means or by some combination thereof.

Gamma irradiation is a useful physical treatment for depolymerizing pectin. Gamma irradiation leads degredation of polymer molecules by the free radical formed (Kange *et al*., 2006). Irradiation induced degredation has been applied to preparing low molecular weight pectin (Cho *et al.*,2003) product with increased amount of mono saccharides.

Extensive investigation has indicated that pectins and MCP (Modified Citrus Pectin) as an important dietary fibers demonstrate the proved physiological activity and therefore have found wide medical applications in preventing gastric diseases, reducing the cholesterol and serum glucose levels (Gulfi *et al.,* 2007 and Umar *et al.,* 2003), repressing the formation of gallstones and hypertension, reducing cancer (Jackson *et al*., 2007), and stimulating the immune response (Inngjerdingen *et al.,* 2007).

Materials and methods

Animals: Female Swiss albino mice weighing 20–25 g were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt).

Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water *ad.* Libitum. Animals were kept under a controlled lighting condition (light: dark, 12 h:12 h).

***Ehrlich Ascites Carcinoma Cells and tumor induction*:** A line of Ehrlich Ascites Carcinoma (EAC) cells was supplied from National Cancer Institute, Cancer Biology Department.Egypt. Solid tumors were produced by intramuscular inoculation with 0.2 ml of EAC, which contained 2.5 x 10^6 viable EAC cells, in the right thigh of the lower limb of each mouse. Mice with a palpable solid tumor , its diameter was 10mm³, that developed within 10 days after inoculation were used in the study.

Chemicals: Citrus Pectin purchased from El-Goumhouria Co., Egypt. All chemical and kits purchased from Segma (USA).

Preparation of citrus pectin and irradiated citrus pectin solutions were prepared by Prof.Dr. Ahmed Ibrahim El Batal. Prof. of Biological Sciences (Applied Microbiology& Biotechnology), Drug Radiation Research Department, Biotechnology Division, National Center for Radiation Research and Technology, Atomic Energy Authority, Egypt as follows:

Preparation of 4% CP: Four grams of citrus pectin was dissolved in100ml distilled water.

Preparation of Irr.CP: Four percent of citrus pectin solution was prepared. This solution was irradiated with a dose of 5 kGy (kilo Gray) gamma radiation. The irradiation process was performed at National Center of Radiation Research and Technology (NCRRT, Cairo), Egypt.

Cell viability assay: EAC viable cells were counted by *trypan blue exclusion* method according to (Ribeiro *et al*., 2006).

Experimental design:

Sixty female Swiss albino mice were divided into 4 groups each contain 15 mice as follows:

Group (1)*:** Served as negative control and orally received saline served as negative control group (NTBM: Non-tumor bearing mice). ***Group (2): Tumor bearing mice without any treatment served as positive control group (TBM) for 6 weeks. ***Group (3):*** Tumor bearing mice received citrus pectin at dose of 3.3gm /Kg body weight/day ($TBM_{(CP)}$) for 6weeks. ***Group (4):*** Tumor bearing mice received Irr.CP at a dose of 3.3gm /Kg body weight /day ($TBM_{(Irr.CP)}$) for 6 weeks.

Blood and tissue sampling:

Directly, after animals were sacrificed after 2 and 6 weeks , blood samples were collected in heparinized tubes and then centrifuged at 3500 rpm for 15 minutes. liver and tumor were dissected out at the end of experiment (after 6 weeks of treatments) and kept in 10% formalin for histopathological examination and apoptosis detection.

Experimental parameters

Lipid peroxide concentrations were determined by measuring the Malonaldialdahyde (MDA) end product according to the method of Yoshioka *et al*., (1979). Reduced glutathione (GSH) estimated as yellow color which developed when 5, 5 dithiol-bis (2-nitrobenzoic acid) is added to sulfhydryl compounds according to the method described by Beutler *et al*., (1963). The glutathione peroxidase (GSH-Px) activity level was assayed as described by Gross *et al.*, (1967) and Necheles *et al.*, (1968). Superoxide dismutase (SOD) activity was estimated by the detection of superoxide anions using nitroblue tetrazoluim formazan color development

as reported by Minami & Yoshikawa, (1979). Total nitrate/nitrite (NO(x)) was measured as stable end product, nitrite, according to the method of Miranda *et al.*, (2001). Catalase activity was measured according to the method of Sinaha, (1972). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma were determined by a colorimetric method as described by (Reitman and Frankel, 1957)using a diagnostic kit supplied by Plasmatek (Germany). Plasma gamma-glutamyl-transferase was determined according to (Szasz, 1969) using a diagnostic kit supplied by (Pointe Scientific, INC Co., USA). Creatinine in plasma was determined by a colorimetric method as described by (Henry *et al*., 1974) using a diagnostic kit supplied by Diamond (Egypt). Urea in plasma was determined by an enzymatic colorimetric method as described by (Palton and Crouch, 1977) using a diagnostic kit supplied by Diamond (Egypt)

Tumor volume determination

Tumor volume was measured twice a week using a Vernier caliper and determined by applying the following equation according to (Jensen *et al*., 2008) :

Tumor volume = 1/2(length × width^2) Where length is the greatest longitudinal diameter and width is the greatest transverse diameter.

Characterization of cell death within tumor tissue (Apoptosis):

Apoptosis was determined using Acridine Orange - Ethidium Bromide Staining (Cho *et al* ., 1999).

Histopathological examination:

Liver tissue samples were fixed in 10% formalin and embedded in paraffin. Sections of tissues were stained with Hematoxylin and eosin.

Statistical analysis:

Statistical analysis was done according to Snedecor and Cochran (1969).

Results

Cytotoxicity of citrus pectin (CP) and irradiated citrus pectin (Irr.CP) against EAC cells:

Data presented in Table 1, shopwed that treatment of EAC cells with citrus pectin at increasing concentrations (0.1-40 mg*/ml*) for one hour incubation showed cytotoxicity with 50% inhibition of cell survival (IC_{50}) at concentration of 26mg/ml.

But, Treatment of EAC cells with Irr.CP at different concentrations (0.1-40 mg*/ml*) for one hour incubation reduce the cell survival. The IC_{50} was at concentration of 3mg/ml of Irr.CP.

Table 1. *in vitro* antitumor activity of CP and Irr.CP

Conc. (mg/ml)		**0**	**0.1**	**0.2**	**0.4**	**4**	**40**
%of viable cells	**CP**	98.6	98.42	88.78	83.98	68.96	39.68
	Irr.CP	98.6	97.22	85.48	72.68	39.68	2.4

Tumor volume:

In the present study, tumor volume (mm^3) in figure 1 revealed significant decrease in CP and Irr.CP treated groups compared to untreated EAC group and continued till the end of

the experiment. While, it was significantly decreased in TBM $_{(MCP)}$ group compared to TBM $_{(CP)}$ group after 4weeks.

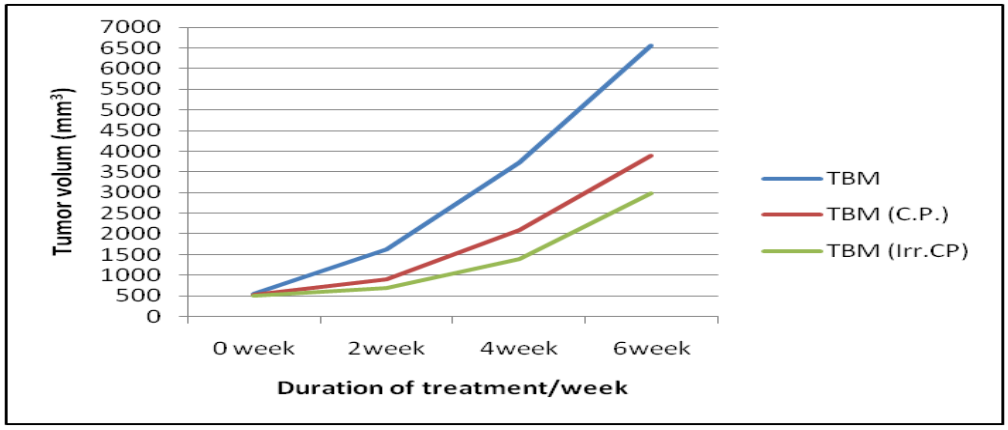

Fig. 1. Effect of CP and Irr.CP on tumor volume of Ehrlich solid tumor

Effect of citrus pectin and Irr. citrus pectin on biochemical parameters:

The results obtained in Table 2 showed that SOD activity was highly significant decreased in TBM ($P < 0.01$) after 2 and 6 weeks compared to NTBM. While it was significantly increased ($P < 0.05$) after 6 weeks in TBM $_{(CP)}$ and TBM $_{(Irr.CP)}$ compared to TBM. But, GPx activity was very highly significant decreased in TBM after 2 and 6 weeks compared to NTBM. While it was significantly increased after 6 weeks in TBM $_{(CP)}$ and TBM $_{(Irr.CP)}$ compared to TBM. Moreover, it was significantly increased in TBM $_{(Irr.CP)}$ after 6 weeks compared to TBM $_{(CP)}$. TBM group showed very highly significant decrease in GSH content after 2 and 6 weeks compared to NTBM. Meanwhile, TBM $_{(CP)}$ and TBM $_{(Irr.CP)}$ revealed significant increase after 2 and 6 weeks compared to TBM. Catalase activity was very highly significant decreased in TBM after 2 and 6 weeks compared to NTBM. While it was significantly increased after 2 and6 weeks in TBM $_{(CP)}$ and TBM $_{(Irr.CP)}$ compared to TBM. Moreover, it was significantly increased in TBM $_{(Irr.CP)}$ after 6 weeks compared to TBM $_{(CP)}$. Furthermore, MDA concentration was very highly significant increased in TBM after 2 and 6 weeks compared to NTBM. While it was significantly decreased after 2 weeks in TBM $_{(CP)}$and after 6 weeks in TBM $_{(Irr.CP)}$ compared to TBM. $NO_{(X)}$ concentration was very highly significant increased in TBM after 6 weeks compared to NTBM. While, it was significantly decreased after 2 and 6 weeks in TBM $_{(CP)}$and after 6 weeks in TBM $_{(Irr.CP)}$ compared to TBM. Furthermotre, it was significantly decreased after2 weeks in TBM $_{(Irr.CP)}$ compared to TBM $_{(CP)}$.

Table 2. Effect of treatment with CP and Irr.CP on SOD, GPx, CAT activities, GSH, MDA and $NO_{(x)}$ concentrations in tumor bearing mice

Group/ Parameters	weeks	NTBM	TBM	$TBM_{(CP)}$	$TBM_{(Irr.CP)}$
SOD(µg/ml packed RBCs)	2	3.34 ±0.09	2.58**± 0.15	2.91 [d] ± 0.12	3.04 [d] ± 0.17
	6	3.11 ± 0.13	2.36**± 0.26	3.00 [a] ± 0.11	3.36 [a] ± 0.13
GPx(mg/dl packed RBCs)	2	0.78 ± 0.01	0.64***± 0.01	0.62 [d] ± 0.01	0.64 ± 0.01
	6	0.78 ± 0.01	0.66***± 0.01	0.69 [a c] ± .01	0.78 [a b] ± 0.01
GSH(mg/dl packed RBCs)	2	137.74 ±1.64	109.96***±2.13	145.42 [a] ±3.25	153.49 [a] ± 5.58
	6	141.16± 5.26	112.29***±3.33	149.69 [a] ±5.89	146.97 [a] ± 3.61
CAT(µM/ml packed RBCs)	2	0.59 ±0.014	0.350***± 0.01	0.46 [a] ± 0.03	0.433 [a] ± 0.02
	6	0.63 ± 0.01	0.503***±0.01	0.55 [a c] ± 0.01	0.60 [a b] ± 0.01
MDA(µM/ml plasma)	2	91.74 ± 1.68	137.73***±4.87	120.41 [a] ±4.02	128.91± 7.36
	6	90.74 ±1.663	111.65***±2.34	105.49 ± 1.57	102.073 [a] ± 4.51
$NO_{(x)}$(µM/L plasma)	2	20.96 ±0.76	31.52***±0.81	31.67 [c d] ±0.69	28.045 [ab] ± 0.79
	6	20.91±0.42	35.44*** ±0.64	21.95 [a] ± 0.72	21.515 [a] ±0.42

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(CP)}$group p<0.05. c, significant from $TBM_{(Irr.CP)}$group p<0.05.

Table 3. Changes in plasma ALT, AST, GGT, urea and creatinine in controls, CP and Irr.CP treated mice groups bearing tumor

Group/ Parameters	weeks	NTBM	TBM	$TBM_{(CP)}$	$TBM_{(Irr.CP)}$
ALT(U/L)	2	50.51 ± 2.80	56.45 ± 4.76	71.07 [a] ± 1.97	69.14 [a] ±0.88
	6	58.25 ± 5.51	73.19 ± 9.69	68.2± 10.56	66.93 ±3.72
AST(U/L)	2	96.50± 4.99	130.45***± 6.16	100.39 [a] ±1.42	118.3 ±11.30
	6	98.59± 1.15	122.14*±3.02	106.09±10.73	101.26 [a] ±7.00
GGT(U/L)	2	31.01± 2.22	52.62***±0.73	28.85 ±2.84	0.77 ±0.08
	6	33.73± 2.14	65.75***± 4.80	34.15±1.65	0.91 ±0.04
Urea(mg/dl)	2	29.65 ±2.52	52.72 ±1.78	26.15 ±0.35	0.70± 0.09
	6	30.60±0.64	42.02 [a] ± 5.45	30.95±2.09	0.82 ±0.05
Creatinine (mg/dl)	2	0.79 ±0.07	50.80 ±1.77	28.90 ±1.515	0.77 ±00.04
	6	0.82 ±0.02	41.92 [a] ± 4.01	28.50 [a] ±1.63	0.76 [a] ±0.04

Each value is the mean ± SEM. Non-significant (N.S): p>0.05; Significant: *p<0.05; highly significant: ** p<0.01; very highly significant: ***p<0.001 from NTBM. a, significant from TBM p<0.05. b, significant from TBM $_{(CP)}$group p<0.05. c, significant from $TBM_{(Irr.CP)}$group p<0.05.

As shown in Table 3, AST activity was very highly significant increased in TBM group after2 weeks became significantly decreased after 6 weeks and GGT activity was very highly significant increased after2 and 6 weeks compared to NTBM group. While,ALT activity was significantly increased in TBM *(CP)* and TBM *(Irr.CP)* after 2 weeks compared to TBM. Also, AST activity was highly significant decreased after 2 weeks in TBM *(CP)* and after 6 weeks in TBM *(Irr.CP)* compared to TBM. GGT activity was significantly decreased

after 6 weeks in TBM $_{(CP)}$ and TBM $_{(Irr.CP)}$ compared to TBM.Urea and creatinine concentrations were significantly decreased after 6 weeks in TBM $_{(Irr.CP)}$ compared to TBM.

Hiopathological examination in liver tissue

Histopathological examination of liver section of TBM group showed numerous neoplastic foci widely spreaded all over the liver in comparison to the control liver(Fig.2a) (normal liver) which either distributed as a focal mass or distributed mainly among the hepatic cords (Fig.2b). But, liver of tumor bearing mice treated with CP showing several neoplastic foci (Fig.2c) with mononuclear cell infiltration in the portal area. While, The liver of tumor bearing mice treated with Irr.CP showed moderate aggregation of neoplastic cells (Fig2d).

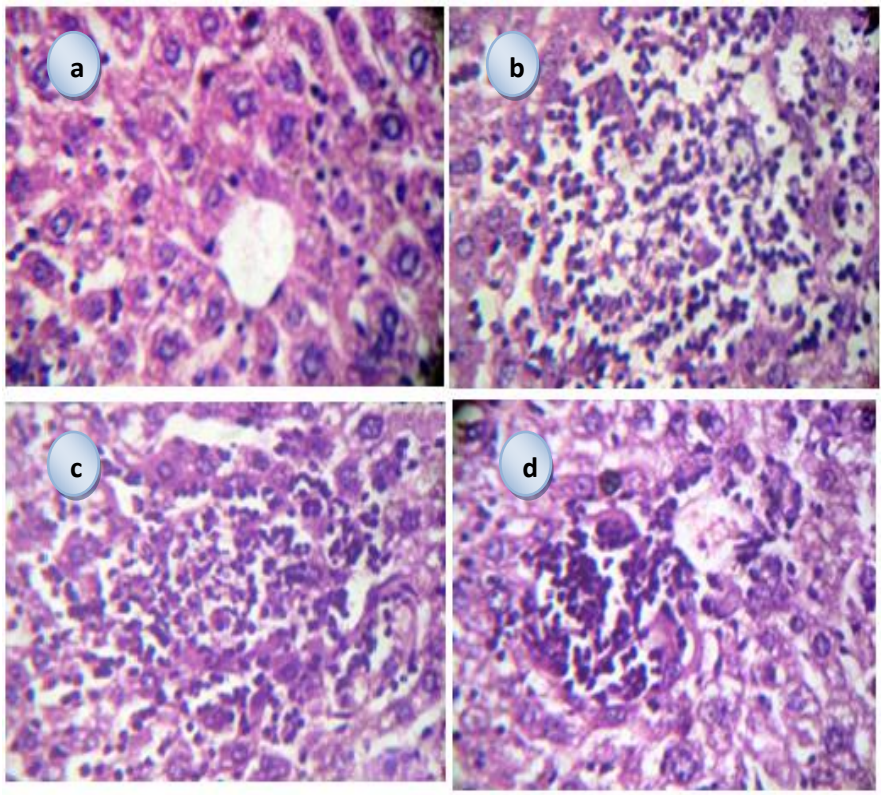

Fig. 2. Photomicrographs of sections in liver stained by H& E (a) normal liver. (b) Liver of mice bearing Ehrlich carcinoma(TBM). (c) Liver of tumor bearing mice treated with citrus pectin. (d) Liver of tumor bearing mice treated with Irr.CP

Characterization of cell death within the tumor (Apoptosis)

Analysis of our photographs revealed normal mice tumor cells stained green with the presence of some level of apoptotic cells as expected (stained orange) presented in figure (3a,b).Also, citrus pectin treatment induce apoptosis all over the sections are punctuated by few of non-apoptotic cells (healthy tumor cells) Fig.(3c,d). But, Fig.(3e,f) revealed the

apoptotic effect of Irr.CP showing induction of apoptosis in different regions with the presence of some level of non-apoptotic cells specially in the core region which appeared more effective than citrus pectin itself.

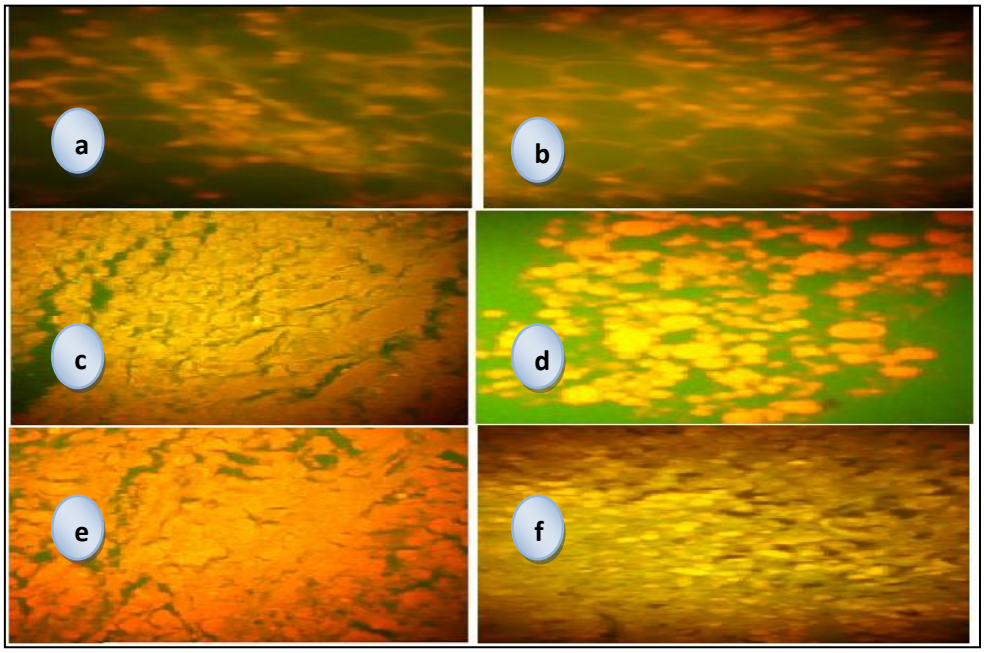

Fig 3. AO/ EB staining sections (a,b) of mice control tumors.(c,d) Sections in tumors of citrus pectin treated mice. (e,f) Sections in tumors of Irr. CP treated mice(AO/EB, X 40)

Discussion

Fragmenting the pectin, may affect the structure of the pectin and thus its function (Jackson *et al.,* 2007).Citrus Pectin and modified Citrus pectin have been found to exhibit antimutagenic activity and inhibit cancer metastasis and proliferation, with no evidence of toxicity or other serious side effects (Chen *et al.*, 2006 & Attari *et al.*, 2009)

Application of CP and Irr.CP on EAC cells showed cytotoxicity with maximum cell mortality (60.32% and 97.6%)respectively at 40mg/ml(CP or Irr.CP) after 1 hour incubation. Our results are in line with Olano -Martin *et al*., (2003) who stated that *in vitro* experiment was shown that dietary pectin and its degraded products induce apoptosis in human colonic adenocarcinoma cells. Galactosyl, a main component of modified citrus pectin (MCP), can specifically inhibit tumor growth and metastasis in vivo and galectin-3-mediated functions in vitro(Nangia-Makker *et al*.,2002). Galectin-3, are closely related to cell to cell adhesion, aggregation of cancer cells in vitro (Dumic *et al.*,2006 & Krzeslak & Lipinska.,2004).

Citrus pectin and irradiated citrus pectin showed marked regression in tumor growth. Although pectins were initially recognized as compounds capable of inhibiting metastatic lesions , recently, pectins have been shown to reduce primary tumor growth (Nangia-Makker

et al. (2002). Also, MCP has been shown to be effective either in vitro or in vivo, or both, against prostate carcinoma, colon carcinoma, breast carcinoma, melanoma, multiple myeloma, and hemangiosarcoma (Glinsky &Raz.,2009). Galectins are specific carbohydrate-binding proteins (lectins) present on the surface of cancer cells. Galectins , especially galectin-3, are closely related to cell to cell adhesion, aggregation of cancer cells in vitro, tumor growth and metastasis in vivo (Dumic *et al*.,2006 & Krzeslak & Lipinska.,2004). It facilitate cell–cell interactions by binding to galactose-containing molecules on neighboring cancer cells (Jakson *et al*.,2007). Moreover, galectins promote angiogenic activity and function as mediators employed by tumor cells to evade the immune response (Azemar *et al*.,2007). Via the mechanism of galectin-3 antagonism, MCP appears to disrupt the processes that allow cancer cells to communicate with one another and block galectin-3 and other molecules from penetrating into nearby healthy tissue to create a new tumor and establish the tumor's blood supply (angiogenesis)(Nicholas.,2009).

Results of antioxidant parameters of TBM group are in agreement with Kumaraguruparan *et al*., (2002) who found that the presence of tumor caused disequilibria of the antioxidant defense system. Moreover, Hayat, (2001) demonstrated that, lipid peroxidation level was significantly increased in blood, liver and tumor tissues of EAC mice when compared with control group. In contrary, Cheeseman *et al*., (1988) who suggested that, there is a decrease rate of lipid peroxidation in liver tumor cell than normal liver cells.
Decline in SOD activity recorded in mice bearing Ehrlich carcinoma was also reported earlier by Sahu *et al*. (1977).They postulated that the loss of Mn-SOD activity could be due to the loss of mitochondria which leads to a decrease in total SOD activity in different tissues of the tumor host. It seems that oxidative damage caused by decreased capacity for H_2O_2 elemination is related to suppressed activity of CAT, as well as to suppressed direct antioxidant action of GSH . This is in agreement with the previous findings that CAT has a more significant role than GPx in protecting erythrocytes against oxidative stress. Some investigators have reported a higher $NO^{\cdot}$ synthase activity in tumors , while some have reported a lower activity. Our result supports the general observation that some malignancies are associated with an increased level of nitric oxide.

Also, our results of treated groups are in harmony with Xiaojian *et al.,* (1997), who reported that pectin could reduce MDA levels and increase SOD in aorta tissue in high fat diet fed rats. In addtition, He and Aoyama (2003)reported that, the addition of pectin to the cystine diet counteracted the activities of the total and Cu,Zn-superoxide dismutase, and of catalase in liver. Moreover, Lien *et al*.,(2008), reported that pectin extracted from citrus and grapefruit peel in laying hens diet increases blood serum SOD activity

But, these results are disagree with Ismail *et al*.,(1999)who reported that, erythrocyte SOD activity was not affected by pectin treatment in hypercholesterolemic rabbits. Marked increase in SOD activity in Irr.CP treated group is in agreement with Ai-zhen and Li-fang., (2007), the bioactivity of SOD and GSH-Px increased in all MCP-fed groups and the level of MDA decreased markedly in hyperlipidemic rats.

It has been suggested that pectin interacts directly with oxidants and free radicals (Khasina *et al.*,2003). The antioxidant activity in pectin could be related to the high galacturonic acid content. It has been reported that a relatively low molecular weight and a high uronic acid content in polysaccharides appeared to increase the antioxidant activity (Chen *et a*l.,2004) and this express the high antioxidant activity of modified citrus pectin (pectin degraded by irradiation) more than citrus pectin without degradation.

Elevation in AST, ALT, GGT , urea and creatinine in plasma of tumor bearing mice agreed with previous studies which have demonstrated that the level of the liver enzymes increased in serum of EAC-bearing mice indicating general toxicity that occurred due to cancer development (Pal *et al*.,2005). The same results were observed by Korver *et al*., (1995) in their study on the liver function in the assessment of head and neck cancer patients. The observed increase in serum urea level in tumor bearing mice are in agreement with the results reported by Hussein and Azab(1997) who observed that, there was a significant increase in plasma urea concentration in tumor-bearing mice, they attributed such increase to the increase in urea production as a result of catabolic effect of tumor. As confirmed by (Kawaguchi *et al.,* 1991) who observed that, creatinine was decreased in tumor-bearing rats as the glomerular lesions progressed, associated with a rise in serum creatinine level. It was previously observed that oxidative damage which appeared as increased lipid peroxidation and inhibition of GSH content, catalase and SOD activity, led to liver and kidney dysfunctions(Borges et al.,2006).

Also, improvement of liver and kidney function in CP and Irr.CP treated group are in agreements with El-Nahal, (2010) who reported that, ALT, AST, GGT activities, serum urea and creatinine were significantly increased in positive control rat groups administrated lead acetate. Low and high esterified pectin significantly decreased the effect of LA on the tested parameters, also, he added that, histopathological examination clearly indicated that high or low esterified pectin eliminated the harmful effect of lead acetate on liver, kidney and brain tissues and showed no significant difference in serum ALT, AST, GGT activities, serum urea and creatinine in treated groups with high and low esterified pectin compared to normal control group.

Our findings in histopathological examination *of liver section* of CP and Irr.CP treated group are in line with Nangia-Makker *et al*., (2002) stated that, there was less tumor burden and metastasis in the MCP-fed nude mice into which human colon carcinoma cells were implanted than in the control mice. Furthermore, Johnson *et al*., (2007) reported that MCP is thought to be useful in the prevention and treatment of metastatic cancer, especially in solid tumors like melanoma and cancers of the prostate, colon, and breast. Scientists believe that MCP works by inhibiting two key processes involved in cancer progression: angiogenesis and metastasis.

In present study, induction of apoptosis due to treatment with Irr.CP which appeared more effective than citrus pectin itself. Several fairly review articles examine in great detail how galactin-3 (Gal-3) protects cancer cells from various forms of apoptosis. Importantly, as Gal-3 exerts its antiapoptotic effects by functioning upon major (i.e., mitochondrial) apoptosis pathways (Nakahara *et al*,2005), it could play a significant role in metastatic cancer cell clonogenic survival. It has been proposed that Gal-3 anti-apoptotic function could be targeted by MCP (Nangia-Makker et al.,2007). It appears that Gal-3, an important regulator of cancer cell apoptosis, suppresses mitochondrial apoptosis pathway (Oka *et al*.,2005 & Fukumori, *et al.*,2006). The enhancement of apoptosis associated with pectin feeding may be caused by modulation of the redox environment that promotes reactive oxygen species-mediated apoptosis (Sanders *et al*., 2004).

Our *in vitro* and in vivo results revealed that treatment with Irr.CP is more effective than treatment with citrus pectin. These results are in agreement with Nicholas.,(2009) who stated that citrus pectin does not have the short polysaccharide chains as MCP, and 'modified' pectin could indicate that the pectin has been altered in some way, but not

necessarily have the shorter polysaccharide chains. MCP provides superior benefits to unmodified citrus pectin because its shorter, galactose-rich polysaccharide chains allow for better absorption and utilization by the body. Further, its galactose-rich side chains allow MCP to bind galactose-binding lectins on the surface of certain cancer cells to help impede cancer adhesion and metastasis (Modified citrus pectin-monograph., 2000).

Conclusions

The overall results indicated that Irr.CP has antitumor activity through induction of apoptosis in Ehrlich carcinoma cells, decrease metastasis and its high antioxidant effect, suggesting that Irr.CP might be a potential alternative agent for cancer therapy.

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2-DE LESS THAN A DAY

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Abstract
Two-dimensional polyacrylamide gel electrophoresis (2-DE) is a milestone for the proteomics research. This method is based on the separation of the proteins according to charge (pI) by isoelectric focusing in the first dimension and molecular size by sodiumdodecylsulfate- polyacrylamide gel electrophoresis in the second dimension.
Because of the efficiency of the separation, this method permits simultaneous fractionation of hundreds or even thousands of proteins.
In the past the 2-DE was a time consuming technique. Now, thanks to developments in the commercial production of the high quality IEF consumables such as ready gels, IPG strips, ready to use sample preparation buffers and high sensitive fluorescent dyes a 2-DE analysis can be accomplished less than 1 day.
Keywords: 2-D electrophoresis

The 2-DE protocol employing 7 cm IPG strips used in our laboratory as follows:

ACTION	DURATION
Tuesday (Start)	**16.00**
Sample rehydration	12 h
Focusing	5 h
Equlibration	30 min
SDS-PAGE	1 h
Staining	3 h
Wednesday (Finish)	**13.30**
TOTAL	21 h, 30 min

Method

I. 2-D electrophoretic analysis of Serum Proteins

a- ***First-dimension (IEF) using the protean IEF cell:*** Immobilized linear pH gradient strips were rehydrated with the serum samples for 12 h at 50 V (2). After rehydration, IEF was conducted by using a Protean IEF Cell for 5 h (3).

b- ***Second-dimension (SDS-PAGE):*** Before the SDS-PAGE, the IPG strips were first equilibrated in 2 ml of equilibration solution 1 for 15 min. Than equilibrated in 2 ml of equilibration solution 2 for 15 min. Finally, the equilibrated IPG strips applied to 4-20% SDS-PAGE gradient gels and were separated at 20 mA constant current/gel for 60 min.

II. Staining of proteins

After fixation in 40% methanol for 30 min, the proteins were stained with syproruby fluorescent protein dye for 150 min. (4).

III. Analysis of gels

The stained 2-D gel was photographed with Gel Doc XR gel imager under UV light and analysed with PDQuest 2D gel analysis software.

Results

All the experiments of the 2DE were accomplished succesfully less than 1 day.

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4. SYPRO® Ruby Protein Gel Stain, Lonza Rockland, Inc.

*In this procedure all equipment's (IEF-Cell, Mini-PROTEAN Tetra Cell, Gel Doc XR, PowerPack Basic) and consumables (IPG strips, 2-D ReadyPrep sample preparation kits, 4-20% gradient TGX gels, kaleidoscope pre-stained molecular weight marker, running and sample buffers) are commercially available (BioRad, USA).

PDQuest: Precise&Reliable Tool for 2-DE Gel Analysis

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Abstract
Two-dimensional gel electrophoresis (2-DE or 2-D electrophoresis) is a form of gel electrophoresis commonly used to fractionate proteins and mixtures of hundreds or even thousands proteins are separated on 2D gels.
The analysis of large number of proteins in a small gel area requires a compatible and reliable software analysis. PDQuest is a 2-DE gel analysis program and engineered for the quantification and analysis of separated protein spots.
This paper describes a simple working guidelines of this programme in 9 steps (Table).
Keywords: 2DE, PDQuest, Gel analysis

Table: Steps of the 2DE gel analysis

Step	Action
1	Cropping the Gel *Open all experiment gels* *Advanced crop*
2	Analysis of the Gels
3	Opening an experiment and loading the cropped gels
4	Spot dedection
5	Optimization of the dedection sensitivity
6	Loading the master (reference) gel
7	Choosing normalization parameters
8	Automatically creation of the analysis set
9	Finalization of the analysis and obtaining the results

Reference

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ASSESSMENT OF GROWTH FACTOR IGF IN CARDIAC DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Abstract
Pluripotent embryonic stem cells (ES) are harvested from the inner cellular mass (ICM) of the preimplantation embryos on E3.5 and cultured thereafter in vitro. ES cells have the ability to remain undifferentiated and proliferate in vitro while maintaining the potential to differentiate into derivatives of all three embryonic germ layers including cardiomyocytes thus becoming an alternative source of functionally intact cardiomyocytes for the treatment of cardiovascular diseases. We used at our experiment the mouse ES cell line CD1EGFP (mouse embryonic stem cells line, gift from, Gocza Elen, Genetic Modification Group, Agricultural Biotechnology Center, Godollo, Hungary) with a normal karyotype, at 12th passages. Is known that insulin-like growth factor-1 (IGF-1) promotes myocyte proliferation. The purpose of this study therefore focused on assessing the effect of IGF on cardiac differentiation of mouse embryonic stem cells. Our results highlight an increase of embryoid bodies with spontaneous contractions and also a positive effect after treatment with cardioactive substances.
Key words: stem cells, differentiation, cardiomyocytes, insulin growth factors

Embryonic stem cells (ESCs), are totipotent cells derived from the inner cell mass of developing blastocysts, are established as permanent lines and characterized by their self-renewal capacity and the ability to retain their developmental capacity in vivo and in vitro (1).

ESCs cells cultivated as embryo-like aggregates, called embryoid bodies (EBs), differentiate in vitro into cellular derivatives of all three primary germ layers of endodermal, ectodermal, and mesodermal origin. Ability of stem cells to differentiate specific depends on a specific set of growth factors, signaling molecules, proteins from the extracellular matrix (ECM) (2,3,4).

Insulin-like growth factor I (IGF I) is essential for normal embryonic growth in mice and was implicated in formation of a functional heart (5,6). IGF-1 induces expression of a number of cardiac-specific transcription factors such as the zinc finger GATA proteins and Nkx-2.5, a coactivator of GATA-4 (7).

The present study we investigated the IGF-1 potential to induce cardiac differentiation of ES cells in the presence of embryoid body formation.

Materials and methods

CD1EGFP cells, kindly provided by ABC (Genetic modification Group, Hungary), were cultured with feeder cells (primary mouse embryonic fibroblast) in Knock'out DMEM (Sigma) supplemented with glutamax (Gibco, 100x), 50 µg/ml streptomycin (SIGMA), 50U/ml penicillin (Sigma), 50mM β-mercaptoethanol (ME) (Sigma), 0.1mM non-essential amino acids (Gibco), 1000 units/ml of leukemia inhibitory factor (Esgro) and 10% ES cell tested fetal calf serum (FCS) (HyClone). For induction and embryoid bodies (EBs) formation,

the ESCs were dissociated and resuspended in differentiation medium Iscove's Modified Eagle Medium (IMDM) supplemented with 0.6m/m% penicillin, 1m/m% streptomycin and 20% FCS was employed. MTG (monothyoglycerol) 3µl/ml was always freshly added to the differentiation medium. EBs was formed in hanging drops of without LIF (400 cells in 20 µL of medium). After 4 days, EBs were plated on gelatin-coated dishes and cultured for 5 days.

IGF-1 (Recombinant Human Insulin-like Growth Factor – 1), (Gibco) was added to culture medium at two final concentrations 10ng/ml and 20 ng/ml. Cultures were examined daily and the percent of beating EBs was recorded for 3 weeks after plating, in both control and IGF-1 treated groups.

Spontaneously beating EBs was investigated at three distinct developmental stages: an early differentiation phase (shortly after initiation of contractions (5+3d), an intermediate phase (day 5+9d) and terminal differentiation phase (5+12d). To assess the functionality of cells derived by differentiation the cultures were treated with chronotrop substances namely Isoprenaline, Phenyleprine and Carbachol, at three developmental stages. For evaluating the cardiac differentiation, the contracting EBs was fixed using 4% paraformaldehyde for immunostaining. Antibodies used in this study included: VEGF, GATA-6 and alfa actinin.

Results and discussions

In the study two concentrations of IGF-1, 10, 20 ng/mL, were administered to promote differentiation into cardiomyocytes. As a preliminary stage of differentiation the CD1EGFP cells were aggregated to obtain embryoid bodies. The EBs are able to differentiate into embryonic germ layers (endoderm, ectoderm and mesoderm). The principle of obtaining EBs is based on preventing ES attachment to the cultivation surfaces. Standard methods of prepare EBs are the hanging drops method and in static suspension culture to allow small scale formation of aggregates (8). These culture systems are capable to maintain a balance between ESCs cell aggregation and prevention of EBs agglomeration (8,9).

After treatment**s** in both groups (treated and untreated) were identified spontaneous and rhythmic contractile activity in the early stage of differentiation (2-4 days after cultivation on gelatin coated plates**).** Cells with spontaneous contraction are located within a well definied portion.

After advancing the differentiation period EBs treated with 20 ng/ml IGF-1 are increasing portion size of EBs with spontaneous contraction, which towards the terminal stage of differentiation including almost all EBs. At EBs treated with 10 ng/ml IGF-1 were observed a decrease of EBs with spontaneous differentiation at terminal stages, same behavior were observed for untreated EBs. The frequency of spontaneous contractions in cardiomyocytes in the experimental groups was lower **(**26.25-36.25**%)** than the control group **(**50.0% - 86.0%). Compared with concentration of 20ng/ ml of IGF-1 the concentration of 20 ng/ml of IGF-1 was most effective to induce differentiation (fig.1).

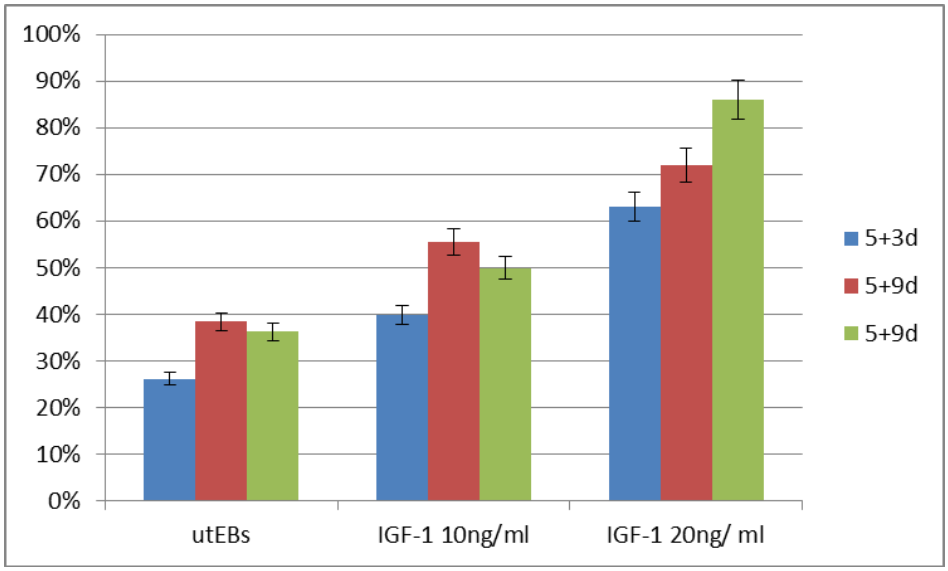

Fig. 1 - Efficiency of cardiomyocyte differentiation in EBs (untreated EBs and EBs treated with insulin growth factor)

To evaluate the functionality and pharmacological response of ESCs-derived cardiomyocytes, the EBs (n=25) with spontaneous beating cardiomyocytes in both control and IGF-1 groups were treated with a 10^{-5} M concentration of isoprenaline, ß1 adrenergic receptors agonist, phenylephrine, α1 adrenergic receptors agonist, carbachol and muscarinic cholinoceptor agonist at 3 distinct developmental stages of differentiation.

Contracting clusters from the embryoid bodies in both control and IGF-1 treatment groups reacted positive or negative chronotropic, from the early stage (day 7 + 3) of differentiation. The presences of cardiomyocytes in both experimental groups were also confirmed by immunocytochemistry (fig.2). Expressions of cardiac specific markers were significantly increased after treatment with IGF-1 (20ng/ml).

After treatment with cardioactive substances the rate of beating was subsequently monitored. After application of isoprenaline positive chronotropic effect was revealed in early stage of differentiation**.** By increasing the level of developmental stage more changes were observed in beating frequency after administration of cardioactive substances. The EBs treated with 20ng/ml IGF-1 also recorded more pronounced response to isoprenaline and a decrease of contraction after treatment with carbachol. Phenylephrine enhanced the rate of beating frequency at all the developmental stages in both groups. The control and IGF-1 treated groups showed a positive staining for VEGF, GATA-6 and alfa actinin.

Cardiac differentiation is a dynamic process consisting of complex growth factors, and various signaling pathways have been implicated in the development of specialized cardiac subtypes. The differentiation of pluripotent stem cells toward cardiomyocytes is still poorly defined. Many differentiation protocols have been described to generate cardiomyocytes from pluripotent stem cells. Some of these protocols and studies demonstrate how the exposure of various growth factors to pluripotent stem cells, at an accurate timing

and dose, is essential for directing the differentiation process from early mesendoderm via mesoderm towards a more specific cardiac fate (10).

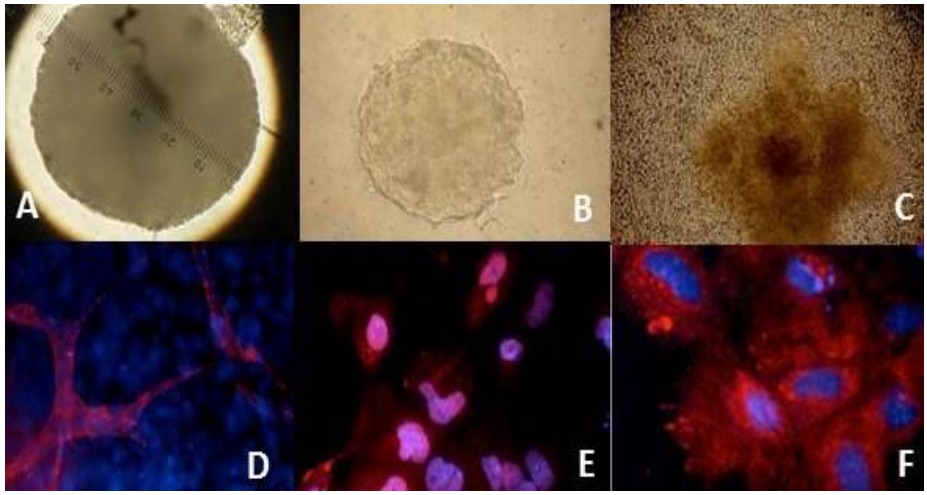

Fig. 2 -The embryoid bodies and positive immunostaining with VEGF, GATA-6 and alfa actinin, **A.** Embryoid bodies aggregated in hanging drops, **B.** EBs after plating on gelatin coated plates, **C.** EBs with spontaneous contracting, **D.** positive staining for VEGF, **E.** GATA-6, **F**. alfa actin

In conclusion, our results demonstrate that the treatment with IGF-1 in suspension period has an inhibitory effect on cardiomyocyte differentiation from ESCs, and also lead to a reduction in the total number of cardiomyocytes per EBs.

Our results demonstrate that CD1EGFP cell line in addition with IGF-1 can be efficiently differentiated into cardiomyocytes. These conclusions are based on the ability of contractility**,** response to cardioactive drugs (isoproterenol, phenylephrine, carbachol) and specific expression of proteins**,** signal molecules and transcription factors**.** The cardiomyocytes cultured in IGF-1 responded better to isoprenaline and phenylephrine, while the rate of beats in response to carbachol reduced more in the cardiomyocytes cultured in IGF-1 in comparison to the ones grown in the control group.

A major challenge of embryonic stem cell biotechnology refers to the identification of soluble growth factors**,** transcription factors and signal molecules able to induce selective differentiation of ESCs cells to specific lines including cardiac differentiation**.** By elucidating these specific mechanisms these cells may be specific tools with major importance for regenerative therapy**.**

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THE MORPHOLOGY OF THE APPENDICULAR SKELETON IN JAGUAR OR AMERICAN LEOPARD (*PANTHERA ONCA*)

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Abstract

In order to described the anatomical configurations were used bone from a specimen of a male jaguar (Panthera onca), with melanism from Bucharest Zoo. Animal weight was about 60 kg, 140 cm in length and 24 years old. As a protected species find it useful to describe the morphology of this, this is useful for veterinarians dealing with health insurance of animals in zoos, parks and protected areas. Bones were obtained after removal of soft tissue, the pieces obtained were subjected to maceration process by which any remaining soft tissue was removed, after which they were washed and dried. Bones were measured and described in accordance with Nomina Anatomica Veterinaria 2005. Should be noted that the jaguar is a protected exotic animal and we can study them only in rare cases. The scapula has a coracoid process longer than that of other great feline. The greater development of the medial border of the distal epiphysis of the humerus in jaguar in relation to pantherins could be related to the presence of stronger flexor muscles. In general, calculated ratios are relatively constant and can provide information about the provenance of the bones from the similar in size feline species.

Key words: jaguar, bones length, scapula, radius

Regardless of origin, the feline were the subject of many legends, but only jaguar dominated the religion and culture of the people from South American continent.

Jaguar (Panthera onca) is one of the biggest cats in Central and South America. Morphology is similar to leopard (Panthera pardus), but the jaguar is the largest and most powerful. On the jaguar's back and sides, large black or dark brown clusters of spots, called rossetes, or irregularly shaped blotches mark a background color that varies from pale yellow to tawny. Melanism appears to be more common among jaguars than any other large cats; on these blackish brown or black individuals the rosette pattern is ofen visible beneath the dark background coat color (1,4).

In the wild, the dimensions and body mass of males range from 41-102 kg (the most massive is found in the Pantanal region - Brazil and Venezuela); the females being slightly lower.

Jaguars are typically found in a variety of tropical and subtropical habitats from the sea level to about 1 200meters elevations (3 800meters in Costa Rica). While the jaguar is commonly thought of as a denizen of dense tropical forest, it also inhabits a wide range of other habitat types, including arid scrub and swampy grasslands, and has been recorded from montane forest, lowland evergreen forest, dry deciduous forest, and mangrove swamps (2,3,5).

Materials and methods

In order to described the anatomical configurations were used bone from a specimen of a male jaguar (Panthera onca), with melanism from Bucharest Zoo. Animal weight was about 60 kg, 140 cm in length and 24 years old. As a protected species find it useful to describe the morphology of this, this is useful for veterinarians dealing with health insurance

of animals in zoos**,** parks and protected areas**.** Jaguar is included in Annex I of CITES (the Convention on International Trade in Endangered Species of Wild fauna and Flora 1973).

Fig. 1 Left scapula in Panthera onca –lateral aspect
L=19 cm; l = 9 cm; L/l = 1,72
a = 6,5 cm; b= 8,5 cm; a/b = 0,76

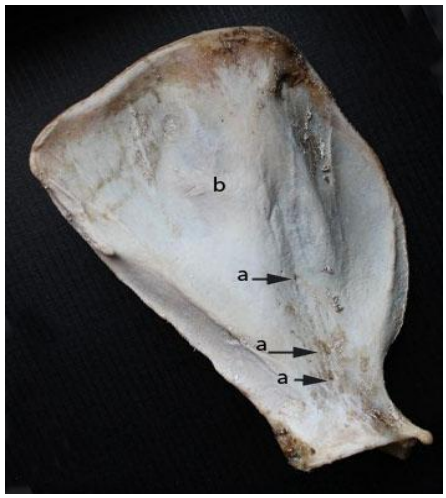

Fig. 2 Left scapula in Panthera onca –medial aspect
a = vascular holes; b-fosa subscapularis

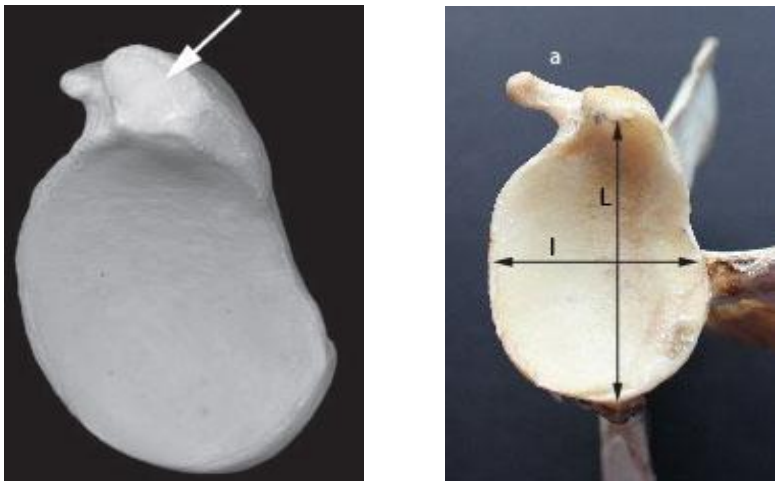

Fig. 3 Glenoid cavity in Panthera pardus – left (after Salesa et col. 2010) and Panthera onca – right (original)
a-coracoid process; L/l = 1,33

Bones were obtained after removal of soft tissue, the pieces obtained were subjected to maceration process by which any remaining soft tissue was removed**,** after which they

were washed and dried**.** Bones were measured and described in accordance with Nomina Anatomica Veterinaria 2005. Should be noted that the jaguar is a protected exotic animal and we can study them only in rare cases.

Results and discussions

Scapula in Panthera onca rear looks like in other big cats. Bone length (measured dorso-ventral) on the dorsal edge (taking the direction of the spina scapularis) to acromion is 19 cm; width at half of shaft is 9 cm, the ratio of these dimensions is 1,72. Distance from the middle cranial edge to the scapular spine is 6,5 cm, and the distance from the dorsal edge of the spina scapularis to the thoracic angle is 8,5 cm, the ratio of the two dimensions of 0,76 cm (Fig. 1). On the average, we observed three vascular holes, the proximal being located approximately halfway between the dorsal edge and the glenoid cavity (Fig. 2). Coracoid process is longer than other cats. The ratio of the length and width of glenoid cavity is 1,33.

Fig. 4 The left humerus in Panthera onca
L= 23,5 cm; l=3 cm; L/l =7,83; a-the crest of great tubercle; b-deltoidian crest; c-great tubercle; d-epicondylar hole; e-trochleea; f-condyle.

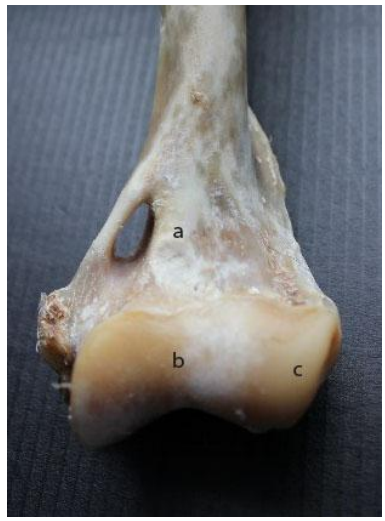

Fig. 5 The distal extremity of humerus in Panthera onca; a- epicondylar hole; b-trochleea; c-condyle.

Humerus has a 23,5 cm in length and 3 cm width at the middle of the shaft , the ratio being 7,83. The crest of great tubercle and deltoid crest meet to the middle of cranial edge of shaft. The ratio of the length and width of proximal epiphysis is 1,19 and of the distal

epiphisis 0.64 (Fig. 4). The epicondylar hole is oval, large, enclosed by a strong bony bridge has diameters ratio of 1.85(Fig. 5).

Radius is 19,5 in length and and 2 cm width at the middle of the shaft, the ratio of the length and width is 9,75. Proximal extremity of the glenoid cavity has two diameter ratio of 1,45 (Fig. 6).

Ulna is heavier than radius and has 24 cm in length. The ratio beetwen the length of the ulna and olecranon height (measured from the base of coronoid process to the tuberosity of the olecranon is 3,33 (Fig. 6). Unlike other cats which have equal the two tubers from the olecranon tuberosity, or sometimes the medial is developed, in Jaguar look is opposite, lateral tubercle being developed (Fig. 7). The opening of trochlear notch has the same size as the distance between the lateral and medial peaks coronoide processes.

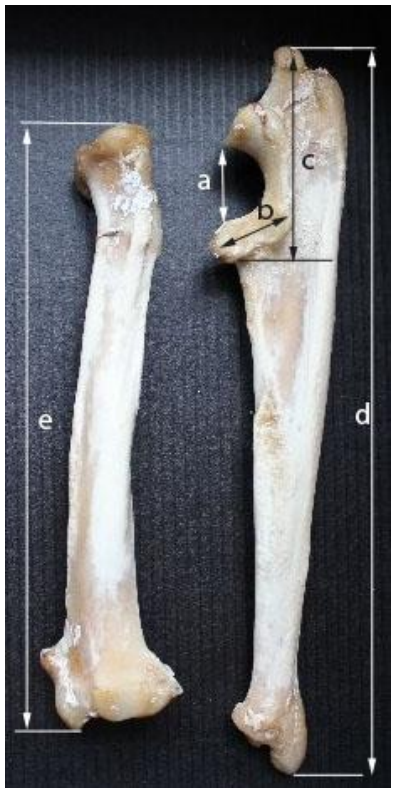

Fig. 6 The left radius and ulna in Panthera onca; a=b; d=24 cm; d/c=3.33; c-condyle; e=19,5 cm.

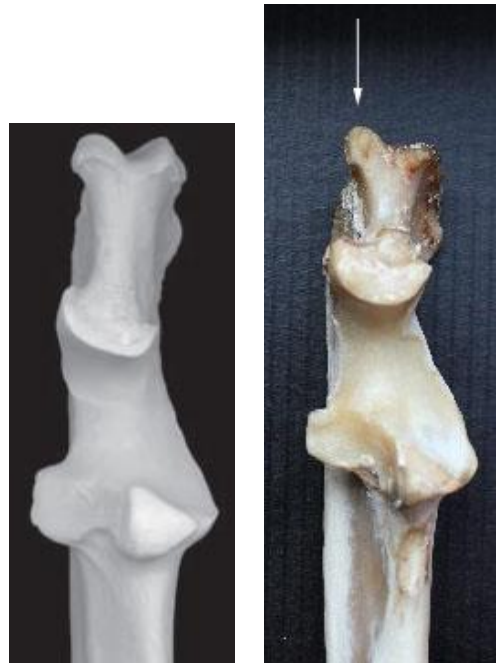

Fig. 7 The olecranon of left ulna, cranial view in Panthera pardus (left) and Panthera onca (right)

Conclusions

1. The scapula has a coracoid process longer than that of other great feline. The coracoid process is the origin area of the muscle coracobrachialis which would be larger than that of a pantherin of similar size;
2. The greater development of the medial border of the distal epiphysis of the humerus in jaguar in relation to pantherins could be related to the presence of stronger flexor muscles;
3. The greater development of the lateral tubercle from the olecranon show that there is another report in the development of triceps muscle portions;
4. In general, calculated ratios are relatively constant and can provide information about the provenance of the bones from the similar in size feline species.

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RESEARCH REGARDING THE STRUCTURE AND SOME SYNTHETIC QUALITY INDICES OF PHARAON QUAIL EGGS DEPOSITED AT THE PEAK PHASE OF THE LAYING PERIOD

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Abstract
From a group of 20 Pharaon japanese quail (Coturnix coturnix japonica) of 95-105 days old and with a body weight of 245.136 grams that were at the peak phase of the laying period, we took 200 eggs, which were studied in terms of weight, structureand synthetic idices that characterize their quality. As working methods we used:gravimetric, biometric (ovometric), mathematical and statistical methods.We obtained the following results: the weight of quail egg mineral shell was of 0.796± 0.007 grams, which represents 6.845±0.054 % from average weight of whole egg (11.668 grams); the albumen weight of these quail eggs was of 7.084±0.06 grams, which represents 60.21±0.27 % from whole egg weight; the yolk quail egg weight was of 3.888±0.046 grams, namely 32.98±0.26 % from whole egg weight. The carotene content of the quail egg yolk was estimated at 15.144±0.113 mcg per gram. The synthetic quality indices of these eggs had values of 0.0334 g/cm^2, for MSI (Mineral Shell Index), of 0.080, for the AI (Albumen Index) and of 0.4497, for the YI (Yolk Index). The Haugh Index (HI) had an average value of 92.31±0.39 HU.

Keywords: egg structure, Pharaon, quail, haugh index

Introduction

Poultry eggs are, for humans, a valuable source of protein, essential amino acids, vitamins of the "B" complex, phosphorus, phospholipids, triglycerides and cholesterol. The last of them are however organic substances incriminated in a number of diseases of the digestive, cardiovascular and nervous systems, leading to some restrictions in the consumption of these products (eggs). Quail eggs contain large amounts of fat soluble vitamins (A, D, E, K) and hydrosoluble vitamins ("B" complex), micro and macro minerals, essential amino acids (lysine, tryptophan, arginine, methionine, cystine, etc.) but are low in cholesterol and triglycerodes and are considered to be a true "cure-all", placed 3rd in the Chinese traditional medicine, after "GinSeng" and viper venom [6]. Quail eggs are very good in the prevention and treatment of many diseases of the heart and blood vessels, stomach and liver, in combating stress and are well known and applied in different treatment regimens [1], [2].

Japanese quails are birds with a high egg production, there are already specializes breeds for this, as there are for meat. "Pharaon", the breed we studied, is a meat-type one, but produces about 200-220 eggs per year. These eggs, produced at the peak phase of the laying period [11], [12] have undergone a larger study which concerned aspects of morphology and structure of eggs, their quality, chemical composition, caloricity and sanitation.

Material and methods

This research has required a variety of biological and non-biological materials. The biological materials represented by Pharaon quail breed, which are birds specialized for meat production, with a body weight of 235-245 grams, the females and 200-210 grams, the males, had an age of about 95-105 days in the peak stage of the laying period, when we harvested the eggs for our study (tab. 1).

From these quails (20 females) we collected, within 10 days, 200 eggs, which have been cleaned, individualized, weighed, measured with a caliper (diameters) and with a braid centimeter (circumferences) and after that they were broken to determine their structure.

The birds from which we collected the eggs were normally developed, clinically healthy, with intact and specific colored plumage and a good appetite. The eggs collected from these quails had weights of 12 to 13 grams, a large diameter (length) of 30-34 mm, a small diameter of 24 to 26 mm and their coloration was specific and characteristic for this species, with a intact mineral shell without defects (fissures, cracks, etc.) (tab. 1).

Table 1. The biological material that we used in our research and the main purpose of our study

Specification		**MU**	**Laying stage observed and purpose**	
			Peak of laying period	**Purpose**
Pharaon quail breed	Bird's age	days	95 - 105	Normal size egg harvested
	Number of individuals	heads	20	Normal dimensions and weight without defects outside of the egg
	Bird's weight	grams	245	
Pharaon quail eggs	The number of harvested eggs	pieces	200	Determination of egg structure and of sintetic quality indices of quail eggs
	Egg weight	grams	12 - 13	
	Egg longitudinal diameter	mm	30 - 34	
	Egg transversal diameter	mm	24 - 26	
	Egg colour	-	Characteristic and normal of egg pigmentation	

Non-biological materials were represented by: digital scales, ovens, DMC Camera, calipers, rulers, forceps, pipettes, measuring glass cylinders of varying capacities, Erlenmeyer and Berzelius glass recipients, glass funnels and Petri dishes.

After breaking the eggs, we removed the mineral shell and the two shell membranes, which were washed with distilled water (to remove any albumen remains), dried in an oven at +65°C, cooled and then weighed with great accuracy [6]. The yolk was extracted from dense and fluid egg albumen, then measured in diameter an height, in milimeters, with a caliper device [6], [7].

The weight of egg albumen (EW) (grams) was determined by mathematical methods, using the following formula: (1) EW = TEW – (MSW + YW), where: TEW = Total Egg Weight (g); MSW = Mineral Shell Weight (g); YW = Yolk Weight (g). The proportion of the three egg components (mineral shell, albumen, yolk) was determined with the following formulas: (2) MSP = $\frac{MSW\ x\ 100}{TEW}$, (3) AP = $\frac{AW\ x\ 100}{TEW}$, (4) YP = $\frac{YW\ x\ 100}{TEW}$, where: MSP, AP, YP = proportion of mineral shell, of albumen and of yolk (%); MSW, AW, YW = weight of mineral shell, of albumen and of yolk (g) [6].

The carotene quantity from yolk was estimated using the color notes given by a "La Roche" scale and then with: (5) CC(mcg) = 2 x LRn + 1, where: CC = carotene content in

mcg (micrograms) per gram of yolk and per whole yolk; LRn = "La Roche" scale notes [11], [12].

The synthetic quality indices that we used for these quail eggs were: (6) MSI = $\frac{MSW}{MSS}$, where: MSI = Mineral Shell Index (g/cm^2); MSW = Mineral Shell Weight (g; mg); MSS = Mineral Shell Surface (cm^2); (7) AI = $\frac{h}{(D+d)/2}$, where: AI = Albumen Index; h = dense albumen height (mm); D = large diameter of total albumen (dense and fluid) (mm); d = small diameter of total albumen (dense and fluid) [11], [12]. (8) YI = $\frac{YH}{YD}$, where: YI = yolk index; YH = Yolk Height (mm); YD = Yolk Diameter (mm) [11], [12]. (9) HI = 100 x Log (h – 1,7 x TEW^{0,37} + 7,57), where: HI = Haugh index (%); h = dense albumen height (mm); TEW = Total Egg Weight (g); 1,7 și 7,57 = mathematical index [6], [7], [11], [12].

All the data obtained after the weighting, measurements and calculation have been included in specific tables and then they were statistically processed. We followed: the mean ($\bar{x}$), standard deviation (s); standard error of the mean ($s\bar{x}$), variance (s^2) and the variance coefficient (V%) [6], [8].

Results and discussions

The results obtained by us in this study refers to, on one hand, at the weight and structure of Pharaon quail breed eggs and, on other hand, to all quality synthetic indices of these eggs. Thus, the eggs studied by us had an average weight of 11.668±0.083 grams (v=10.08 %), and from this weight, the mineral shell (with the two shell membranes) represents 6.845±0.054 %, which means that the shell weighs an average of 0.796±0.007 grams (tab. 2) (fig. 1 and 2). The data variability on weight and the proportion of the mineral shell had average values (v=12.36 %; v=11.21 %) (tab. 2).

Table 2. Statistical indicators for Pharaon quail eggs weight and structure

Specification	MU	n	Statistical indicators calculated			Variation limits	
			$\bar{x}\pm s\bar{x}$	s	V(%)	minimum	maximum
Whole egg weight(TEW)*	grams	200	11.668±0.083	1.1762	10.08	8.41	15.10
Egg shell weight	grams	199	0.7958±0.007	0.0984	12.36	0.5519	1.092
Egg shell proportion (from TEW)	%	199	6.845±0.054	0.7675	11.21	4.7934	8.8425
Albumen weight	grams	166	7.084±0.0615	0.7924	11.19	4.8283	9.0729
Albumen proportion (from TEW)	%	166	60.211±0.270	3.480	5.78	45.907	73.949
Yolk weight	grams	166	3.888±0.046	0.5932	15.26	2.550	5.670
Yolk proportion (from TEW)	%	166	32.984±0.259	3.3364	10.11	26.051	46.283

*TEW =Egg Toal Weight

The albumen of the quail eggs studied by us is their major component, with an average weight of 7.084±0.0615 grams and with limits of minimum 4.8283 grams and maximum of 9.0729 grams (tab. 2).

Reported at egg total weight, the albumen weight represented 45.91 % - 73.95 %, with a statistical mean of 60.21±0.27 % (v=5.78 %) (tab. 2) (fig. 1 and 2).

Concerning the Pharaon quail egg yolk, it has an average weight of 3.888±0.046 grams and the 200 variables considered in our statistical study have shown variation limits of 2.55 grams to 5.67 grams (tab. 2) (fig. 2).

Reported at the whole egg weight, the yolk weight has a mean proportion value of 32.984±0.259 % (v=10.11 %) (tab. 2) (fig. 2).

To calculate the synthetic quality indices of the eggs we measured their albumen and yolk (diameter and height). Thus, the large diameter of dense and fluid albumen has an average of 76.419±0.975 mm (v=16.49 %), while the small diameter of the albumen (dense and fluid) has ranged between 28.85 mm and 79.45 mm, the average of the 167 values considered statistically being 53.726±0.787 mm (v=18.94 %) (tab. 3). The height of the dense albumen has values between 3.00 and 7.35 mm, with a statistic mean of 5.092±0.076 mm (v=19.39 %) (tab. 3). The yolk had a diameter value between 20.40 and 29.70 mm and its statistic mean was of 24.827±0.128 mm (v=6.65 %) (tab. 3). The yolk of these eggs had an average height of 11.125±0.049 mm (v=5.76 %) (tab. 3). Also, we appreciated, by "La Roche" scale the color of egg yolk, giving notes according to this scale from 5.00 to 8.50, their mean being 7.02±0.056 (v=10.30 %). Subsequently, we estimated the amount of carotene in the yolk, so that when the report was made from 1 gram of yolk, the values we found were between 11.00 and 18.00 mcg, with an average value of 15.144±0.113 mcg (tab. 3). Compared to the total egg weight (TEW) the carotene content of quail eggs we studied ranged between 32.12 and 87.04 mcg, with an average content of 58.975±0.862 mcg (v=18.82 %) (tab. 3).

Table 3. Statistical indicators for the albumen and dimensions of Pharaon quail eggs produced at the peak phase of the laying period

Specification		MU	n	Statistical indicators calculated			Variation limits	
				$\bar{x}\pm s_{\bar{x}}$	s	V(%)	minimum	maximum
Large diameter of dense and fluid albumen		mm	167	76.419± 0.975	12.6012	16.49	49.350	111.200
Small diameter of dense and fluid albumen		mm	167	53.726± 0.787	10.1733	18.94	28.850	79.450
Dense albumen height		mm	167	5.092 ± 0.076	0.9876	19.39	3.000	7.350
Yolk diameter		mm	167	24.827± 0.128	1.6504	6.65	20.400	29.700
Yolk height		mm	167	11.125± 0.049	0.6406	5.76	9.150	12.400
Yolk colour		LRn*	167	7.072 ± 0.056	0.7286	10.30	5.000	8.500
Egg carotene content	per gram of yolk	Mcg	167	15.144± 0.113	1.4573	9.62	11.000	18.000
	per whole yolk	mcg	167	58.975± 0.862	11.1005	18.82	32.120	87.040

*LRn = La Roche note

Regarding the albumen index (AI), which is the ratio between height and diameter of its two component parts (dense and fluid albumen) and which shows the eggs age and freshness as a factor influencing their quality, we obtained values ranged from a minimum of 0.0392 and a maximum of 0.1476, with an average of 0.080±0.0015 (v=24.87 %) (tab. 4). It is estimated [12] that this index indicates that the albumen of eggs decreases with the aging process of eggs and is presented in the scientific literature with values of 0.0480 – 0.0636, depending on the phase of the laying curve [6]; [8]; [9]; [10]. The values we obtained for this index allows us to say that the eggs studied here were relatively fresh during their processing moment.

Table 4. Statistical indicators for the quality sintetic indices of Pharaon quail eggs produced at the peak phase of the laying period

Specification		**MU**	**n**	**Statistical indicators calculated**			**Variation limits**	
				$\bar{x}\pm s_{\bar{x}}$	**s**	**V(%)**	**Min.**	**Max.**
Quality sintetic indices	Mineral shell index	g/cm^2	167	0.0334± 0.0002	0.00349	10.47	0.0243	0.0422
	Albumen index	-	167	0.080± 0.0015	0.0199	24.87	0.0392	0.1476
	Yolk index	-	167	0.4497± 0.003	0.0355	7.89	0.3741	0.5379
	Haugh index	HU *	167	92.31± 0.39	4.9811	5.40	80.30	103.08

*HU = Haugh units

Regarding the synthetic index for assessing the quality of Pharaon quail eggs, they were based on specific mathematical relationship using preliminary data that have been already commented.

Thus, the mineral shell index (MSI) has values of 0.0243 g/cm^2 to 0.0422 g/cm^2, with a statistical mean of 0.0334±0.0002 g/cm^2 (v=10.47 %) (tab. 4).

Concerning the yolk index (YI), which is the ratio between the height and the diameter of this egg component and which also offers us clues regarding the freshness of eggs and their incubation quality. This index had values between 0.3741 and 0.5379, with a statistical mean of 0.4497±0.003 (v=7.89 %) (tab. 4). Based on these values we can say again that the eggs collected during the peak phase of the laying period deposited by Pharaon quail females were fresh.

Finally, as a corollary of quail eggs quality and more, we calculated and appreciated the Haugh Index (HI), which has values between 80.30% and 103.08%, with a statistic mean of 92.31±0.39 UH (%) (v=5.40 %) (tab. 4).

All values obtained by us on the structure and the synthetic quality indices of Pharaon quail eggs are presented in table 5, as a mirror of the overall assessment of the quality of these eggs.

Table 5. The structure and some quality synthetic indices of Pharaon quail eggs produced at the peak phase of the laying period

Specification		MU	Obtained values	Specification		MU	Obtained values
Whole eggs weight		g	11.668	Yolk diameter		Mm	24.827
Egg structure	Mineral shell weight	g	0.796	Yolk height		Mm	11.125
	Mineral shell proportion	%	6.845	Yolk color		LRn*	7.072
	Yolk weight	g	3.888	Egg carotene content	per gram of yolk	Mcg	15.144
	Yolk proportion	%	32.984		per whole yolk	Mcg	58.975
	Albumen weight	g	7.084	Quality synthetic indices	Mineral shell index	g/cm^2	0.0334
	Albumen proportion	%	60.21		Albumen index	-	0.080
Albumen dimensions	Large diameter of dense and fluid albumen	mm	76.419		Yolk index	-	0.4497
	Small diameter of dense and fluid albumen	mm	53.726		Haugh index	HU**	92.31
	Dense albumen height	mm	5.092				

**LRn = La Roche note; **HU = Haugh units

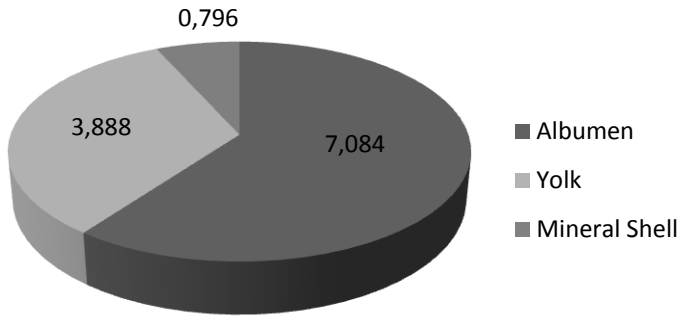

Fig. 1. The weight (grams) of the three quail egg components: albumen, yolk and mineral shell

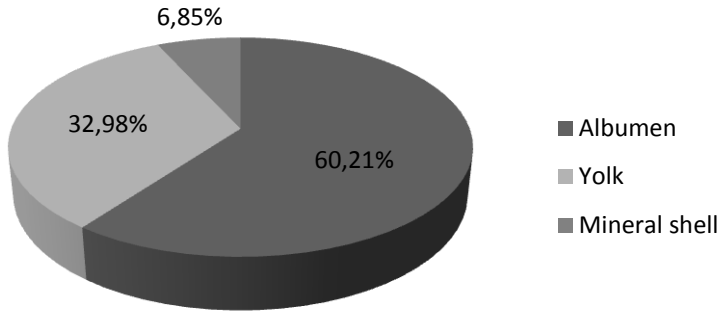

Fig. 2. The proportion (%) of the three quail egg components: albumen, yolk and mineral shell

Conclusions

1. The Pharaon quail eggs produced at the peak phase of the laying period had the following structure: 6.845 % mineral shell (0.796 g); 60.21 % albumen (7.084 g) and 32.98 % yolk (3.888 g).
2. The albumen of the quail eggs studied by us had dimensions of: 76.419 mm, for the large diameter; 53.726 mm, for the small diameter and 5.092 mm, for the albumen dense part height.
3. The yolk of Pharaon quail eggs produced at the peak phase of the laying period had an average diameter of 24.827 mm and a height of 11.125 mm.
4. The yolk carotene content of these quail eggs had values of 15.144 mcg/g or of 58.975 mcg/whole egg (yolk).
5. The mineral shell index of the quail egg studied by us was of 0.334 g/square centimeters; the albumen index had a value of 0.080 and the yolx index was of 0.4497.
6. The Haugh synthetic index of these quail eggs had an average value of 92.31 % (HU).

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RESEARCH REGARDING THE MORPHOLOGY OF QUAIL EGGS FROM PHARAON BREED, PRODUCED IN THE PEAK PHASE OF LAYING PERIOD

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Abstract
From a number of 20 domesticated quails from the Pharaon breed, with a age of 95-105 days and a average weight of 245.136±5.378 grams, within the peak of the laying period, have been harvested 200 eggs. These eggs have been studied in terms of their weight and dimensions; volume; surface; density and shape. A couple of methods have been used for the determinations: gravimetry, biometry, mathematical methods (volumetry, density, Anova Single Factor). The following results have been obtained: the eggs produced by the quails in the peak period of laying had a average weight of 11.668±0.083 grams; the longitudinal diameter was 32.841±0.113 mm, and the transversal diameter was 25.703±0.0627 mm. The large circumference of these eggs was of 9.1927 cm, and the small one of 8.1037 cm. The shape index had values of 1.278/1 (78.38%). The eggs surface had a average value of 23.862±0.115 cm^2, and the volume was of 11.295-11.317 cm^3, depending on the measuring method used. The density of studied quail eggs was 1.0328±0.0043g/cm^3, and their specific weight was 0.9714 cm^3/g.

Keywords: eggs; quail; Pharaon; volume; weight

Introduction

Poultry eggs represent one of the most complete and complex source of proteins, aminoacids, lipids and vitamins for the human body, having a height digestibility. The quail eggs are no exception, being very rich in B vitamins (tiamin, riboflavin, cabalamin, etc), phosphorus, iron, and poor in cholesterol (10 times lower then the hen eggs), having a caloricity of 17-19 kcal/egg [1]. The domestic quails can produce in one year 300-350 eggs with a average weight of 10-12 grams [3];[4]. The production is influenced by breed and other factors. There are already breeds and genetic lines of quails specilized in meat and eggs production. The Pharaon breed, that makes the subject of this study, is a meat breed, but it produces also eggs (220 eggs/year) with a average weight of 12-14 grams [5];[7]. The body weight of Pharaon quails is 235 g for females and 190-200 g for males [6];[7];[15]. The laying is starting at the age of 50-55 days [6]. In this context is included the present study, in which we followed the structure, morphology and chemical composition of Pharaon quail eggs produced in the peak of laying period.

Material and method

The materials used in this study have been biological and non-biological. The biological ones were represented by quails from Pharaon breed and eggs harvested from them. Thus, the 20 japanese quails from Pharaon breed, had body weights of 211.53 – 301.0 grams and ages of 95 – 105 days, being in the peak phase of laying period. The birds were normal developed, with a healthy plumage cover of a specific color. The appetite was normal. From these quails 200 eggs have been harvested, cleaned, individualized, weighed and measured with a caliper (diameter) and a measuring tape (circumferences). The eggs were introduced in a 200 ml measuring glass cylinder with water, for the volumes determination. After, these eggs were broken for the determination of structure and chemical composition. The weight of these eggs was of 8-15 grams, and the dimensions were 28-38 mm

(longitudinal diameter) and 23-28 mm (transversal diameter). The mineral shell was intact, without cracks and defects, with a specific color. The non-biological materials were represented by digital scales, ovens, photo camera, computer, instruments (calipers, magnifying glasses, measuring tape, tweezers, ruler), glass instruments (measuring cylinder, Berzelius and Erlenmeyer glasses, funnels, Petri dishes, pipettes), different chemical reagents. The eggs (whole eggs and the three components) were weighted on a Shimatzu-UX 4200H digital scale, with a precision of 4 decimal places. The measuring of the eggs was made with a Toya-15100 caliper and a measuring tape. For the circumference and shape indexes, volume, surface, density and specific weight of eggs, a series of mathematical relations were used as follows:

(1) $TD\bar{x} = \frac{TD1+TD2}{2}$, where $TD\bar{x}$ is the average of the two transversal diameters (mm);

(2) $SI = \frac{LD}{TD\bar{x}}$, where SI is the format index (x/1); LD is the longitudinal diameter (mm)[13];[14];

(3) $SI(\%) = \frac{TD\bar{x} \times 100}{LD}$; (4) $CI = \frac{LC}{SC}$, where CI is the circumference index (x/1); LC is the large circumference (cm); SC is the small circumference;

(5) $CI(\%) = \frac{SC \times 100}{LC}$; (6) $V = 0{,}519 \times LD \times TD\bar{x}^2$, where V is the egg volume (cm^3), 0,519 is the calculation coefficient for volume [2];

(7) $S = 4{,}56604 \times W^{0{,}67352}$, where S is the surface of the egg (cm^2); W is the egg weight; 4,56604 and 0,67352 are calculation coefficients;

(8) $D = \frac{W}{V}$, where D is the density of eggs (g/cm^3); (9) $SW = \frac{V}{W}$, where SW is the specific weight (cm^3/g).

All data obtained from weighings, measurements and calculations, were put in tables and statistically processed, the following indices being obtained: mean ($\bar{x}$); standard error of mean ($s\bar{x}$); standard deviation (s); variance (s^2); variation coefficient (v%)[12].

By comparison, this study took in consideration some results previously obtained [7];[8];[10]; [11] on the quail eggs from O-Baloteşti breed.

Some morphological indices of the quail eggs obtained from the two breeds were compared in the end of this study, the percentage differences being established.

Results and discussions

The results obtained refers to weight, dimensions, surface, volume and density of quail eggs from Pharaon and O-Baloteşti breeds, produced in the peak phase of laying period. Thus, the mean weight of the Pharaon quail eggs was 11.668±0.083 grams (v=10.08%), the limits of the 200 values introduced in calculations being of 8.41 and 15.10 grams (table 1) (fig. 1). The longitudinal diameter of these eggs had an average of 32.841±0.113 mm (v=4.87%), having limits of 28.50 mm and 37.50 mm (table 1) (fig. 1).

The two transversal diameters had means of 25.703±0.063 mm, with variation limits between 23.20 mm and 28.35 mm (v=3.45 – 3.47%) (table 1). The large circumference of these eggs had a statistic mean of 9.1927±0.0254 cm (v=3.91%), and a small circumference of 8.1037±0.023 cm (v=4.02%) (table 1).

Table 1. Statistical indicators for the weight and dimensions of Pharaon quail eggs, produced in the peak phase of the laying period

Specification		MU	n	Calculated statistical indicators			Variation limits	
				$\bar{x}$±s$\bar{x}$	s	v(%)	min	max
Eggs weight		grams	200	11.668±0.083	1.1762	10.08	8.41	15.10
Eggs dimensions	Longitudinal diameter (LD)	mm	200	32.841±0.113	1.6003	4.87	28.50	37.50
	Transversal diameter 1 (TD1)	mm	200	25.703±0.063	0.8930	3.47	23.20	28.35
	Transversal diameter 2 (TD2)	mm	200	**25.703±0.063**	0.8881	3.45	23.25	28.30
	Mean of the two transversal diameters (TD$\bar{x}$)	mm	200	25.703±0.062	0.8879	3.45	23.22	28.32
	Large circumference (LC)	cm	200	9.1927±0.025	0.3593	3.91	8.19	10.05
	Small circumference (SC)	cm	200	8.1037±0.023	0.326	4.02	7.08	9.48

The shape of these quail eggs is ranged in the cartesian oval, this aspect being illustrated by the two values of the shape index, but also by the two values of the circumference indices (table 2) (fig. 1). The shape index had a mean value of 1.278/1 (v=4.05%) or 78.38±0.0225% (v=4.06%). For the circumference index, the mean values have been of 1.135/1 (v=2.96%) or 88.18±0.19% (v=2.98%) (table 2).

The egg surface of the Pharaon breed produced in the peak phase of the laying period had a average value of 23.862±0.115 cm^2, with limits of 19.169 and 28.419 cm^2 (v=6.81%) (table 3) (fig. 2).

Table 2. Statistical indicators for the shape index and circumference of Pharaon quail eggs, produced in the peak phase of the laying period

Specification	MU	n	Calculated statistical indicators			Variation limits	
			$\bar{x}$±s$\bar{x}$	s	v(%)	min	max
Circumference index (LC/SC)	x/1	200	1.135±0.0024/1	0.0336	2.96	1.00/1	1.270/1
Circumference index ((LC x 100)/SC)	%	200	88.184±0.186	2.6304	2.98	78.23	99.55
Shape index (LD/ TD$\bar{x}$)	x/1	200	1.278±0.0037/1	0.0518	4.05	1.130/1	1.478/1
Shape index ((TD$\bar{x}$ x 100)/LD)	%	200	78.377±0.225	3.1814	4.06	67.66	88.50

The volume of quail eggs from the Pharaon breed, calculated by the relation (6), had a mean value of 11.295±0.084 cm^3 (v=10.47%) (table 3). At the same eggs, the volume calculated by their sinking in water, in the measurement glass cylinder, had values between 8.1 and 14.4 cm^3, the mean of the 200 determined values being of 11.317±0.081 cm^3 (10.09%) (table 3) (fig. 2).

The differences found between the two measurements methods are very small, having a mean of 0.231±0.012 cm^3, which is 2.045% from the average volume calculated by the (6) relation. The density of quail eggs from Pharaon breed, produced in the peak phase of the

laying period, had a mean value of 1.0328±0.0043 g/cm^3 (v=5.94%) (table 3) (fig. 2), and the specific weight of these eggs was of 0.9714±0.004 cm^3/g (v=5.68%).

Table 3. Statistical indicators for the surface, volume, density and specific weight of Pharaon quail eggs, produced in the peak phase of the laying period

Specification		MU	n	Calculated statistical indicators			Variation limits	
				$\bar{x}\pm s_{\bar{x}}$	s	v(%)	min	max
Surface		cm^2	200	23.862± 0.115	1.6239	6.81	19.169	28.419
Volume	Calculated*	cm^3	200	11.295± 0.084	1.1832	10.47	8.1885	14.8150
	Determined**	cm^3	200	11.317± 0.081	1.1421	10.09	8.100	14.400
	Difference between the two volumes	cm^3	200	0.231±0. 012	0.1700	73.59	0.0011	0.7977
Density		g/ cm^3	200	1.0328± 0.0043	0.0613	5.94	0.782	1.436
Specific weight		cm^3/g	200	0.9714± 0.0039	0.0552	5.68	0.6961	1.2778

*volume calculated by relation (6); **volume determined by measurement cylinder.

The quail eggs from the O-Balotești, produced in the peak phase of laying period, had a mean weight of 10.696±0.086 grams, the limits of the 123 values taken in calculations being of 8.37 and13.10 grams (v=8.88%) (table 4) (fig. 1). For the longitudinal diameter, the mean was 31.471±0.12 mm (v=4.10%), and for the two transversal diameters, the mean values were 25.011±0.073 mm and 24.973±0.07 mm (v=3.21-3.23%) (table 4) (fig. 1).

The surface of these eggs had a mean value of 22.509±0.121 cm^2 (v=5.98%) (table 4) (fig. 2), and the calculated volume by relation (6) had a mean value of 10.224±0.083 cm^3 (v=9%) (table 4) (fig. 2).

The quail eggs (O-Balotești breed) density, had a mean value of 1.0465±0.002 g/cm^3 (v=2.21%) (table 4) (fig. 2). At these eggs, the shape index had mean values of 1.260±0.004/1 (v=3.87%), respectively 79.455±0.276% (v=3.85%) (table 4).

Comparatively, the shape indices, weights, dimension, surface and density of the quail eggs from the two studied breeds, we can say that the ones from the Pharaon breed are superior to those from the O-Balotești breed. Thus, the weight of the Pharaon eggs is higher with 0.972 grams, which represents a plus of 9.09% (table 5) (fig. 1), but the variability of this breed is higher (with 1.2 percentage points) then the O-Balotești breed.

Table 4. Statistical indicators for the weight, dimensions, volume and density of O-Balotești quail eggs, produced in the peak phase of the laying period

Specification		MU	n	Calculated statistical indicators			Variation limits	
				$\bar{x}\pm s\bar{x}$	s	v(%)	min	max
Weight		grams	123	10.696± 0.086	0.949	8.88	8.37	13.10
Dimensions	Longitudinal diameter (LD)	mm	123	31.471± 0.120	1.292	4.10	28.00	35.60
	Transversal diameter (TD1)	mm	123	25.011± 0.073	0.807	3.23	22.95	27.00
	Transversal diameter (TD2)	mm	123	24.973± 0.07	0.801	3.21	22.95	27.00
	Mean of the two transversal diameters (TD$\bar{x}$)	mm	123	24.987± 0.072	0.796	3.18	23.20	27.00
Surface (S)		cm^2	123	22.509± 0.121	1.346	5.98	10.099	25.825
Volume (Vc)*		cm^3	123	10.224± 0.083	0.920	9.00	7.906	12.650
Density (D)		g/cm^3	123	1.0465± 0.002	0.023	2.20	0.968	1.103
Specific weight (SW)		cm^3/g	123	0.956± 0.002	0.021	2.21	0.907	1.033
Shape index (SI)		x/1	123	1.260± 0.004/1	0.0487	3.87	1.166/1	1.402/1
Shape index (SI%)		%	123	79.455± 0.276	3.058	3.85	71.35	85.77

*Vc is the volume calculated by relation (6)

Regarding the studied eggs dimensions, the ones from Pharaon breed are bigger with 4.35% for LD and with 2.86% for TD$\bar{x}$, in comparison with the ones from O-Balotești breed (table 5) (fig. 1). The eggs from the two breeds do not differ much, in terms of shape, the shape indices having similar and complementary values. There are some notable differences between the surface and volume of these two eggs categories. The surface of Pharaon eggs is higher with 6.01% then the ones from O-Balotești, and the volume of Pharaon eggs is with 10.47% higher than the O-Balotești eggs (table 5) (fig. 2). Regarding the density and specific weight, the differences are small (1.31%-1.61%) (table 5).

Table 5. The main morphological statistical indices of O-Balotești and Pharaon quail eggs, produced in the peak phase of the laying period

Specification		MU	Studied quail breeds				
			O-Balotești		Pharaon		
			Absolute values	Relative values (%)	Absolute values	Relative values (%)	±%P/B*
Weight		grams	10.696	100.00	11.668	109.09	+9.09%
Dimensions	Longitudinal diameter (LD)	mm	31.471	100.00	32.841	104.35	+4.35%
	Transversal diameter 1 (TD1)	mm	25.011	100.00	25.703	102.77	+2.77%
	Transversal diameter 2 (TD2)	mm	24.973	100.00	25.703	102.92	+2.92%
	Mean of the two transversal diameters (TD$\bar{x}$)	mm	24.987	100.00	25.703	102.86	+2.86%
Shape index	LD/ TD$\bar{x}$	x/1	1.260/1	100.00	1.278/1	101.43	+1.43%
	(x 100)/LD	%	-	79.455	-	78.377	-1.078pp*
Surface		cm^2	22.509	100.00	23.862	106.01	+6.01%
Volume***		cm^3	10.224	100.00	11.295	110.47	+10.47%
Density		g/cm^3	1.0465	100.00	1.0328	98.69	-1.31%
Specific weight		cm^3/g	0.956	100.00	0.9714	101.61	+1.61%

*Pharaon – O-Balotești breeds comparison; **percentage points; volume calculated by relation (6).

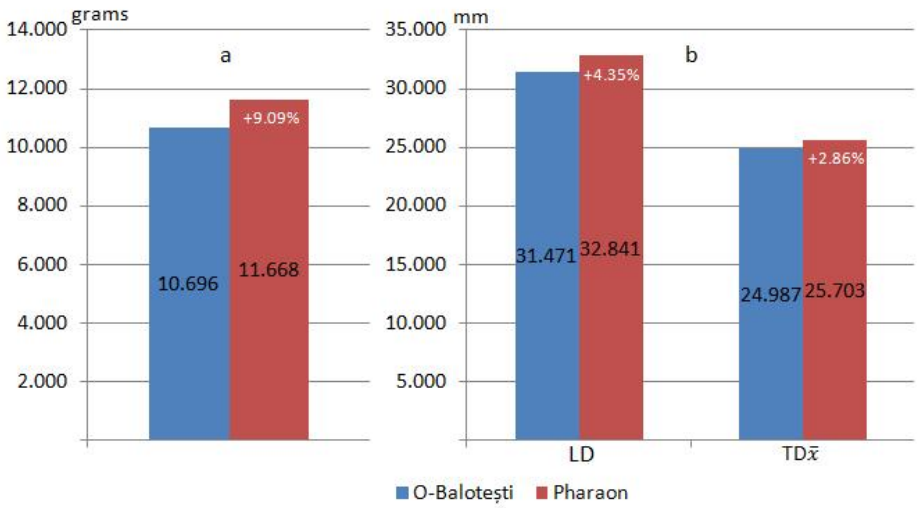

Fig. 1. Weight (a) and dimensions (b) of O-Balotești and Pharaon quail eggs, produced in the peak phase of the laying period

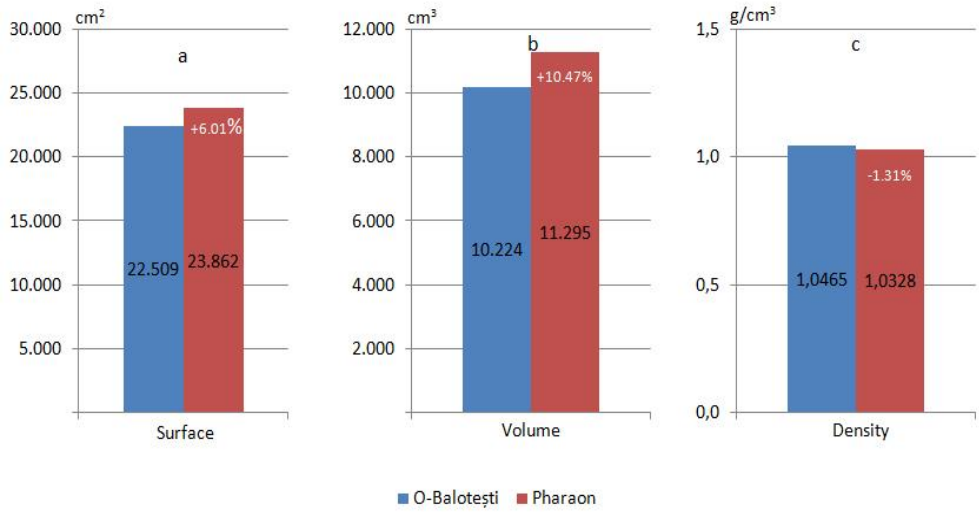

Fig. 2. Surface (a), volume (b) and density (c) of O-Baloteşti and Pharaon quail eggs, produced in the peak phase of the laying period

Conclusions

1. The average weight of Pharaon breed quail eggs, produced in the peak phase of laying period is 11.668 grams.
2. The dimensions of these eggs are 32.841 mm for the longitudinal diameter; 25.703 mm for the transversal diameter; 9.193 cm for the large circumference and 8.104 cm for the small circumference.
3. The shape index of these quail eggs had mean values of 1.278/1, respectively 78.38%.
4. The surface of Pharaon breed quail eggs has a mean value of 23.862 cm^2.
5. The volume has a mean value of 11.295-11.317 cm^3, depending on the method of determination.
6. The density and specific weight of these quail eggs has a mean value of 1.0328 g/cm^3, respectively 0.9714 cm^3/g.
7. Compared to the O-Baloteşti quail eggs, the Pharaon quail eggs have a higher weight by 9.09%; are bigger with 2.86-4.35%; have a larger volume by 10.47% and a larger surface by 6.01%.

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OVARIAN FOLLICULAR ATRESIA IN ONE MONTH OLD HYBRID MERINO EWES. HISTOLOGICAL STUDY

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Abstract
Ovaries harvested from young ewes, 1-30 days old, were processed for histological investigation. The presence of a large number of primordial follicles in the ovary was highlighted in the first decade of life, along with atretic follicles whose number and size were very different from one animal to another. The follicular atresia maintains at a high level throughout the whole study period, thus at the age of 30 days, the number of primordial follicles is significantly decreased. There are large differences from one animal to another, which does not allow a trenchant percentage expression of the decrease of the primordial follicles through follicular atresia in the first month after birth, but only an approximation of it, somewhere between 30-60%.
Keywords: atresia, ewe, follicle, ovary, primordial.

Introduction

Follicular atresia is a natural mechanism, which ensures fertility control, both quantitatively and qualitatively. The germ cell surplus is consumed during development, prenatal, neonatal, prepuberal, puberal and reproductive life of animals through atresia (Sharma, 2000; Bhardwaj and Sharma, 2011). Follicles in any developmental stages can undergo atresia, and its progress is more complex, the more evolved the follicle is. It appears that atresia intervenes in the competition mechanisms between follicles, through the programmed death of some follicles while others are favoured to continue their development (Ross and Wojciech, 2006). During foetal life, apoptosis is restricted to oocytes, whereas in adult animals, this phenomenon is frequently observed in the granulosa cells of the secondary and antral follicles (Hussein, 2005). In primordial and primary follicles' case, the degenerative processes simultaneously involve the oocyte and granulosa cells (Ross and Wojciech, 2006). Some authors claim that only 0.001% (Tabarowski *et al.*, 2005; Sharma and Bhardwaj, 2009), of the 60,000 – 200,000 primordial follicles (depending on the species) present in the neonatal ovary (Hafez and Hafez, 2000), ovulate. The primordial follicles' number considerably decreases in the first month of life. 50% of the primordial follicles, from a mouse ovary, are lost in the first month after birth (Peters and McNatty, 1980). In order to see if the situation is comparable in other species, we considered the investigation on sheep ovaries as purposeful.

Material and methods

Ovaries from seven young ewes with ages between 1 and 30 days (3 days, 7 days, 8 days, 10 days, 14 days, 20 days and 30 days) were harvested in order to carry out histological examinations. The harvested specimens were fixed in 10% formalin for seven days, dehydrated with ethanol, clarified with n-Butanol and paraffin embedded. Five µm thick sections were made and stained with *Modified Goldner's Masson Trichrome method. Examination of the histological sections was made using an Olimpus BX41 microscope.*

Results and discussion

Ovaries harvested from young ewes in the first decade of life, display a large number of primordial follicles, characteristically grouped at the periphery of the cortex (Fig. 1). Along them, there are also other categories of follicles (primary, small antral, large antral), which suggests that the organ is active even at such an early age.

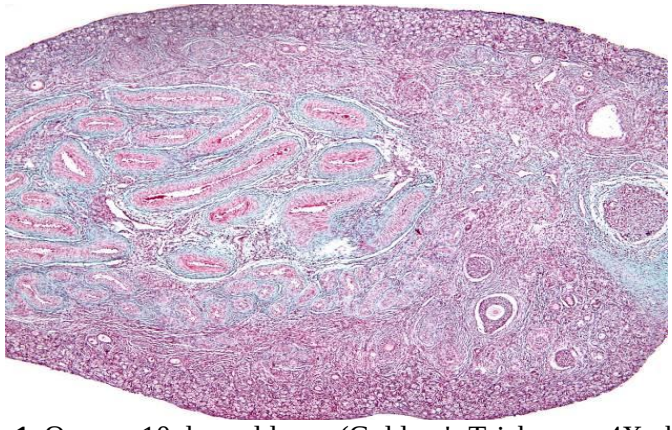

Fig. 1. Ovary - 10 days old ewe (Goldner's Trichrome, 4X obj.)

It is worthy of note that sometimes there are large differences between one animal and another, concerning both the developing follicle number and size. In some ovaries, the number of antral follicle is relatively small (Fig. 1), while in others is larger or even very large (Fig. 2).

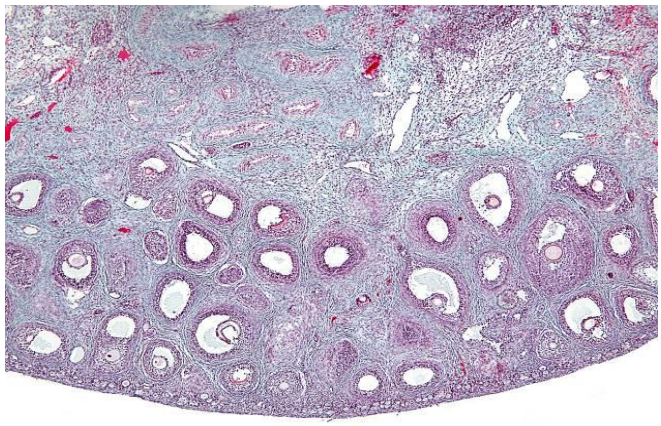

Fig. 2. Ovary - 10 days old ewe (Goldner's Trichrome, 4X obj.)

The large majority of antral follicles have different degrees of degeneration in the oocyte, and in those with a disintegrated oocyte, zona pellucida appears collapsed and in many cases curved (Fig. 3).

Different degrees of oocyte degeneration are actually present in the majority of the developing follicles, regardless of their size (Fig. 4). Changes are also present in the granulosa layer, whose cells appear *dissociated by oedema and some undergo apoptosis* (Fig. 5).

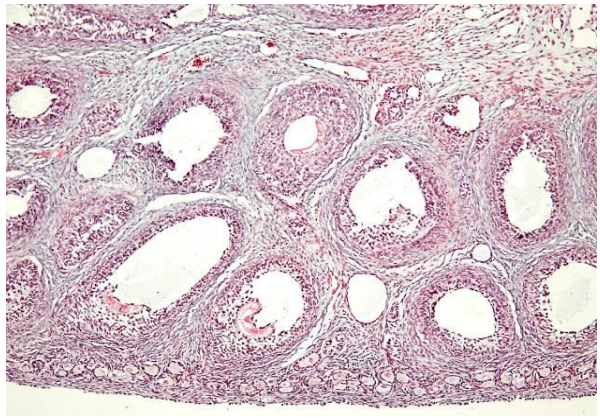

Fig. 3. Ovary - 3 days old ewe (Goldner's Trichrome, 10X obj.)

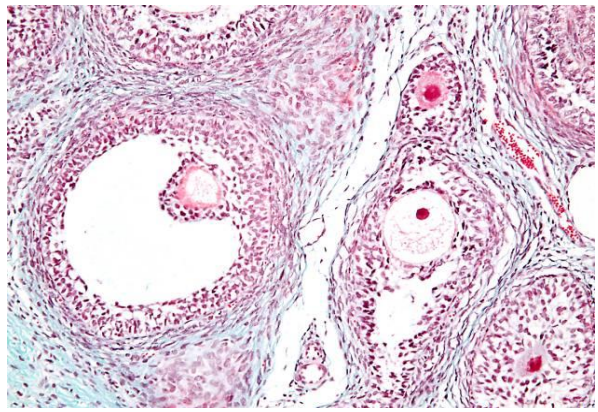

Fig. 4. Ovary - 3 days old ewe (Goldner's Trichrome, 20X obj.)

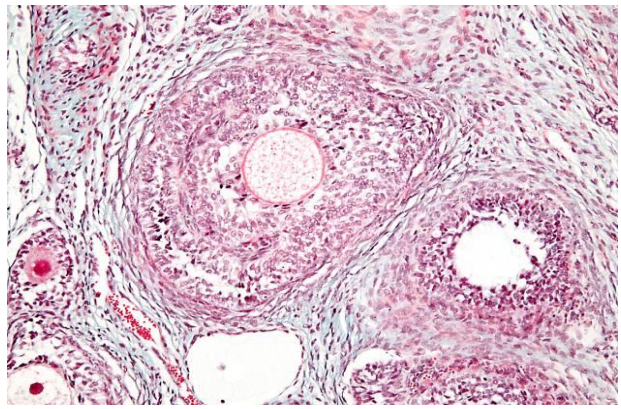

Fig. 5. Ovary - 3 days old ewe (Goldner's Trichrome, 20X obj.)

Ovaries harvested in the second decade of the first month after birth have numerous developing follicles, from all categories, beside a large number of primordial follicles (Fig. 6). Differences from one animal to another are obvious in this period as well, in some cases there are ovaries in which the primordial follicle consumption is clearly more advanced and most of the times the existent developing follicles usually have a large size (Fig. 7).

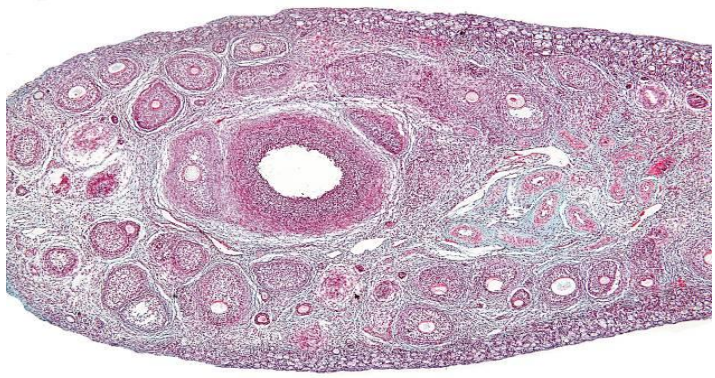

Fig. 6. Ovary – 14 days old ewe (Goldner's Trichrome, 4X obj.)

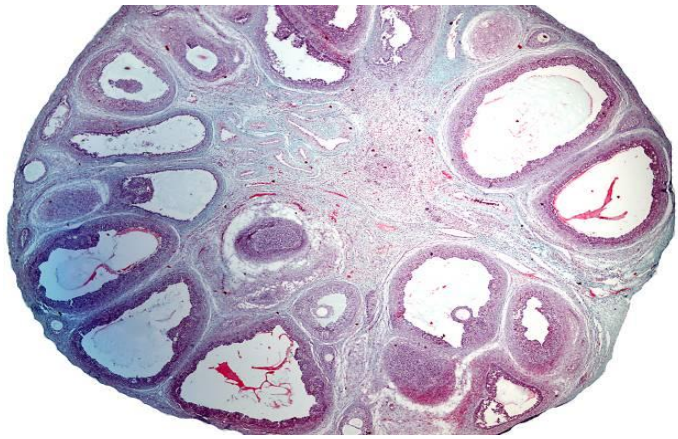

Fig. 7. Ovary - 20 days old ewe (Goldner's Trichrome, 4X obj.)

At the end of the first month of life, the ovarian activity is present, but seems to be in a slightly decrease in comparison to the second decade. Primordial follicles are still well represented although they have numerically decreased significantly in comparison to the first decade (Fig. 8). There are differences from one animal to another and even from one area to another of the same ovary.

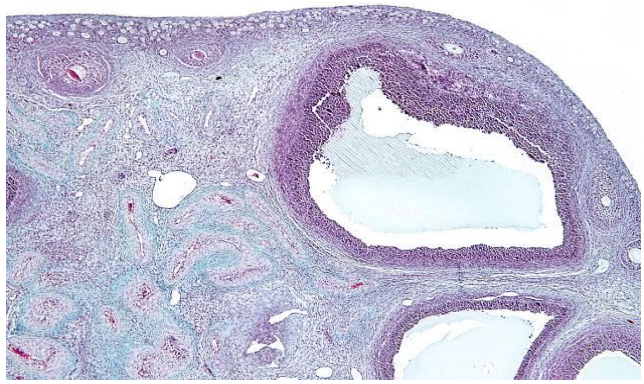

Fig. 8. Ovary - 30 days old ewe (Goldner's Trichrome, 4X obj.)

Our study confirms the fact that a large number of follicles from the primordial follicle stock, present in the ovaries at birth, are eliminated through follicular atresia processes. Developing follicles, in any developmental stage can undergo atresia, and their number and the proportion between small and large atretic follicles is very different from one animal to another, even in those of comparable age. Indubitably, in the first month of life, young ewes lose a very large number of follicles through atresia, which makes the follicle stock to decrease significantly at the end of the first month, in comparison to the stock at birth. This aspect is in accordance at a certain extent with the data in the speciality literature reported by Peters and McNatty (1980), who claimed that 50% of the primordial follicles in a mouse ovary are lost in the first month of life. In the case of young ewes the primordial follicle stock decreases very much in the first month of life also, but the large differences between the ovaries of animals of comparable age, makes us sit on the splice in making a trenchant percentage expression, which however would not be valid for all the animals. This decrease is situated between 30-60%.

Conclusions

1. Ovaries of the young ewes contain a large number of primordial follicles and also atretic follicles at birth, whose number and size are very different from one animal to another.
2. Follicular atresia progresses at a high rate throughout the period taken into study, so that the primordial follicle stock decreases significantly after 30 days of life.
3. The large differences between the ovaries of animals of comparable age, do not allow a percentage expression of the follicular stock decrease in young ewes in the first month of life, but only an approximation, between 30-60%.

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ETIOMORPHOPATHOLOGY OF BRONCHOPNEUMONIAS IN SWINE RAISED IN INTENSIVE SYSTEMS

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Abstract
Necropsic and histopathological examination of lungs of sacrificed or dead pigs were performed. Lungs from 72 pigs of various age and sex were examined. Macro- and microscopical examinations revealed several types of inflammatory pneumopathies (necrotic, catarrhal, fibrnious, purrulent, gangrenous and lymphohystiocytic bronchopneumonia).
Key words: bronchopneumonia, pig, intensive system

Introduction

Research was performed on dead pigs from a swine farm for human consumption. The farm works in closed circuit. Biological research material is represented of PIC hybrid.

Investigations showed several morphological types of inflammatory pneumopathies, some of them as a result of the simultaneous intervention of several pathogens.

Bronchopneumonies of swine in the intensive system are mainly due to the intervention of the shelter microflora, as well as to physical microclimate factors.

The etiology of the catarrhal bronchopneumonia is represented by several infectious, physical or chemical factors that reach the lungs through the airwaves, so lesions are localized in the cranial, cardiac and anterior third of the lungs (7, 8).

Fibrinous bronchopneumonia of the swine is produced by Pasteurella and Actinobacillus pleuropneumoniae, and has an intense stadial development on a lobular level (1, 3, 7).

Purrulent bronchopneumonia appeared in both morphological aspects (diffuse and focalized) and has a predominantly infectious etiologyŞ streptococci, staphylococci, Pseudomonas and last bt not least, Arcanobacterium pyogenes (2, 4).

Lymphohystiocytic bronchopneumonia has a mixt etiology (viruses and bacteria with pulmona affinity) and the fundamental lesion consists of lymphohystiocytic hyperplasia with specific distribution according to the pathogen involved (5, 6, 7).

Material and method

Investigations were performed in 2009-2011 on pig bodies from an intensive farm in Braila county.

Necropsic and histological examination of the lungs was performed. The lungs were prelevated from 72 pigs of various age and sex. After prelevation, samples were conserved in 10% formaldehyde, paraffin embedded and sectioned at 5 μm. Slides were stained in Haematoxylin-Eosine – Methyl Blue (Trichromic Masson) and Periodic Acid - Fuxine Schiff (PAS).

Results and discussions

The following morphological types of bronchopneumonia were identified:

Necrotic bronchopneumonia

The isolated agent was *Fusobacterium necrophorum*.

Macroscopically, the lesion was identified as grey-yellow necrotic foci surrounded by a red area with a diameter of 1,5 cm.

Histologically, the presence of alterative phenomena was observed, that evoluated to coagulation necrosis. After the initial phase of structured necrosis, the astructural phase of coagulation necrosis is defined by the presence of the central debris. Exsudative changes were identified, as leukocytic exsudate forming a cellular barrier arround the destroyed area, sustained by vasodilatation and perifocal congestion (Fig.1).

In acute necrotic inflamation the foci were surrounded by a red area corresponding to the congestive-haemorrhagic area peripheric to the necrotic tissue.

Macroscopically, necrotic foci were also identified in other organs (liver, pericard, miocardium).

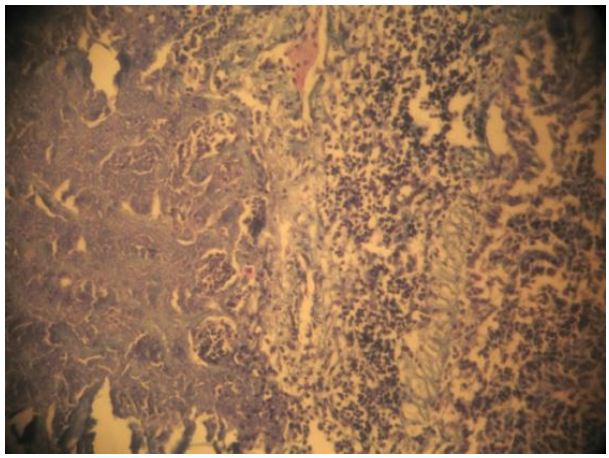

Fig. 1. Pig. Necrotic bronchopneumonia (area of unstructured necrosis – necrotic debris, leukocytic barrier). HEA, x100

Catarrhal bronchopneumonia is a pathological process determined by irritative or infecting action of the inhaled air.

The acute stage (filling stage) was initially noticed as inflammatory edema appearing as compact dark red foci, consistent, shiny and with positive docimasy, localised predominantly in the anterior lobes of the lungs (Fig. 2). Histologically, septal hyperemia and filling of the alveolae with epithelial cells migrated from the interstitium was noticed (Fig. 3). Many of these cells have a clear macrophagic aspect. The acute process affects all pulmonar components. The congestion is followed by mucus hypersecretion of goblet cells, loss of cilli and of epithelial cells from their basal membranes. Intensification of diapedesis and infiltrative granulocytosis of the chorion and of the epithelium favors the apparition of the catar: abundent PAS-positive mucus, epithelial cells, macrophagues, epithelial cells.

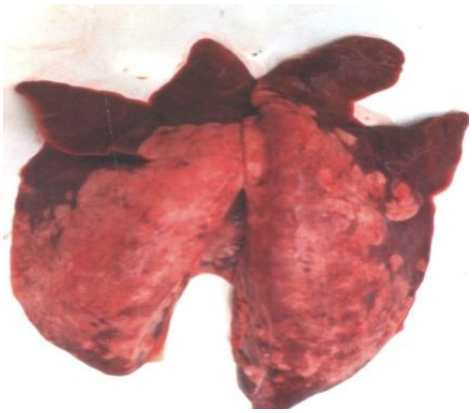

Fig. 2. Pig. Catarrhal bronchopneumonia – filling stage

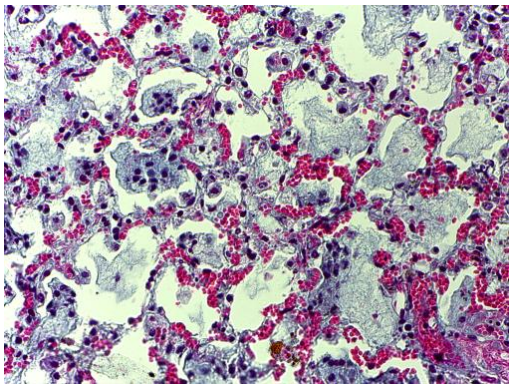

Fig. 3. Pig. Catarrhal bronchopneumonia – filling stage. HEA, x100

In chronic evolutions, the hyperplasia of the epithelium and of bronchic glands occurs, with mucus hypersecretion and formation of purrulent catar (that can cause obstructions), lymphohystiocytic hyperplasia in the chorion or in peribronchic spaces, with plasmocytic or eosinocytic differentiation; prolifferations can take a lobular aspect, like real lymphoid follicles. In time, wall scerosis of fibrosis of peribronchic spaces can occur.

The acute process usually diffuses endo- or peribronchically to inferior airwaves and affects alveolar territories too. This process leads to the tumefaction of alveolar cells (type I and II pneumocytes) or to congestive, exsudative and prolifferative septal processes.

In the first case, true catarrhal bronchopneumonia is installed which, besides the above mentioned bronchic changes, leads to thining of alveolar walls and blockage of their lumen with pneumocytes, macrophagues, rare neutrophiles and serous or serofibrinous exsudate.

In aubacute evolutions the affected territories are decreased in volume, firm, with red granular section surface.

Chronic evolutions are diagnosed by the net contour of pulmonary lobes, their grey-whitish colour and firm consistence, noticeable during sections (Fig. 4).

Adjacent territories develop lesions of passive atelectasy or compensator emphysema.

The fact that in slides obtained rom the sacrificed pigs shoe several cases of bronchitis with minimal or absent alveolar lesions suggest either the begining of the process or alveolar lesions way below the obtained sections.

Catarrhal bronchopneumonia as small foci is developed as a consequence to secondary lesions.

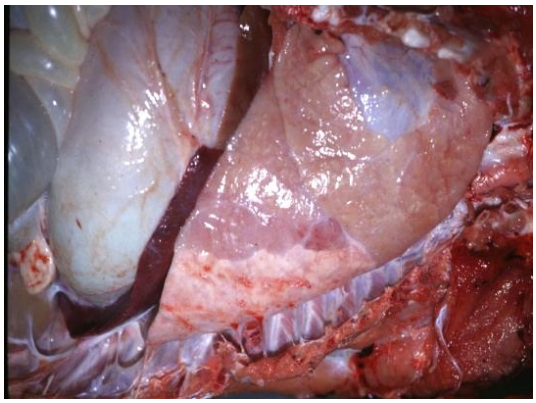

Fig. 4. Pig. Catarrhal bronchopneumonia – pancreatization stage

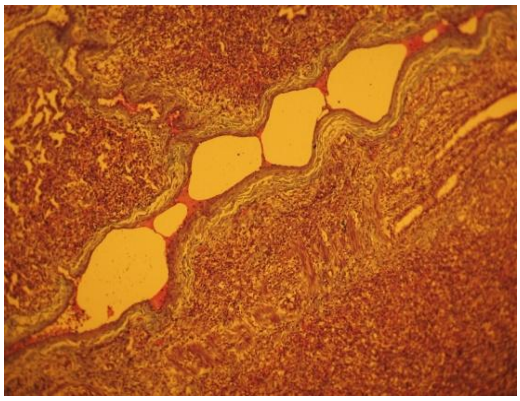

Fig. 5. Pig. Fibrous bronchopneumonia. HEA, x100

Fibrinous bronchopneumonia in swine from this farm was attributed to infections with *Actinobacillus pleuropneumoniae*.

Macroscopically, affected pulmonary territories were distended, with a polichrome aspect quite specific, due to the accentuated stadialization of the inflammation on a lobular level (Fig. 6).

Histologically, the lesion was noticed during the first three stages (filling, red hepatisation and grey hepatisation)

I. – ***filling stage***, macroscopically characterised by a red-violet colour, pasty consistence and moist section surface, presenting the changes of inflammatory edema: septal hyperemia and accumulation of serous exsudate with descuamated cells in the alveolae;

II. – ***red hepatisation stage*** is manifested through red-grey colour, pronounced compactisation and dry aspect of the section surface; histologically, the lumen of the deformed alveolae and interlobular spaces are filled with reticular oxyphile fibrin (fig. 7);

III. – during the ***grey hepatisation stage***, in which affected areas appear grey-yellowish, massive meutrophilic exsudation occurs (Fig. 8);

IV. – in the ***resolution stage*** in animals that survive, the proteolitic action of leukocytic enzymes leads to the disappearance of fibrin deposits with the reopening of air spaces.

Stadialisation of fibrinous bronchopneumonia can go down to sublobular levels, groups of only a few alveolae showing different stages of inflamations.

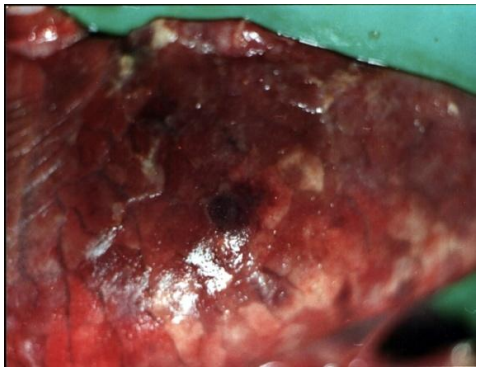

Fig. 6. Pig. Fibrinous bronchopneumonia

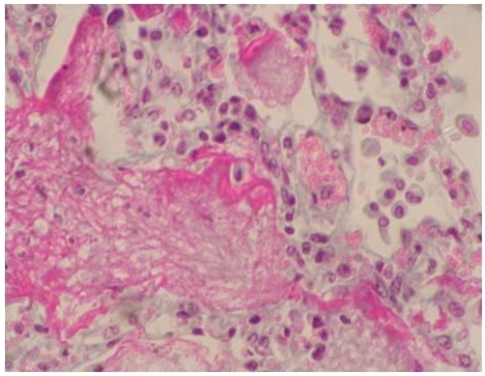

Fig. 7. Pig. Fibrinous bronchopneumonia – red hepatisation stage
PAS, x400

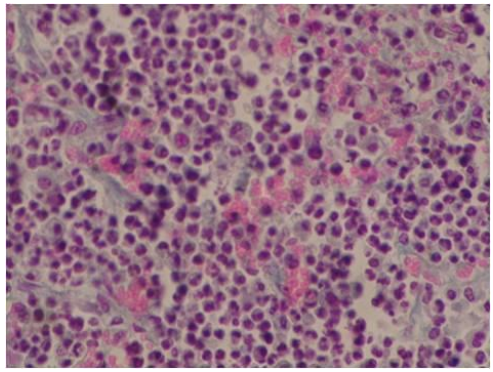

Fig. 8. Pig. Fibrinous bronchopneumonia – grey hepatisation
Stage HEA, x400

Purrulent bronchopneumonia was noticed in both morphological forms of evolution:

- diffuse purrulent bronchopneumonia
- focalised purrulent bronchopneumonia

The diffuse form was considered a following stage of acute catarrhal bronchopneumonia, due to the intervention of pyogen germs.

Macroscopically, the lung was increased, with high consistence and with abundant expression of puss on section surfaces (Fig. 9).

Histologically, the purrulent exsudate, predominantly leukocytic, was noticed in the interstitium and in the bronchiae (Fig. 10, Fig.11, Fig.12).

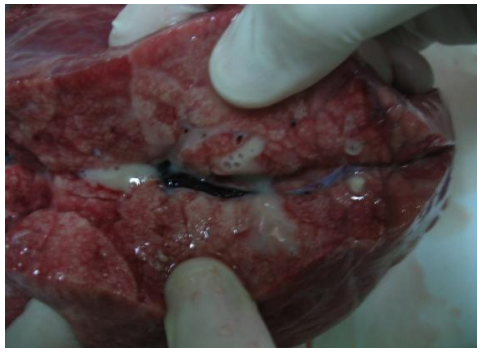

Fig. 9. Pig. Diffuse purrulent bronchopneumonia

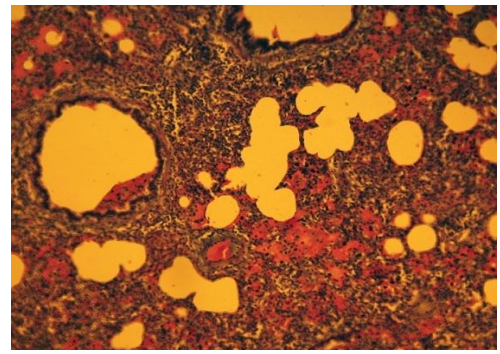

Fig. 10. Pig. Diffuse purrulent bronchopneumonia. HEA, x100

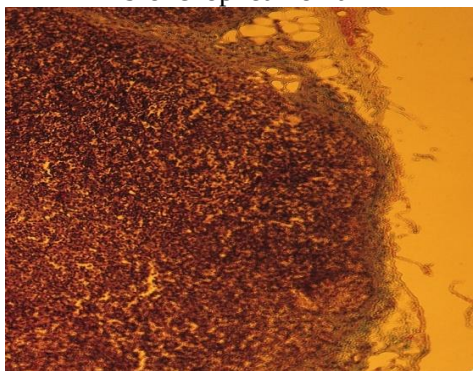

Fig. 11. Pig. Diffuse purrulent bronchopneumonia. HEA, x100

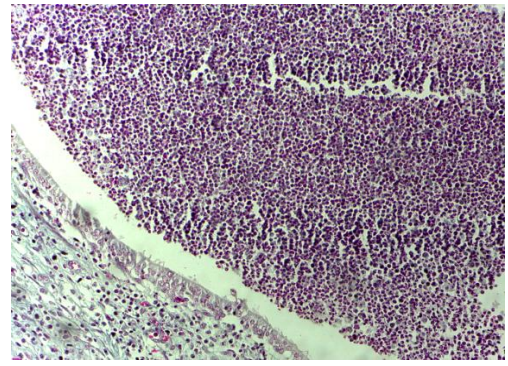

Fig. 12. Pig. Purrulent bronchopneumonia (intrabronchic purrulent exsudate) HEA, x100

The focalised form was noticed as disseminat abcesses (Fig. 13).

A well configured mature abcess shows variable diemeters, a wall and an internal cavity which contains the puss, with different aspects according to the etiologic agent: streptococci, staphyloccoci, *Pseudomonas spp.*, *Arcanobacterium pyogenes.*

Histologically, the wall of the mature abcess is formed of 3 concentric areas, with clear limits (Fig. 14):

- **Internal area** that separates the cavity of the abcess appears in small resolution as a clear area of macrophagues with vesiculous nuclei and vacuolised ramified cytoplasm;
- **Median area** of unspecific hyperplasia made of lymphocytes, hystiocytes, plasmocytes and many new formed capillaries;
- **External area**, very well developped in old abcesses, predominantly fibrous, made of fibroblasts and reticulin and collagen fibers.

Purrulent inflammation in abcesses usually appears as multiple abcesses, diseminated in the mass of the organ.

The evolution of the abcesses is favorable in the case of sterile abcesses, out of which the small ones heal and big ones either capsulate or suffer dystrophic calcification.

In the case of active abcesses caused by highly pathogenetic and toxigenous bacteria, tissular necrosis spreads before the constitution of a thock wall, so the thin capsule will crack due to the internal pressure of the puss, which will spread in the pleural cavity.

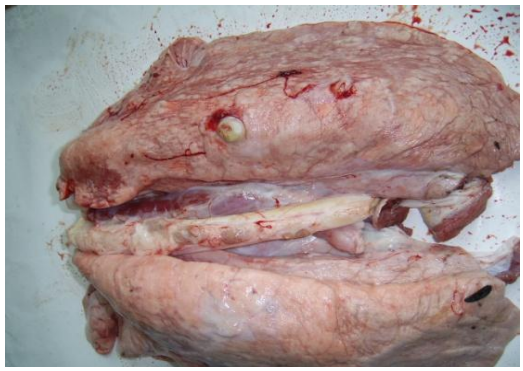

Fig. 13. Pig. Purrulent bronchopneumonia. Pulmonary abcess

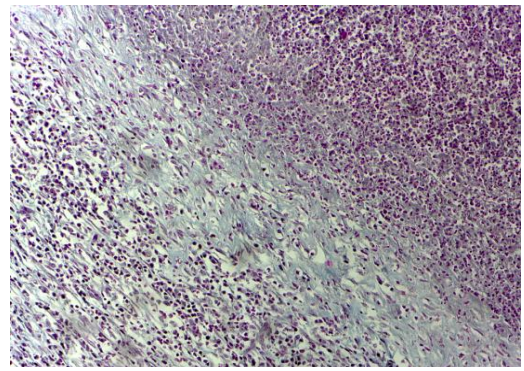

Fig. 14. Pig. Pulmonary abcess, HEA, x100; Cavity: purrulent exsudate Wall: macrophagues, lymphohystiocytic prolifferation, peripheric connective tissue prolifferation

Gangrenous bronchopneumonia was macroscopically identified as turgescent pulmonary territories, blue-green or black, gassy on palpation. When sectioned they produce a fowl smell and, most of the times, the organ was hardly recognizable (Fig. 15).

Etiopathogenesis of gangrenous bronchopneumonia is explained by food aspiration. The lesions affected anterior lobes and were the consequence of inhalation of plant parts or food debris.

Histologically, normal tissue was practically melted and replaced by cellular debris, fibrin, plasma and bacterial colonies. The main difference to the purrulent bronchopneumonia is the absence of leukocytic exsudate (Fig. 16).

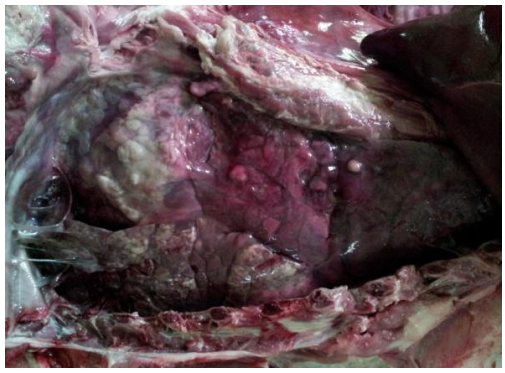

Fig.15. Pig. Gangrenus bronchopneumonia

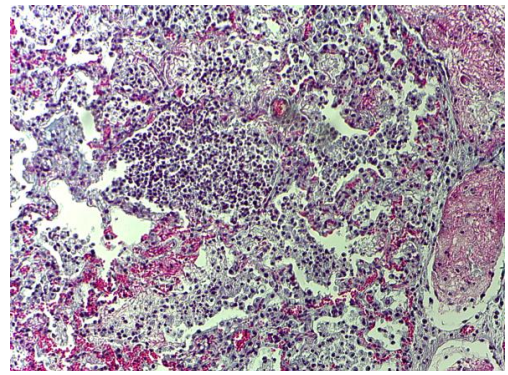

Fig. 16. Pig. Gangrenus bronchopneumonia (fibrin, leukocytes, bacterial colonies). HEA, x100

Lymphohystiocytic bronchopneumonia

Under the influence of biologically active substances (interleukins) eliberated from the tissues in the initial phases of the inflammatory process, hyperplasia and differentiation of local mesenchymal cells occur towards hystiocytes and lymphocytes.

Lymphohystiocytic bronchopneumonia appears as septal hyperplasia with nodular or, rarely, diffuse character, on varriable territories, in most pulmonary virrosis.

In our case, lymphohystiocytic bronchopneumonia appeared in enzootic swine pneumonia caused by *Mycoplasma suipneumoniae*, a very frequent disease in great intensive farms. boală foarte frecventă în marile unităţi de creştere a suinelor.

Lesions were localised in apical and cardiac lobes and sometimes in the anterolateral area of diafragmatic lobes (fig. 17).

During the first phases of the process the lesions are slightly retracted, dark red, shiny; later, affected territories become grey and rough, with positive docimasy in all phases. Macroscopically, changes are similar to those encountered in catarrhal bronchopneumonia.

Histological examination showed septal diffuse prolifferations and uniform peribronchiolar and periartheriolar hyperplasia, a very important diagnostic element.

Lymphocytes were noticed due to their intense purple nuclei in HEA or Giemsa stain, with a very high nucleo-cytoplasmatic ratio (8/1-9/1) Cytoplasm appears as a narrow basophile perinuclear band. Hystiocytes have big clear vesiculous nuclei and ramified and poorly delimited cytoplasm. Plasmocytes, eosinocytes, connective cells and fibers are poorly represented.

Lymphohystiocytic brinchipneumonia is mainly attributed to mycoplasmic infections, probably associated to respiratory adenoviruses and manifested through peribronchiolar lymphohystiocytic hyperplasia with assimetric disposition that invaded respiratory mucosa and were accompanied by epithelial prolifferations (Fig. 18).

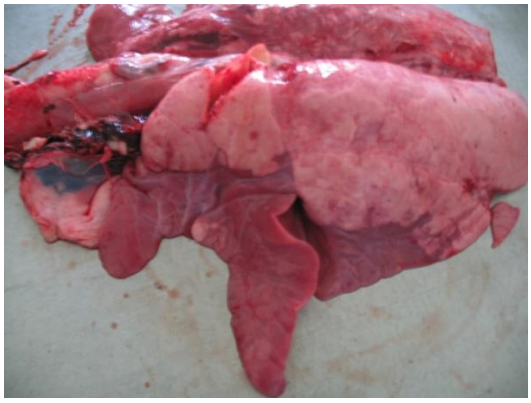

Fig. 17. Pig. Enzootic pneumonia. Lymphohystiocytic bronchopneumonia of the left lung (apical, cardiac and anterior 1/3 of the diafragmatic lobe)

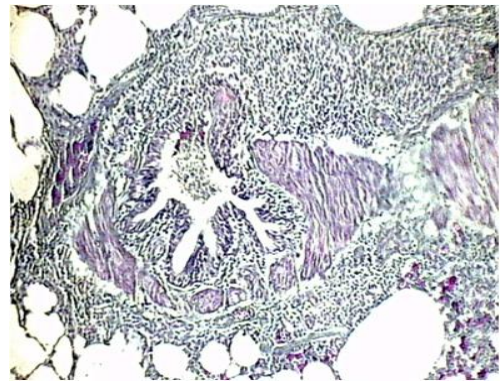

Fig. 18. Pig. Enzootic pneumonia. Lymphohystiocytic bronchopneumonia. Lymphohystiocytic peribronchial prolifferations. HEA, x200

Conclusions

1. Necrotic bronchopneumonia was sporadically encountered in this livestock.
2. In several cases, catarrhal bronchopneumonia as small foci appeared as a complex od lesions secondary to other affections.
3. Diffuse purrulent bronchopneumonia appeared as a complication with pyogen germs of catarrhal bronchopneumonia..
4. Gangrenous bronchopneumonia was sporadically encountered and was due to food aspiration.
5. Macroscopical examination of lungs diagnosed with lymphohystiocytic bronchopneumonia, changes are identical to those encountered in catarrhal bronchopneumonia.
6. Lymphohystiocytic bronchopneumonia was first attributed to a mycoplasmic infection associated with respiratory adenoviruses and had a lesional manifestation of lymphohystiocytic hyperplasia with a symmetric character arround the bronchiae, that invaded respiratory mucosa and were accompanied by epithelial prolifferations.

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CORELATION BEETWEN SUBAREOLAR AND PERITOUMORAL BLUE DYE INJECTION TO IDENTIFY SENTINEL LYMPH NODES IN CANINE MAMMARY GLANDS NEOPLAZIA

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Abstract
Identification and assessment of mammary glands of carnivores is still a challenge to modern medicine. Therefore, injecting blue dye Evans is the most accepted and used method for identifying the lymphatic system. However, the optimal site of injection continues to be cause of debate. The present research aims to determine whether the dye subareolar injection leads to the identification of the same lymph nodes as peritumoral injection. Seven female dogs with mammary glands neoplazia, located at thoracic, abdominal cranial and inguinal mammary, were used for these purpose. Dye injection was made subareolar and peritumoral in four sites, in neoplastic mammary glands and in four cases it was injected the same neoplastic mammary gland, in both ways, subareolar and peritumoral. Contralaterally it was subareolary injected the healthy mammary glands. Also, peritumoral blue dye injection was made in T1, A1, and Ingiunal mammary glands. Thirty three lymph nodes belonging axillary, superficial ingiunal,cranial sternal and popliteus lymphcenter were identified. The 12th lymph node was stained after subareolar blue dye injection, while after peritumoral blue dye injection the 11th lymph node was colored. Combined blue dye injection (subareolar and peritumoral), made in four neoplastic mammary glands, identified eight lymph nodes. After peritumoral blue dye injection in cranial thoracic mammary gland, there was no staining of axillary lymph nodes, but the cranial sternal lymph node was colored. Also, in one case of combined blue due injection in inguinal neoplastic mammary gland, it was well stained a rich lymphatic plexus at the medial aspect of tigh along the popliteal lymph node. Our result demonstrates that there exists a high concordance between the two types of blue dye injection, to identify the sentinel lymph node, especially when they are used together. Our findings suggest that the breast parenchyma and the subareolar plexus drain in the same lymph node. In conclusion, the two techniques are complementary and used together, lead to an accurate identification of sentinel lymph node of female dog mammary gland.

Keywords: sentinel lymph nodes, blue dye, mammary neoplazia

Introduction

Mammary gland neoplazia is an undeniable reality in both, human and veterinary medicine. The specialists encounter a lot of problems in early diagnosis of tumor disease. Moreover, determination the sentinel lymph node and assessing its metastatic invasion are the first steps in treatment management (1,2). The presence of lymph node metastasis is the most important prognostic factor, in human breast cancer and female dog neoplazia. Certification of lymphatic extension is associated with decreasing survival rates, less than 5 years, for about 50% of cases (1).

Therefore, management of mammary neoplazia treatment, requires careful staging, by identifying the first station of lymphatic drainage of the tumor lymphatic basin and appreciation the lymphatic dissemination of metastatic cells in these lymph nodes. Sentinel lymph nodes, or node, are the first lymph nodes from the lymphatic basin of the primary tumor, who receive lymphatic vessel's related to primary tumor. In recent years, surgical treatment of mammary gland neoplazia in female dog, assumes primary tumor excision, sometimes entire ipsilateral mammary chain associated with excision of draining lymph

nodes, if they are found intraoperatory. Therefore, is strongly needed to identify the sentinel lymph node, because the consequences of an unjustified lymph node excision are more critical.

The concept of sentinel lymph node belongs to Cabanas, implemented in 1977 in penile cancer. Morton et al. first used the concept of lymphatic mapping in patients with cutaneous melanoma, by using a dye injection around the primary tumor, to determine location of sentinel lymph node (3, 4). Later, Giuliano et al. have successfully applied that method (peritumoral injection) to identify sentinel lymph node in women breast cancer. Krag et al, have used the technique of peritumoral injection using a radioisotope injection, followed by identification with a gamma probe to achieve axillary lymph nodes biopsy, to assess their status.

According to numerous studies, if the biopsies show no presence of metastases, is considered that the entire drainage basin is negative, and the surgical act is limited to primary tumor excision. In that way are avoided the side effects of lymph node excision. In case of a positive sentinel lymph node, is presumed lymphatic invasion, and there is required extensive lymph node excision for a full staging and initiation of appropriate therapeutic attitudes (5). Thus, in mammary neoplasia in the female dog, identification of location of the sentinel lymph nodes, assess their status, given the particular species and the variability of this system in carnivores, is the key to an successful treatment. Normally, mammary lymph drainage is made in relation to location of the mammary gland, with two-way drainage: by axillary lymph nodes in cranial way for toracal mammary gland, and caudally by superficial inguinal lymph nodes for the caudal abdomimnal and inguinal mammary glands (6,7). Cranial abdominal mammary gland has in many cases two-way drainage, cranial and caudal. Pereira et al showed in a recent study that neoplasia, drainage may be altered, and noting the involvement of other lymph nodes not belonging to another lymph center, who are not belong the common norms.

Material and methods

We have used a number of seven female dogs with cranial thoracic, cranial abdominal and inguinal mammary gland tumors, clinically and histopathologically diagnosed. Evans blue dye solution in concentration of 0.5% was injected as follows: subareolar in healthy mammary gland on the contra lateral site of mammary tumor, for T1, A1 and I mammary, only peritumoral in T1, A1 and I mammary gland, and the combined injection (subareolar and peritumoral) in the A1 and mammary glands. The total amount injected was 1 ml blue dye Evans (0.25 ml / injected point), in four point-cranial, caudal, medial and lateral.

Subareolar injection was followed by gentle massage of the injected area for 3 min. to facilitate the penetration and dispersion of blue dye in lymph vessels. Peritumoral injection was not massaged after the injection.

Results were performed 24 hours after injection. Loco regional and stratigraphic dissection plane by plane was made, given the injection mode, to conserve the optimum colored lymphatic vessels. Lymphatic's were followed to the first lymph node, vascular characteristics were recorded alongside the number of lymph nodes identified.

Table 1. The type of injection and injected mammary gland are shown in table below

Mammary injected	**T1 (2 subjects)**		**A1 (3 subjects)**		**I (3 subjects)**	
Site of injection	Right	Left	Right	Left	Right	Left
Tipe of injection	SA	PT	SA	SA+PT	SA+PT	SA
	PT	SA	PT	SA+PT	PT	SA+PT
			PT	SA	SA	PT

Results

Thirty-three sentinel lymph nodes were identified at stratigraphic dissection: eleven belonging axillary lymph center, twenty to inghino femoral lymph center, through superficial inguinal lymph nodes, a cranial sternal lymph node belonging ventral thoracic lymph center and one popliteal lymph node. After subareolar injection of dye in six mammary glands, were identified a number of twelve lymph node (average 2/subiect); after peritumoral injection in six mammary glands were identified eleven lymph nodes (average 1.8 / subject), while the combined subareolar and peritumoral injection of 4 mammary glands identified nine lymph nodes (average 2.2 / subject).

Surprisingly, in a case of peritumoral injection in T1 cranial thoracic mammary gland, the ipsilateral axillary lymph node was not stained, but were observed lymphatic vessel. After subareolară injection in contralateral gland tumor on the opposite side, (heterolateral T1), the sentinel lymph nodes stained were the axillary lymph nodes and accessories (Fig. 1).

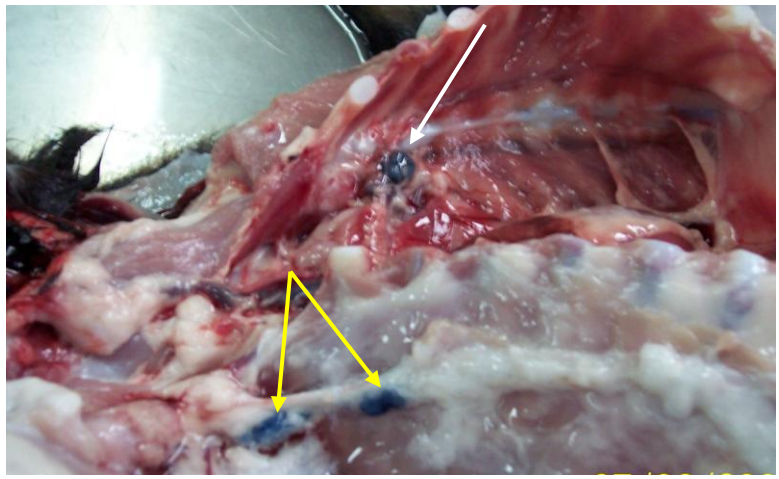

Fig. 1 The axillary lymph nodes centre (yellow arrow) and cranial sternal lymph node (white arrow), at peritumoral injection of T1, to the left side

Another feature of the present research, was observed after concomitant peritumoral and subareolar injection in inguinal mammary gland, namely colouring superficial inguinal lymph nodes and popliteal lymph node, with evidence of a rich lymphatic plexus at the medial aspect of thigh.

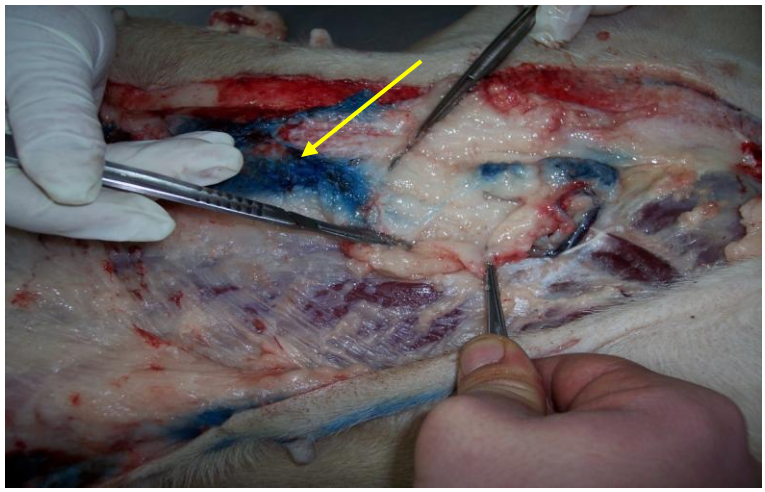

Fig. 2 Peritumoral injection of inguinal mammary gland (yellow arrow) and inguinal sentinel lymph nodes

Also, in this case, with tumor in both inguinal mammary glands, were prominently colored a few lymphatic vessels that cross the median plane, making a real communication between the two contralateral lymph nodes (Fig. 2).

Regarding the connections between two ipsilateral mammary glands, their existence was noted only in one case of peritumoral injection of mammary glands A1, between the injected gland and A2. In this case, no axillary lymph nodes was identified as sentinel lymph nodes of the udder cranial A1, but caudally superficial inguinal lymph nodes were stained.

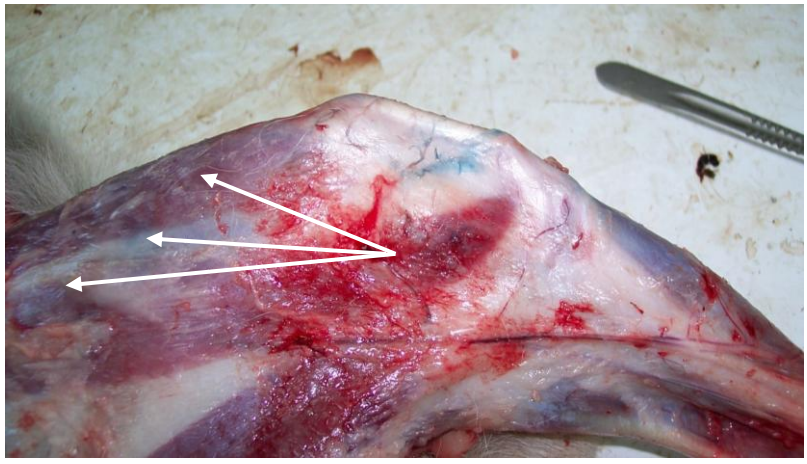

Fig. 3. Lymphatic Vessels at the medial aspect of the thigh (white arrow)

Table 2. Our results are collated in the table below

Mammary injected						
SLN and type of injection	**T1 (2 subjects)**		**A1 (3 subjects)**		**I (3 subjects)**	
	Axillary LN	**Cranial Sternal LN**	**Axillary LN**	**Inguinal LN**	**Popliteus LN**	**Inguinal LN**
Subareolar	3		2	3		4
Peritumoral	1	1	2	3		5
Subareolar +peritumoral			3	2	1	3
Total of AxLN	11					
Total of IngLN	20					
Cr St LN	1					
Popliteus LN	1					
Total of SLN	33					

Discussion

In malignant tumors, cancer cells, direct locally spread by soft tissue infiltration, or at distance by invasion of vascular structures, to migrate through the lymphatic or blood vessels torrent in different sites. Lymphatic invasion, compared with blood invasion, which causes any site metastasis, lymphatic migration is usually stepwise, through successive lymph nodes, which can temporarily slow or even stop the progression of cancer disease. In malignant tumor of mammary gland of the female dog, as in the human breast cancer, the basic problem is to determine whether cancer is a systemic disease from the onset, in which case the emphasis should be put on a systemic treatment, but, if, before metastasizing, there is an local infiltrating, focus should be put on active loco regional treatment of disease (8,9). These findings can be considered contradictory, mammary neoplasia best summarizes these paradigms. Detection of sentinel lymph node in breast tumors and evaluation of their status by biopsy, had a major importance in the evaluation and staging of malignancy. Excision of entire mammary chain, and the lymph nodes associated with its drainage, without prior investigation, often leads to major associated complications: lymphedema, seroma, paresthesia of the ipsilateral limb, and not least, pain.

From the perspective of an anatomist, identification of the first lymphatic stations in mammary neoplasia in the female dog, using the blue dye injection, is based on normal drainage variants, in two direction: cranial by axillary lymph center (for T1, T2 and A1 mammary glands) and caudal through superficial inguinal lymph nodes (for A1, A2 and I mammary glands) (10,11). Up to drain the lymph nodes, drainage way must be reviewed by the lymphatic vessels. Sappey, since 1870 showed that there is a rich subareolar lymphatic plexus, which have no relation to deep vessels in female breast, a concept that has changed radically (12).

The fact that we chose subareolar and peritumoral injection is in addition to Patsikas et al. research, who used injection in neoplastic mammary gland parenchyma, to identify sentinel lymph nodes(14,15, 16) According to the descriptive anatomy, lymphatic vessels start as a network, a capillary network with a diameter of 20-70 μm. These vessels drain into precolector vessels, which have a diameter of 300 μm, containing valves, equipped with fine bundles of muscle fiber and drained into the skin. Based on this finding and the fact that the

mammary gland, in concordance with embryology, is a modified gland, lymphatic vasculature can systematize the udder

Lymphatic vasculature originates in the interstitial spaces of the mammary gland. Mammary gland has a superficial lymphatic network, which collects lymph from the skin and subcutaneous tissue covering the mammary gland, and deep lymphatic network (the actually mammary gland). Between the two networks is done two types of lymphatic anastomoses: one in the areola and the second at the breast periphery (13). These anastomoses are performed with tumor cells invading the superficial lymphatic network.

After subareolar injection, the dye was taken by superficial lymphatic vessels, which they could view right next to the epidermis, perpendicular to its surface, continue with lymphatic capillaries located deep in the epidermis. This finding leads us to say that subareolar network practically drains the deep lymphatic network of the mammary gland too. Much larger lymphatic vessels were observed around the nipple and its base. These lymph vessels joined in 2 or 3 collectors who had gathered in the cranially axillary lymph nodes in for T1 and A1, or caudally into superficial inguinal lymph nodes for A1 and inguinal mammary glands. By section, the vessel's presented a rather tortuous route portion on the superficial subcutaneous tissue.

Other reason of choice subareolar injection, in addition to the above anatomically specification, is related to the facility of injection in this location, on the one hand, and on the other hand, when a tumor can not be palpated, there is a risk of failure sentinel lymph node identification. Also, we can not omit the fact that the route of blue dye to the first lymph node station, is shorter after subareolar injection (17). We believe that, subareolar injection is in deeply concordance with the requirements of anatomical lymphatic drainage of the mammary glands.

Deep injection reasoning, more specifically peritumoral, derived from descriptive anatomy of deep parenchyma of mammary gland. Poirier et al., described in women, that the glandular lymphatic vessels, arise from the glandular lobes of perilobular bags, which are continuing with superficial subareolar plexus, which drain into the axillary lymph nodes.

Present research does not comply fully with the other descriptions, because the peritumoral injection of cranial thoracic mammary gland, were not identified as sentinel lymph nodes, the axillary nodes, but it was well colored the cranial sternal lymph node. Our results provide an anatomical explanation for the present experiment. This is attributed to two possibilities: 1. tumor compression could be achieved by a lack of lymphatic vessels immediately proximal tumor staining, making drainage through the lymph vessels deeper specified above. This is quite plausible, considering the numerous anastomoses which are made through the perforations of thoracic fascia (18, 19).

The lymphatic system, according to the anatomists, is classified, in the superficial and deep lymphatic system, in dependent relationship with the deep fascia. Mammary gland is encased by a fibrous capsule, dependent of superficial fascia, which in mammary gland have a superficial and one deep layer. The two layers merge at the periphery of the gland, continuing cranially to cranial thoracic mammary gland with a conjunctive fine portion, imprecisely defined, constituting a sort of suspensory ligament of the gland (T1), and merges caudally with thoracic fascia . For abdominal and inguinal mammary glands, mammary fascia merges with abdominal fascia..

Superficial fascia has a very fine structure, is fenestrated, but well defined, adhering closely with glandular mass. The fascia is more developed from the caudal portion of the

cranial thoracic mammary gland, and passes between the network of small vessels in the chorion and the vascular-lymphatic. The importance of this fascia is that it does not form a security barrier to tumor invasion, is sometimes fenestrated, facilitating communication between breast lobules. Also, it is ran through by arteries, veins, nerves and, most importantly, the lymphatic vessels connecting the superficial plexus of subcutaneous lymph gland. Stiles has described in very rare cases, presence of small islands of glandular parenchyma, accompanying fibrous extensions of the fascia, which sometimes penetrate between the muscle fibers of the pectoral muscle, explaining the release of tumor at this level and beyond in the mediastinum. .

A second possibility could be linked to the variability of the lymphatic system of carnivores, which was revealed in our previous research, along with the hypothesis that in some cases there is anastomosis of the two drains mammary lymphatic plexus, the deep and superficial one. Variability in this situation is related to the presence of penetrating lymphatic vessels, which for the T1 mammary gland, go with the internal mammary artery branches, which can not be predicted or detected clinically (20,21).

Therefore the possibility that sentinel lymph nodes are located intramammary is also unpredictable (22), once again our claim that unjustified excision of lymph nodes without a prior evaluation is unrecomended is as documented as possible (23). As well, after the concomitant subareolar and peritumoral injection of the inguinal mammary gland, alongside the superficial inguinal lymph nodes, there was noticed an emphasized coloring of a superficial lymphatic plexus, at the medial aspect of the thigh. The popliteal lymph node was also colored.. This is in concordance with the research of Dessiris and Patsikas, which gave similar results to intraparenchymal injection of the inguinal mammary gland..

Conclusions

The results of our research have shown that, there are practically sufficient arguments to consider that the two subareolar and peritumoral injection methods are truly important in identifying sentinel lymph nodes in mammary gland neoplazia in carnivores. Subareolar injection is easy to perform and can accurately identify sentinel lymph nodes of mammary glands, especially in the presence of impalpable tumors. Peritumoral injection, when it's possible to perform is, just as important, if we consider the variability and plasticity of the lymphatic drainage of the mammary glands in carnivores, meaning that it can identify the sentinel lymph nodes with other location than standard joint location.

We believe that the two techniques are complementary for successfully locating sentinel lymph nodes. Therefore, our recommendation is that for an accurate identification of sentinel lymph nodes in neoplastic mammary glands in carnivores the two modes of injection (peritumoral and subareolar) should be applied.

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RESEARCH REGARDIN THE SURFACE ON TRANSVERSAL SECTION AND DENSITY OF SUPERFICIAL PECTORAL MUSCLE OF MEAT TYPE HYBRID COBB-500, SLAUGHTERED AT DIFFERENT AGE STAGES

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Abstract

For our study we collected histological samples from the superficial pectoral muscle of 30 COBB-500 meat type hybrid chicken, slaughtered at the age of 35, 42 and 49 days. The samples were procesed using the paraffine sectioning method, alowing us to obtain 50 histological slides which were studied using a MC3 type microscope. We measured the following parameters: myocyte diameters, perimeter and their density. We also determined the surface on transversal section of these muscular fibers. The results were as follows: the average myocytes diameter, at the age of 35 days had a value of 28.012µ, for males and of 24.752µ, for females; the average perimeter of myocytes was of 88.004µ, for males and of 78.117µ, for females; the surface of myocytes had a mean value of 605.46μ^2, for males and of 483.8 μ^2, for females; the density of these fibers varied between 874.46 and 1033.31f.m./mm^2, regarding of the sex of chickens. At the age of 42 days, the myocytes had an average thickness of 34.73µ, for males and 29.40µ, for females, their perimeter was of 92.373µ-109.104µ, depending on sex. The density of these myocytes had values of 672.76 f.m./mm^2,for males and of 721.07 f.m./mm^2,for females.At the age of 49 days,the myocytes had a average thickness of 37.44µ, for males and of 32.47µ,for females,with an average perimeter of 102.01-117.63µ,depending on sex.These myocytes density had values of 596 f.m./mm^2, for males and of 637 f.m./mm^2,for females.There were differences of 7.10-26.79%,between the two sexes, regarding the studied parameters,at all the three slaughter age. These differences were highly statistical significant. Also, most of the differences between those three slaughter ages of the chickens, both in male and female, are very statistically significant.

Keywords: COBB-500, densitaty, myocytes, superficial pectoral, perimeter

Introduction

Poultry meat has remarkable dietetic qualityes and a high trofic-biologycal value, which makes this product very requested not only among costumers but also by the meat industry. Chicken meat is the most consumed of all poultry meat and it is obtained from specific highly performant hybrids. Among these there is COBB-500 meat type hybrid which we analysed in this study [14] and which has great performances in meat industry domain [14]. Because the problems that regard the meat quality of this hybrid are far from being resolved, new studies are required. The quality of meat can be influenced by many factors including some histological aspects (thickness, density, profile) of muscular fibers from the muscles that compose the slaughtered bird carcasses [7];[9];[10];[11]. The researches that have been done until now [7];[9];[10];[11], have followed the technological and histological characteristics of pectoral and calf muscles of chickens that have been slaughtered at the technological age of 42 days, while, in this paper, we extended the study for other slaughtering ages.

Material and methods

The biological material that we used was represented by a number of 30 COBB-500 meat type hybrid chickens (15 males and 15 females), which have been slaughtered at the age of 35, 42 and 49 days. The chickens weight for each slaughter age was of 1406-1614 grams; of 2168-2296 grams and of 2350-2378 grams, according to sex [9]. After the slaughter stage

we extracted some histological samples from the middle area of the superficial pectoral muscle (Pectoralis superficialis) (PS) [1];[4];[5];[15], which have been processed by the stalk paraffin technique [2];[3];[15], to obtain 50 hystological slides with PS muscle transversal sections. These sections were colored using bycromic "E-H" method. The slides have been analised with a MC3-type photonic microscope, using a micrometric measuring device and a micrometric scale to count the myocytes. On microscopic field we photographed, measured and counted the Superficial Pectoral muscle myocytes. We measured the large diameter (LD) and the small diameter (SD) of muscular fibers that compose the primar muscular fascicles (PMF). To calibrate the microscope were calculated used two micrometric values (MV): 9.011μ and 4.441μ, corresponding to the two associations of oculars and objectives that we worked with [9];[10];[11]. Through calculations we determined the mean diameter (MD) of myocyte area and the muscle fiber density. We used the following mathematical formulas:

(1) Large diameter of myocytes: $MD_{(\mu)}$=Number of Micrometer Divisions. x M.V., where: V.M.=micrometric value of associations OC10 x OB20 = 4.441μ;

(2) Small diameter of myocytes (SM): $MD_{(\mu)}$=Number of Micrometer Divisions. x M.V., like formula (1);

(3) Mean diameter of myocytes: $D\bar{x} = \frac{LD+SD}{2}$;

(4) Myocytes perimeter: $P_{m.f.}=\frac{LD+SD}{2}\ x\ \pi\ (\mu^2)$;

(5) myocytes transversal section surface: $S_{s.t.}=\frac{LD\ x\ SD}{4}\ x\ \pi\ (\mu^2)$;

(6) Surface of primary muscle fascicles (PMF): $S_{PMF}=\frac{LDPMF\ x\ SDPMF}{4}\ x\ \pi\ (\mu^2)$, where: LDPMF=large diameter of primary muscle fascicles (μ); SDPMF=small diameter of primary muscle fascicles (μ);

(7) Myocytes density: $D_{m.f..}=\frac{Nr.m.f.\ x\ 10}{PMFS}$ (f.m./mm^2), where: Nr.m.f.=number of muscular fibers from one primary muscle fascicle; PMFS=primary muscle fascicles surface.

All data obtained from these micrometric measurements and calculations have been statistically processed and then interpreted. We calcuated the general statistical estimators such as: statistical mean and its standard error, standard deviation, variance and the coefficient of variation and also the Fischer (F) and Tukey (W) values to test the statistical significance of differences between the two sexes and between the three ages of slaughter, for all studied parameters. For these calculations we used a Anova Single Factor (ASF) statistical algorithm, included in Microsoft Excel software package [8].

Results and discussions

For bird hybrids bred for meat production, the pectoral muscles are highly important and developed and they represent about 68-71 % from the weight of chest with bone and skin (the region of the carcasses). In the chest there are two superficial pectoral muscles which represent 80-85 % of the breast and two deep pectoral muscles. In all these muscles the striated ones predominate assisted by several types of connective tissue (loose, fat, tendon) [2];[3]. The striated muscular tissue has a morpho-functional unit represented by myocyte (rabdocyte), a giant multinucleated cell, which is a few centimeters long and has a variable thickness and a cylindrical shape. Our results showed an average thickness ($D\bar{x}$) of these myocytes of 28.012±0.46μ, at the age of 35 days for males and one of 24.752±0.43μ, at the same age but for females (tab. 1).

The perimeter of these myocytes was 88.004±1.445μ, for males and 78.117±1.362μ for females. These values were found in chickens slaughtered at 35 days. The surface cross section of these myocytes had an average value of 605.457±19.49μ^2, for males and of 483.801±15.413μ^2, for females (tab. 1.). The density of superficial pectoral muscle myocytes was 874.46±18.95 m.f./mm^2, for males and 1033.31±11.75 f.m./mm^2 for females. At the age of 42 days, the muscle fibers of superficial pectoral muscle had an average thickness of 34.729±0.368μ, for males and of 29.403±0.486μ, for females and the perimeter of these myocytes ranged from 92.373-109.104μ, depending on sex (tab. 1).

In transversal section, the superficial pectoral muscle myocytes had an area of 930.579±19.487μ^2, for males and of 681.309±20.99μ^2, for females and their density had a values ranging between 672.76±16.30 and 721.073±13.446 m.f./mm^2 (tab. 1). At the age of 49 days the superficial pectoral muscle myocytes were also thicker: they had an average mean diameter (D$\bar{x}$) of 37.443±0.70μ, for males and of 32.472±0,76μ, for females. Their perimeter had values of 117.631±2.198μ, for males and of 102.013±2.388μ, for females with a cross section area of 843.319±38.56μ^2 – 1101.254±44.528μ^2, depending on gender (tab. 1). The density of these fibers was 595.782±17.978 f.m./mm^2, for males and 637.346±9.453 f.m./mm^2, for females (tab. 1).

Table 1. Statistical indicators for the muscular fibers thickness, perimeter and density from superficial pectoral muscle of COBB-500 avian meat hybrid, according to age

Specification			MU	N	Statistical indicators			Variation limits	
Slaughter age	Sex	Studied parameters			$\bar{x}$±s$\bar{x}$	s	V(%)	Min.	Max.
35 days	Male	Large diameter	μ	80	32.933± 0.604	5.406	16.42	17.764	42.189
		Small diameter	μ	80	23.086± 0.468	4.189	18.15	13.323	35.528
		Average diameter	μ	80	28.012± 0.460	4.114	14.69	15.543	38.858
		M.f.perimeter	μ	80	88.004± 1.445	12.924	14.69	48.831	122.077
		Cross sectional area	μ^2	80	605.457 ±19.49	174.315	28.79	185.880	1177.226
		Myocytes density	m.f./mm^2	30	874.46± 18.95	103.785	11.87	654.959	1094.043
	Female	Large diameter	μ	80	28.059± 0.494	4.416	15.74	13.324	35.528
		Small diameter	μ	80	21.448± 0.418	3.741	17.44	9.770	35.528
		Average diameter	μ	80	24.752± 0.429	3.838	15.51	11,769	35.528
		M.f.perimeter	μ	80	78.117± 1.362	12.178	15.59	36.973	111.614
		Cross sectional area	μ^2	80	483.801 ±15.453	138.217	28.57	106.886	991.360
		Myocytes density	m.f./mm^2	30	1033.31 ±11.75	64.375	6.23	922.637	1160.788

42 days	Male	Large diameter	µ	80	40.177± 0.482	4.308	10.72	31,087	52.670
		Small diameter	µ	80	29.281± 0.310	2.778	9.49	20.473	36.239
		Average diameter	µ	80	34.729± 0.368	3.293	9.48	25.780	43.455
		M.f.perimeter	µ	80	109.104 ±1.156	10.344	9.48	80.990	136.518
		Cross sectional area	μ^2	80	930.579 ±19.487	174.296	18.73	499.862	1429.592
		Myocytes density	m.f./mm^2	30	672.76± 16.30	89.309	13.27	503.01	853.32
	Female	Large diameter	µ	80	32.994± 0.534	4.780	14.49	17.764	42.101
		Small diameter	µ	80	25.80± 0.48	4.291	16.63	11.547	34.285
		Average diameter	µ	80	29.403± 0.486	4.347	14.78	15.388	38.193
		M.f.perimeter	µ	80	92.373± 1.527	13.656	14.78	48.344	119.987
		Cross sectional area	μ^2	80	681.309 ±20.993	187.765	27.56	174.397	1133.669
		Myocytes density	m.f./mm^2	30	721.073 ±13.446	73.648	10.21	606.304	867.689
49 days	Male	Large diameter	µ	80	44.210± 0.831	7.431	16.81	22.205	62.174
		Small diameter	µ	80	30.676± 0.725	6.484	21.14	17.764	44.410
		Average diameter	µ	80	37.443± 0.70	6.258	16.71	22.205	51.071
		M.f.perimeter	µ	80	117.631 ±2.198	19.659	16.71	69.759	160.446
		Cross sectional area	μ^2	80	1101.254±44.528	398.268	36.16	387.25	2672.819
		Myocytes density	m.f./mm^2	30	595.782 ±17.978	98.470	16.53	388.78	826.830
	Female	Large diameter	µ	80	36.860± 0.82	7.348	19.84	17.764	48.851
		Small diameter	µ	80	28.084± 0.813	7.270	25.89	15.543	42.189
		Average diameter	µ	80	32.472± 0.76	6.800	20.94	17.768	45.520
		M.f.perimeter	µ	80	102.013 ±2.388	21.364	20.94	55.807	143.005
		Cross sectional area	μ^2	80	843.319 ±38.56	344.884	40.90	247.840	1618.686
		Myocytes density	m.f./mm^2	30	637.346 ±9.453	51.777	8.12	567.631	753.860

Table 2. The density, perimeter and surface of superficial pectoral muscle myocytes of COBB-500 avian meat hybrid, according to sex and slaughtering age

Muscle	Fiber parameters studied	MU	Slaughter age and sex								
			35 days			42 days			49 days		
			Male (M)	Female (F)	±%F/M* (pp**)	Male (M)	Female (F)	±%F/M* (pp**)	Male (M)	Female (F)	±%F/M* (pp**)
Superficial pectoral muscle (Pectoralis superficialis)	Large diameter	μ	32.933	28.059	-14.80	40.177	32.994	-17.88	44.210	36.860	-16.63
	Small diameter	μ	23.086	21.448	-7.10	29.281	25.800	-11.89	30.676	28.084	-8.45
	Average diameter	μ	28.012	24.752	-11.64	34.729	29.403	-15.34	37.443	32.472	-13.28
	Myocytes perimeter	μ	88.004	78.117	-11.23	109.104	92.373	-15.33	117.631	102.013	-13.28
	Surface	μ^2	605.457	483.801	-20.09	930.579	681.309	-26.79	1101.254	843.319	-23.42
	Myocytes density	m.f./mm$_2$	874.46	1033.31	+18.16	672.76	721.073	+7.18	595.782	637.346	+6.98

*procentual comparation between female and male; **pp=procentual points

Table 3. The density, perimeter and surface of superficial pectoral muscle myocytes of COBB-500 meat-type hybrid, slaughtered at different ages

Studied muscle	Fiber parameters studied	**Slaughtering age and sex**					
		35 days					
		Male (M)		***Female (F)***		***Average value***	
		a.v.**	**r.v.*****	**a.v.**	**r.v.**	**a.v.**	**r.v.**
Superficial pectoral muscle (Pectoralis superficialis)	Large diameter (μ)	32.933	81.97 ***-18.03***	28.059	85.04 **-14.96**	30.496	83.36 **-16.64**
	Small diameter (μ)	23.086	78.84 **-21.16**	21.448	83.13 **-16.87**	22.267	80.85 **-19.15**
	Average diameter (μ)	28.012	80.66 -19.34	24.752	84.27 **-15.73**	26.382	82.31 **-17.69**
	Myocytes perimeter(μ)	88.004	80.66 **-19.34**	78.117	84.57 **-15.43**	83.060	82.45 **-17.55**
	Transv.section surface of m.f. (μ^2)	605.457	65.06 **-34.94**	483.801	71.01 **-28.99**	544.629	67.58 **-32.42**
	Myocytes density (m.f./mm^2)	874.460	129.98 **+29.98**	1033.31	143.30 **+43.30**	953.885	136.87 **+36.87**
		42 days*					
	Large diameter (μ)	40.177	100.00	32.994	100.00	36.585	100.00
	Small diameter (μ)	29.281	100.00	25.800	100.00	27.540	100.00
	Average diameter(μ)	34.729	100.00	29.373	100.00	32.051	100.00
	Myocytes perimeter(μ)	109.104	100.00	92.373	100.00	100.738	100.00
	Transv.section surface of m.f. (μ^2)	930.579	100.00	681.309	100.00	805.944	100.00
	Myocytes density (m.f./mm^2)	672.76	100.00	721.073	100.00	696.916	100.00
		49 days					
	Large diameter (μ)	42.210	110.04 **+10.04**	36.860	111.72 **+11.72**	40.535	110.80 **+10.80**
	Small diameter (μ)	30.676	104.76 **+4.76**	28.084	108.85 **+8.85**	29.380	106.68 **+6.68**
	Average	37.443	107.81	32.472	110.55	34.957	109.07

	diameter(μ)		**+7.81**		**+10.55**		**+9.07**
	Myocytes perimeter(μ)	117.631	107.82 **+7.82**	102.013	110.44 **+10.44**	109.822	109.02 **+9.02**
	Transv.section surface of m.f. (μ^2)	1101.254	118.34 **+18.34**	843.319	123.78 **+23.78**	972.286	120.64 **+20.64**
	Myocytes density (m.f./mm^2)	595.782	88.56 **-11.44**	637.346	88.39 **-11.61**	616.564	88.47 **-11.53**

*technological slaughter age of this type of meat hybrid; **A.v.=absolute values; ***R.v.=relative values; pp=procentual points.

From the data presented in table 1, it appears that, in females, the muscle fibers from superficial pectoral muscle are thinner (finer) than in males, but their density is higher than the one of males. And this applies to all three slaughter ages of chickens. Thus, at the age of 35 days, the female superficial pectoral muscle myocytes are thinner with 11.23-11.64% (fig. 1) than males, but their density is superior with 18.16 % (tab. 2) (fig. 4).

Table 4. Statistical semnification of differences between the two sexes, regarding the myocytes thickness, perimeter and transversal section surface of the superficialpectoral muscle of COBB-500 hybrid

Slaughter age	Studied parameters of muscular fibers	Differences between compared sexes average values	Tukey values (w=0.01)	Statistical semnification	At 1; 158 GL. for:			
					P	p ≤ 0.05	p ≤ 0.01	p ≤ 0.001
					Fα	**3.84**	**6.64**	**10.83**
35 days	Large diameter (μ)	M-F*=4.874	2.009	***	$\hat{F}$	**38.999**		
	Small diameter (μ)	M-F=1.638	1.616	**	$\hat{F}$	**6.808**		
	Average diameter (μ)	M-F=3.260	1.619	***	$\hat{F}$	**26.864**		
	Myocytes perimeter(μ)	M-F=9.887	5.110	***	$\hat{F}$	**24.801**		
	Transv.section surface of m.f. (μ^2)	M-F=121.656	64.017	***	$\hat{F}$	**23.925**		
	Myocytes density (m.f.**/mm^2)	M-F=158.850	59.378	***	**Fα*** **	**4.008**	**7.103**	**12.034**
					$\hat{F}$	**50.755**		
42 days	Large diameter (μ)	M-F*=7.183	1.852	***	$\hat{F}$	**99.678**		
	Small diameter (μ)	M-F=3.481	1.471	***	$\hat{F}$	**37.089**		
	Average diameter (μ)	M-F=5.326	1.569	***	$\hat{F}$	**76.300**		
	Myocytes perimeter(μ)	M-F=16.731	4.930	***	$\hat{F}$	**76.300**		
	Transv.section surface of m.f. (μ^2)	M-F=249.270	73.724	***	$\hat{F}$	**75.734**		
	Myocytes density (m.f./mm^2)	M-F=48.273	56.281	**n.s.**	**Fα*** **	**4.008**	**7.103**	**12.034**
					$\hat{F}$	**5.225**		
49 days	Large diameter (μ)	M-F*=7.350	3.007	***	$\hat{F}$	**39.571**		
	Small diameter (μ)	M-F=2.592	2.803	**n.s.**	$\hat{F}$	**5.667**		
	Average diameter (μ)	M-F=4.971	2.659	***	$\hat{F}$	**23.150**		
	Myocytes perimeter(μ)	M-F=15.618	8.355	***	$\hat{F}$	**23.149**		
	Transv.section surface of m.f. (μ^2)	M-F=257.935	151.504	***	$\hat{F}$	**19.202**		
	Myocytes density (m.f./mm^2)	M-F=41.564	54.090	**n.s.**	**Fα*** **	**4.008**	**7.103**	**12.034**
					$\hat{F}$	**4.187**		

*M=male; F=female;**m.f.=muscular fibers; ***Fα la 1;58 GL (n = 30).

At the age of 42 days, the differences between females and males were mantained and even increased, being 15.33-15.44 %, for the average thickness of myocytes; of 26.79 %, for the cross section area (tab. 2) (fig. 1,3). As for density, here the difference between the sexes is 7.18 % (tabelul 2) (fig. 4).

At the age of 49 days, we found gender differences in all parameters studied as follows: 13.28 % for myocytes thickness; 23.42 %, for the cross-sectional area and 6.98 %, for their density (tab. 2) (fig. 1, 4, 5).

As the slaughter age of 42 days is considered to be the technological age and the reference in our study, we also made some comparisons between the 3 age of slaughter of chickens. Thus, the average thickness of superficial pectoral muscle myocytes was 26.382µ (the mean of sexes), this being 17.69 % lower than the equivalent (mean of sexes) found for the age of 42 days (32.051µ) (tab. 3) (fig. 1). It also notes that at the age of 49 days the muscle myocytes are thicker with 9.07 % (mean of sexes) than those of the same muscle at 42 days (tab. 3). So there is an increased thickness of muscle fibers with increasinf age of the bird (from 26.382µ to 32.051µ to 34.957µ) (tab. 3.). The situation is similar for muscle fibers perimeter (tab. 3) and their cross-sectional area, but the differences are greater in the latter case (32.42 % and 20.64 %) (tab. 3) (fig. 2, 3).

Table 5. Statistical semnification of differences between the three slaughtering ages, regarding the myocytes thickness, density, perimeter and transversal section surface of the superficial pectoral muscle of COBB-500 hybrid

SEX	Studied parameters of muscular fibers	Differences between compared slaughter age* average values	Tukey values (w=0.01)	Statistical semnification	At 2; 237 GL. from:			
					P	p ≤ 0.05	p ≤ 0.01	p ≤ 0.001
					Fα	2.99	4.60	6.91
MALE	Large diameter (µ)	V_1-V_2=7.244	2.699	***	$\hat{F}$	76.077		
		V_1-V_3=11.277		***				
		V_2-V_3=4.033		***				
	Small diameter (µ)	V_1-V_2=6.195	2.182	***	$\hat{F}$	58.193		
		V_1-V_3=7.590		***				
		V_2-V_3=1.395		n.s.				
	Average diameter (µ)	V_1-V_2=6.717	2.176	***	$\hat{F}$	84.518		
		V_1-V_3=9.431		***				
		V_2-V_3=2.714		***				
	Myocytes perimeter(µ)	V_1-V_2=21.100	6.835	***	$\hat{F}$	84.519		
		V_1-V_3=29.627		***				
		V_2-V_3=8.527		***				
	Transv.section surface of m.f. (μ^2)	V_1-V_2= 325.122	124.564	***	$\hat{F}$	69.404		
		V_2-V_3= 495.797		***				
		V_2-V_3= 170.675		***				
	Myocytes density (m.f./mm^2)	V_1-V_2=201,70	75.448	***	**Fα*** **	**3.1140**	**4.8945**	**7.5575**
		V_2-V_3= 278.678		***				
		V_2-V_3=76.978		***	$\hat{F}$	**65.533**		

FEMALE	Large diameter (μ)	V_1-V_2=4.935	2.610	***	$\hat{F}$	48.470		
		V_1-V_3=8.801		***				
		V_2-V_3=3.866		***				
	Small diameter (μ)	V_1-V_2=4.352	2.456	***	$\hat{F}$	31.995		
		V_1-V_3=6.636		***				
		V_2-V_3=2.284		n.s.				
	Average diameter (μ)	V_1-V_2=4.651	2.377	***	$\hat{F}$	45.392		
		V_1-V_3=7.720		***				
		V_2-V_3=3.069		***				
	Myocytes perimeter(μ)	V_1-V_2=14.256	7.481	***	$\hat{F}$	43.844		
		V_2-V_3=23.896		***				
		V_2-V_3=9.640		***				
	Transv.section surface of m.f. (μ^2)	V_1-V_2= 197.508	110.712	***	$\hat{F}$	44.894		
		V_1-V_3= 359.518		***				
		V_2-V_3= 162.010		***				
	Myocytes density (m.f./mm^2)	V_1-V_2= 312.237	49.512	***	**Fα*** **	**3.1140**	**4.8945**	**7.5575**
		V_1-V_3= 395.964		***				
		V_2-V_3=83.727		***	$\hat{F}$	**319.968**		

The density of myocytes is higher at the age of 35 days (953.885 m.f./mm^2) and then decreases with age, at 696.916 m.f./mm^2 and 616.564 m.f./mm^2, there is an inverse proportion between their thickness and their density (tab. 3) (fig. 5). The differences between the sexes and between the 3 slaughter ages, for all studied parameters, were tested in erms of their statistical significance (tab. 4) (tab. 5). Thus, it appears that at the age of 35 days, all differences between males and females at all 6 studied parameters are very statistically distinct and significant ($\hat{F}$>Fα; D<$W_{0.01}$) (tab. 4).

At the age of 42 days, only the difference between the sexes for the density of myocytes was statistically insignificant ($\hat{F}$<Fα; D>$W_{0.01}$), the other five differences were highly significant (tab. 4). Finally, at the age of 49 days, there are two statistically significant differences, namely that for small diameter and the density of myocytes ($\hat{F}$<Fα; D>$W_{0.01}$), the remaining differences between males and females are statistically significant (tab. 4). Between the three slaughtering ages (V_1=35 days; V_2=42 days; V_3=49 days), for males, we found that most of the values are statistically significant ($\hat{F}$>Fα; D<$W_{0.01}$), except for the small diameter of myocytes (tab. 5). For the female sex the situation is repeated, except for one difference (for small diameters) which is insignificant, but the remaining values are highly statistically significant (tab. 5).

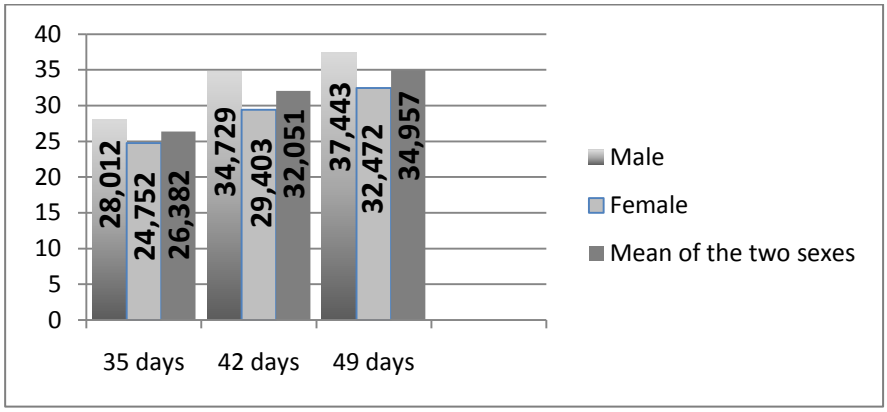

Fig.1. The average thickness of Superficial pectoral muscle myocytes regarding the three slaughter age of meat type hybrid COBB-500

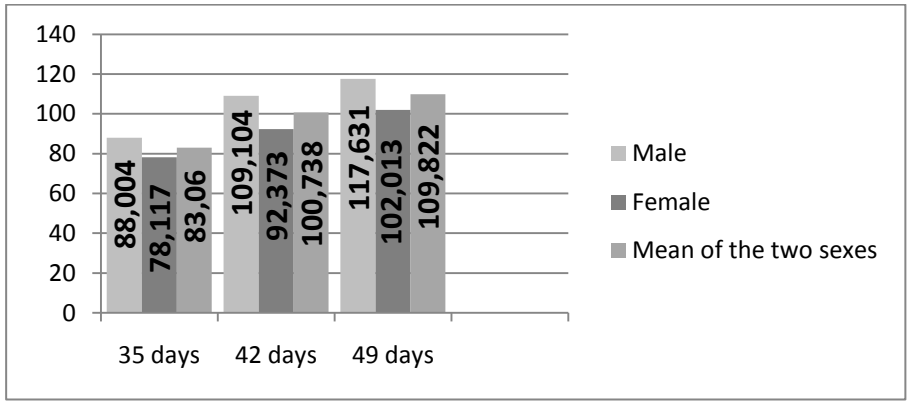

Fig.2. Superficial pectoral muscle myocytes perimeter concerning sex and slaughter age of meat type hybrid COBB-500

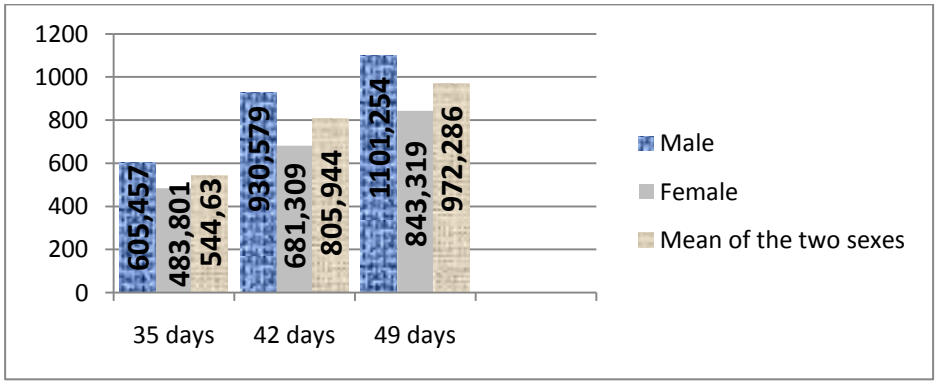

Fig.3. Tranversal section surface of Superficial pectoral muscle myocytes of meat type hybrid COBB-500 concerning sex and slaughter age

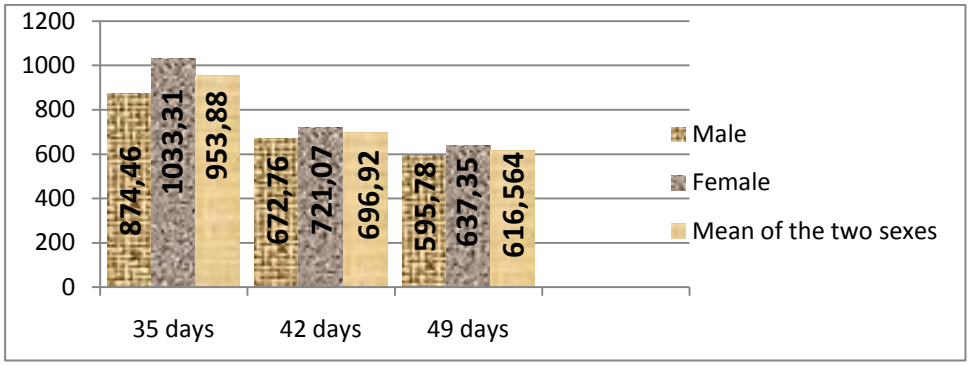

Fig.4. The density of Superficial pectoral muscle myocytes of meat type hybrid COBB-500 concerning sex and slaughter age

Conclusions

1. The average thickness of myocytes from superficial pectoral muscle, for COBB-500 meat-type hybrid increased with age, from 28.012µ, at 35 days, up to 37.443µ, at 49 days, for males and from 24.752µ, at 35 days up to 32.472µ at 49 days, for females.
2. The average thickness of muscle myocytes of the studied hybrid was lower in females, with 11.64-13.28-15.34 % compared to males for all 3 slaughter ages (35, 42 and 49 days).
3. The perimeter of myocytes from superficial pectoral muscle had values (mean of sexes) which increase from 83.06µ, at 35 days up to 109.82µ, at 49 days, the values from females being lower than those of males with 11.23-13.28-15.33 %.
4. The cross section surface of myocytes from superficial pectoral muscle studied has values of 544.63μ^2, at 35 days; of 805.94μ^2, at 42 days and of 972.29μ^2, at 49 days, being directly linked to their average diameter and circumference.
5. The superficial pectoral muscle myocytes density of COBB-500 meat-type hybrid had values of 953.88 m.f./mm^2, at 35 days; of 696,92 m.f./mm^2, at 42 days and of 616,56 m.f./mm^2, at 49 days, being inversely proportional with their thickness.
6. Most of the differences between males and females and between the 3 age of slaughter have been shown to be distinct and highly statistically significant in almost all parameters studied.

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INFLAMMATORY MEDIATORS AS THE POSSIBLE MECHANISM OF THE GASTRIC ULCERATION IN "SHAY RAT MODEL": STUDYING THE GASTROPROTECTANT ROLE OF COPPER-GLYCINATE AND -NICOTINATE COMPLEXES

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Abstract:
At a molecular level, the multifaceted-multifactorial pathogenesis of ulcer disease reflects imbalanced aggressive ulcerogenic and defensive cytoprotectant factors. The role of cytokines and growth factors in that process is not well-understood. As antiulcer agents predictably with no side-effects and natural biological occurrence, copper complexes have a wide-range of other relevant pharmacological effects. Investigating commonplace pathogenetic mechanisms particularly involving inflammation and oxidative stress that lead to microcirculatory failure - a characteristic of the early disease in other models and human - and their modulation by new therapeutic approaches is a necessisity. Quantitatively investigated plasma and gastric juice oxidative stress biomarkers [total peroxides (TP), total antioxidants (TAO) and oxidative stress index (OSI)], interleukin (IL)-6, and the soluble IL-6 receptor alpha (sIL-6Rα) content as compared to the descriptive histopathology in Shay Rat gastric ulcer model untreated or treated with either copper glycinate or copper nicotinate complexes or omeprazole as a therapeutic control was conducted. Histopathologically, gastric fundus showed ulcerative damage, inflammatory infilteration and microcirculatory failure - as congesition. Each of the 3 treatments greatly prevented the ulceration with minor difference is their efficiency. Lowered total antioxidants, increased total peroxides and accordingly the oxidative stress index, and higher IL-6 and sIL-6Rα contents were detected. Each of the 3 treatements markedly ameliorated these changes towards a cytoprotective trend (lowered TP, OSI, IL-6 and sIL-6Rα, and improved TAO). The inflammatory biomarkers correlated significantly and positively with each other and with the ulcer index, whereas, TAO correlated significantly and negatively with them. This is a first reporting of these mechanistic effectors for ulceration in the Shay Rat model and for copper complexes used.

Key words: Interleukin 6, Interleukin 6 soluble receptor α, oxidative stress, Shay rat gastric ulcer; copper nicotinate complex, copper glycinate complex, omeprazole

Introduction

At a molecular level, the multifaceted-multifactorial pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive ulcerogenic (proinflammatory cytokines, oxidative stress, acid, pepsin, ulcerogenic eicosanoids, extracellular matrix degradation, microcirculatory failure, *H. pylori* infection and cell shedding by apoptosis, etc) and decreased defensive cytoprotectant factors (antioxidants, mucin, cell proliferation, cytoprotective prostaglandins, angiogenic and mitogenic/motogenic growth factors, etc) (Szabo et al., 2007). The role of cytokines in that process is not well-understood. This necessitates investigating commonplace pathogenetic mechanisms particularly involving inflammation and oxidative stress leading to microcirculatory failure that characterizes early development of the disease and their modulation by new therapeutic approaches - including the organic copper complexes.

We investigated the antiulcer anti-inflammatory cytoprotectant effects of copper complexes with specific organic moieties utilizing "Shay Rat" gastric ulcer model, viral hepatitis patients and rat model for rheumatoid arthritis (el-Saadani et al., 1993 and el-

Saadani, 2004). However, their mechanism of action was still speculative due to the very limited number of mechanistic studies.

As antiulcer agents predictably with no side-effects and natural biological occurrence, copper complexes have a wide-range of other relevant pharmacological activities, namely; anti-inflammatory; antioxidants; healing promoter; angiogenic; analgesic; antipyretic; radioprotectant; antimicrobial; and antimutagenic. These complexes other than the ever existence of copper in their form, they facilitate copper absorption, tissue distribution and utilization (Sorenson, 1989; Saczewski et al., 2006).

Oxidative stress is a serious imbalance between production of reactive species and antioxidant defense in favor of the former, leading to potential damage (Halliwell and Whiteman, 2004). Several lines of research provided evidence for the involvement of free radicals and oxidative stress in the pathogenesis of experimental and human gastric ulceration (Rastogi et al., 1998; Das et al., 1998). Gastric mucosal lesion revealed decreased gastric blood flow, significant increase of neutrophil infiltration, increased local and plasma proinflammatory cytokines and significant oxidative stress; ameleoratable by antioxidants (Bhattacharya et al., 2006).

IL-6 - particularly in the gut - is a regulatory proinflammatory cytokines secreted by polymorphs and macrophages, mast cells, monocytes/macrophages, keratinocytes, endothelium and fibroblasts (Mora et al., 2006). IL-6 is crucial to epithelialization and influences granulation tissue formation, as proved from the wound healing studies of mice null for the IL-6 gene (Gallucci *et al.*, 2000). IL-6 activity is exceptionally agonistically modulated by its soluble receptor α (sIL-6Rα); through transsignaling even on the receptor non-expressing cells (Scheller et al., 2006). The sIL-6Rα in physiological fluids comes from proteolytic shedding of membrane-bound IL-6Rα (by TNF-α-converting enzyme, matrix metalloproteinase and other calcium dependent proteinases activation) and/or from an alternatively spliced mRNA species. Increased sIL-6Rα levels correlate a number of clinical conditions. This shedding is inducible by IL-1β, TNF-α, cellular cholesterol depletion, lipopolysaccharide endotoxin, specific viral infections, Ca^{2+} ionophore, C-reactive protein, phorbol ester, parathyroid hormone and ceramide (Jones et al., 1998; Franchimont et al., 2005).

In the model used, preventable ulceration involved fundic mucosa only with viable areas among ulcerations. Therefore, mucosal damage through oxidative stress and IL-6-mediated inflammation was proposed as possible commonplace mechanisms for the model and for the gastroprotective effect of the glycinate and nicotinate copper complexes used. Histopathology and omeprazole as therapeuric control were used. Ulcer index; IL-6, sIL-6Rα, and, global biomarkers of oxidative stress [total peroxides (TP), total antioxidants (TAO) and oxidative stress (OSI)] in the plasma and gastric juice were investigated and correlated to each other.

Material and methods

Synthesis and dosage of copper complexes: The copper (II)-(glycinate)$_2$ H_2O complex and The Copper (I)-Cl-(nicotinic acid)$_2$ complex were synthesized and their elementary analysis confirmed as before (Sorenson 1976; Goher 1987; el-Saadani et al., 1993). The ED_{50} of the copper(II)-glycinate complex - 5 mg/kg body weight - was chosen (Kishore et al., 1982) and copper(I)-nicotinic acid complex dose was chosen on previous experience to be 5 mg/kg body weight.

The "Shay Rat" fundic ulceration model and sampling: Wister Albino male rats weighing 200 - 250 g. were used to induce the starvation-pyloric ligation-fundic ulceration model (Shay et al., 1945) with modifications to make it more humane. Specifically, we used Ketamine (75 mg/kg)/Diazepam (5 mg/kg) intramuscular general anesthesia (Waynforth and Flecknell, 1992) and avoided subcutaneous saline injection to prevent drenching pneumonia. The anesthesia-induced 2 hrs post-surgery sleeping reduced number and severity of ulceration into ~50% of the original drastic model. Animal husbandry and surgery were conducted in the Experimental Surgery Unit, Department of General Surgery, Faculty of Medicine, Assiut University, Assiut, Egypt - permitted by the local ethics committee of conduct of scientific research. Rats in individual-drop-through cages were divided into 4 groups - 16 animal each - assigned as follow: ulcerated vehicle-treated control group I; ulcerated copper glycinate-treated experimental group II; ulcerated copper nicotinate-treated experimental group III; and ulcerated omeprazole-treated therapeutic control group IV. Each of the copper complexes was injected intragastrically after laparotomy and pyloric ligation and before abdominal wall wound closure. Omeprazole dissolved in dimethylsulfoxide (DMSO) as a single dose was administrated subcutaneously 1 hr before surgery at 20 mg/kg (Warzecha et al., 2001). 19 hrs post operation, plasma was separated from jugular EDTA-blood samples in pyrogen-free tubes, and stored aliquotted at -40 °C. After sacrificing rats, gastric juice collected was centrifuged at 3000 rpm/4 °C and stored aliquotted at -40 °C. The stomach was opened to score the fundic mucosal ulcerations (Peskar et al., 1986).

Investigations: Reagent grade chemicals were used (Sigma Chemical Co., St. Louis, MO, USA). Plasma and juice TP were measured colorimetrically as H_2O_2 equivalent by xylenol orange reagent (Harma et al., 2005). TAO was measured using the 2,2`-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) reagent (Erel, 2004). OSI was calculated as a ratio of TP content in μM to TAO in mM multiplied by 100 of each sample (Harma et al., 2005). Total rat IL-6 content was ELISA quantitatively assayed (Quantikine, cat. # R6000B, R&D Systems, Inc., Minneapolis, MN, USA; minimum detection limit is 21 pg/mL). The total sIL-6Rα was quantitated using DuoSet sandwich ELISA development kit (Cat.#DY1830; R&D Systems Europe, Ltd, Abingdon, UK; minimum detection limit was 12 pg/mL).

H&E histopathological staining: Full-wall specimens of stomach fundus were stained with standard H&E (Drury and Wallington 1980).

Statistical Analysis: Prism 3.0 statistical package (Graph Pad Software, Inc, San Diego, CA, USA) was used to analyze mean ± standard error of the mean (SEM), one way ANOVA for significance, and intragroup correlation among the investigated parameters by the non-parametric Spearman's analysis. The level of significance was set at $P<0.05$. All juice investigated parameters were corrected for the juice volume. Immunoassays were conducted only using 10 rats per group due to assay kit limitations.

Results

The histopathological Findings: Figure 1a, b, c and d, and, their inserts revealed the gross ulceration confined to the fundus, including epithelial discontinuation and inflammatory cell infiltration. Changes were extensive in control ulcerated untreated rats but was markedly alleviated by different treatments used.

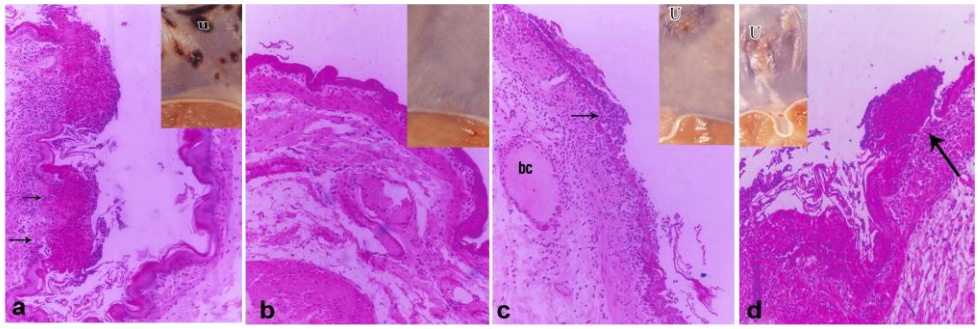

Fig. 1. Figure 1a is a photomicrograph of the fundus non-glandular stomach of the positive ulcerated control animals with inflammatory infilteration, multiple lymphoid follicles invading mucosa and discontinuous epithelium at the lesion side (arrow) while the other side looks more or less healthy representing inter-lesion areas; (H&E x100)

Inserted is a gross photo of the fresh stomach showing multiple deep ulcer patches (u). Figure 1b is for the Copper Glycinate group II with all layers looking healthy (H&E x100). Inserted is a gross photo of the fresh stomach appearing ulcer-free reflecting the preventability of ulceration. Figure 1c is for the Copper Nicotinate group III with minimal epithelium discontinuation (arrow) and inflammatory cells infiltration with blood capillary (bc) (H&E x100). Inserted is a gross photo of the fresh stomach with mild ulcer erosions (u). Figure 1d is for the Omeprazole therapeutic control group IV is epithelial discontinuation and lymph follicle aggregation (arrow) (H&E x100). Inserted is a gross photo of the fresh stomach with a few fundic congestion, erosions and ulcerations (u).

The ulcer index of different treatment groups: As shown in Table 1, the ulcerated control animals showed significant ulceration that was equipotently significantly prevented by copper glycinate treatment, copper nicotinate or omeprazole without significant difference among them.

Table 1. The fundic ulceration index in the studied groups of "Shay Rats" gastric ulceration model. ns = non-significant difference

Ulcer Index; n = 16	**Control**	**Glycinate**	**Nicotinate**	**Omeprazole**
Range	18.0 – 61.0	1.0 – 9.0	1.0 – 10.0	4.0 – 14.0
Mean ± SEM	**33.810 ± 3.478**	**3.625 ± 0.569**	**4.875 ± 0.724**	**7.863 ± 0.747**
P< vs. Control	-	0.001	0.001	0.001
P< vs. Glycinate	-	-	ns	ns
P< vs. Nicotinate	-	-	-	ns

The change in the oxidative stress biomarkers in gastric juice and plasma:

A. Effect on total peroxides (Figure 2): The ulcerated untreated control animals showed high gastric juice total peroxide content of 116.900 ± 8.914 μM/L (range, 88.39-175.50 μM/L). In comparison, the total peroxide content was significantly reduced after treatment with copper glycinate into 45.540 ± 4.943 μM/L (range 16.42-70.10 μM/L;

P<0.001), followed by the copper nicotinate treatment into 47.540 ± 5.902 μM/L (range 22.52-79.85 μM/L; P<0.001). Surprisingly, omeprazole caused highest significant reduction in the total gastric juice peroxide content (41.660 ± 6.688 μM/L; range 14.84-76.12 μM/L; P<0.001). However, there was no significant difference among the three treatment groups. The ulcerated untreated control animals showed a plasma total peroxide content of 35.540 ± 2.922 μM/L (range, 27.61 – 67.61 μM/L). In comparison, the total peroxide content was non-significantly reduced after treatment with copper glycinate into 31.400 ± 0.773 μM/L (range, 27.98 – 35.59 μM/L), whereas, the copper nicotinate caused mild significant reduction to 29.480 ± 0.186 μM/L (range, 28.59 – 30.60 μM/L; P<0.05). Again, omeprazole caused most marked significant reduction in the total plasma peroxide content to 28.840 ± 0.104 μM/L (range, 28.40 – 29.50 μM/L; P<0.05). However, there was no significant difference among the three treatments. The modest increase in plasma total peroxide content compared with its massive increase in the gastric juice may indicate that the stress-induced biological changes in the present model were mainly gastric.

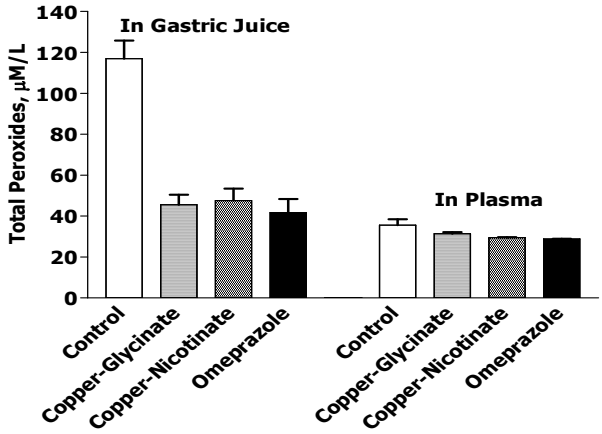

Fig. 2. The change in the total gastric juice and plasma peroxide content in the studied groups of "Shay Rats" gastric ulceration model. For the mean ± SEM, range and ANOVA inter-group significance of difference, see the text above

B. Effect on total antioxidant activity (TAO; Figure 3): The ulcerated untreated control animals showed a reduced gastric juice TAO of 0.121 ± 0.008 mM/L (range, 0.075 - 0.158 mM/L). In comparison, copper glycinate caused marked increase in gastric juice TAO reaching 0.840 ± 0.037 mM/L (range, 0.680 - 1.015 mM/L; P<0.001), followed by the copper nicotinate (0.617 ± 0.024 mM/L; range, 0.467 - 0.718 mM/L; P<0.001), then omeprazole (0.468 ± 0.038 mM/L; range, 0.217 - 0.696 mM/L; P<0.001). There was a significant difference among the three treatment groups (P<0.001).

The ulcerated untreated control animals showed a reduced plasma total antioxidant activity of 0.799 ± 0.017 mM/L (range, 0.691 - 0.863 mM/L). In comparison, copper nicotinate caused marked increase in plasma TAO reaching 1.515 ± 0.074 mM/L (range 0.955 - 1.800 mM/L; P<0.001), followed by the copper glycinate (1.188 ± 0.063 mM/L; range, range, 0.957 - 1.652 mM/L; P<0.001) and, omeprazole (0.962 ± 0.042 mM/L; range,

0.776 - 1.195 mM/L; P<0.05). There was a significant difference between glycinate treated group and each of nicotinate and omeprazole treated groups (P<0.001 and 0.01, respectively), whereas, nicotinate was, also, significantly higher than omeprazole treated group (P<0.001).

Plasma TAO changes were modest compared to the gastric juice content confirming the locality of most of the model-induced stress.

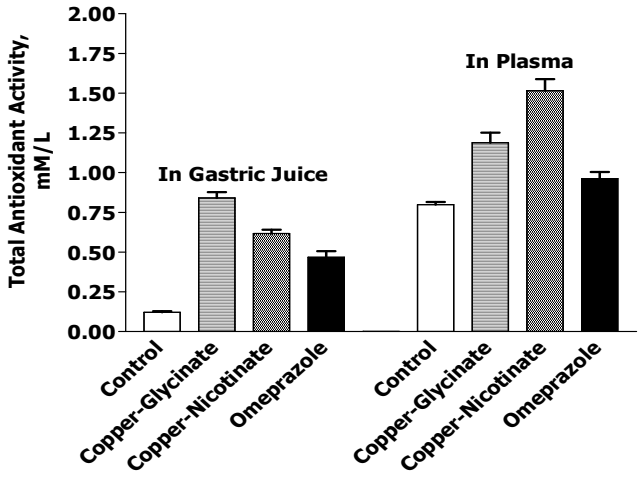

Fig. 3. The change in the total gastric juice antioxidant activity (TAO) in the studied groups of "Shay Rats" gastric ulceration model. For the mean ± SEM, range and ANOVA inter-group significance of difference, see the text above

C. Effect on the oxidative stress index (OSI; Figure 4): The ulcerated untreated control animals showed massive oxidative stress reaching 100.7 ± 6.349% (range 61.160 - 123.70%). In comparison, copper glycinate caused the lowest OSI reaching 5.587 ± 0.756% (range, 2.287 - 10.32%; P<0.001), followed by the copper nicotinate (7.495 ± 0.728%; range, 3.567 - 11.12%; P<0.001) and omeprazole (9.846 ± 2.002%; range, 2.132 - 27.85%; P<0.001). There was no significant difference among the three treatments. The ulcerated untreated control animals showed very modest oxidative stress in plasma reaching 4.460 ± 0.351% (rane, 3.271 - 8.016%). This finding confirms the notion that the model is a local gastric affliction rather than a generalized body change. In comparison, copper nicotinate caused the lowest OSI reaching 2.043 ± 0.134% (range, 1.588 - 3.203%; P<0.001), followed by the copper glycinate (2.738 ± 0.143%; range; 1.693 - 3.721%; P<0.001), and then, omeprazole (3.082 ± 0.132%; range, 2.376 - 3.802%; P<0.001). Thus, nicotinate caused a significantly lower OSI than each of glycinate and omeprazole treated groups (P<0.05 and P<0.01, respectively). There was no significant difference between glycinate and omeprazole treatments.

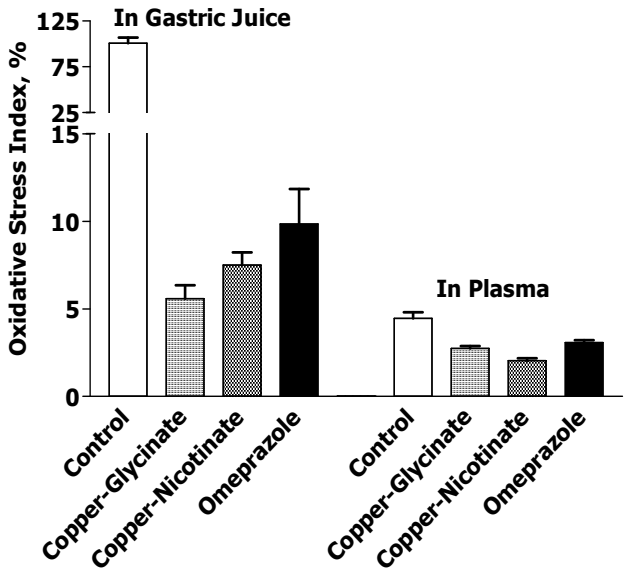

Fig. 4. The change in the oxidative stress index (OSI) in gastric juice of the studied groups of "Shay Rats" gastric ulceration model. For the mean ± SEM, range and ANOVA inter-group significance of difference, see the text above

The change in the total IL-6 in gastric juice and plasma (Figure 5): The ulcerated untreated control animals showed extensive induction of IL-6 in gastric juice reaching 327.600 ± 24.770 pg/mL (range, 187.10-501.20 pg/mL). In comparison, each of the copper glycinate (133.400 ± 9.158; range, 90.13-191.70 pg/mL), copper nicotinate (160.800 ± 6.920 pg/mL; range, 120.40-198.00 pg/mL) and omeprazole (162.800 ± 6.083 pg/mL; range, 120.30-190.30 pg/mL) treatment caused highly significant reduction in the IL-6 gastric juice content ($P<0.001$). There were no significant differences amongst the three treatments. The ulcerated untreated animals showed increased level of IL-6 in the plasma reaching 112.000 ± 10.560 pg/mL (range, 68.47-192.60 pg/mL). In comparison, each of the glycinate (47.880 ± 4.552 pg/mL; range, 18.97-76.27 pg/mL), nicotinate (48.900 ± 5.029 pg/mL; range, 24.20-83.46 pg/mL) and the omeprazole (59.15 ± 4.42 pg/mL; range, 25.44-80.25 pg/mL) treated groups showed significant reduction ($P<0.001$) in plasma level of IL-6. There was no significant difference amongst the three treatments. Again, the magnitude of change in the plasma IL-6 content reflects the locality of the model affliction with the used modifications.

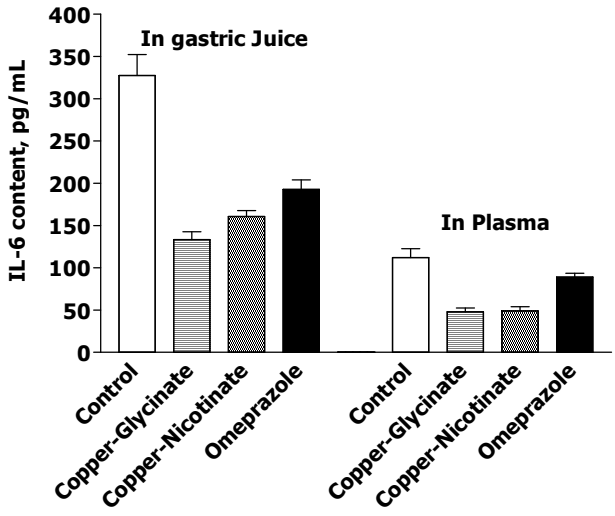

Fig. 5. The change in the IL-6 content of gastric juice and plasma in the studied groups of "Shay Rats" gastric ulceration model. For the mean ± SEM, range and ANOVA inter-group significance of difference, see the text above

The change in total sIL-6Rα in gastric juice and plasma (Figure 6): The ulcerated untreated control animals showed extensive induction of sIL-6Rα in gastric juice reflecting a trans-signaling inflammatory pathogenetic role reaching 574.400 ± 22.080 ng/mL (range, 429.500–731.400 ng/mL). In comparison, each of the copper glycinate (120.000 ± 6.944; range, 81.040–162.500 ng/mL), copper nicotinate (99.450 ± 4.423; range, 78.550–132.000 ng/mL) and omeprazole (346.200 ± 10.400; range, 296.200–409.700 ng/mL) treatment caused highly significant reduction in the sIL-6Rα gastric juice content ($P<0.001$). The glycinate and nicotinate groups were non-significantly different and each was significantly lower than omeprazole group ($P<0.001$). The ulcerated untreated animals showed increased level of sIL-6Rα in the plasma reaching 218.200 ± 8.486 ng/mL (range, 186.300–293.600 ng/mL). In comparison, each of the glycinate (72.160 ± 2.353; range, 46.44–87.19 ng/mL), nicotinate (79.120 ± 4.357; range, 60.510–127.200 ng/mL) and the omeprazole (173.500 ± 6.744; range, 112.800–212.800 ng/mL) treated groups showed significant reduction ($P<0.001$) in plasma level of sIL-6Rα. The glycinate and nicotinate groups were non-significantly different and each was significantly lower than omeprazole group ($P<0.001$). Again, the magnitude of change in the plasma sIL-6Rα reflects the locality of the model-induced stress.

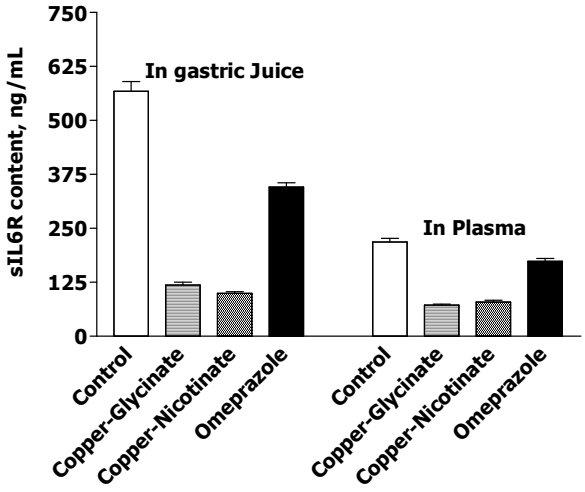

Fig. 6. The change in the sIL-6Rα content of gastric juice and plasma in the studied groups of "Shay Rats" gastric ulceration model. For the mean ± SEM, range and ANOVA inter-group significance of difference, see the text above

The results of the intragroup correlation studies:

Table 2 presents correlations among investigated parameters in the control ulcerated untreated rats; UI correlated significantly and positively with each of; total peroxides in juice and plasma, juice and plasma IL-6, juice and plasma OSI, but negatively with TAO in the juice and plasma. Similar trend of correlation was notice for the juice and plasma biomarkers namely; total peroxides, OSI, IL-6.

Table 2. Correlation of the investigated parameters in the Control ulcerated untreated "Shay Rats"

Control	jTP	jTAO	jOSI	jIL-6	jsIL-6R	pTP	pTAO	pOSI	pIL-6	psIL-6R
UI	0.635 0.01	-0.653 0.001	0.775 0.001	0.776 0.001	0.668 0.01	0.582 0.01	-0.746 0.001	0.747 0.001	0.849 0.001	0.799 0.001
jTP		-0.650 0.01	0.500 0.05	0.756 0.001	0.426 ns	0.757 0.001	-0.575 0.01	0.643 0.01	0.646 0.01	0.616 0.01
jTAO			-0.700 0.01	-0.781 0.001	-0.499 0.05	-0.738 0.001	0.569 0.05	-0.613 0.01	-0.610 0.01	-0.530 0.05
jOSI				0.705 0.001	0.523 0.05	0.391 ns	-0.618 0.01	0.461 0.05	0.689 0.01	0.439 0.05
jIL-6					0.505 0.05	0.681 0.01	-0.751 0.001	0.657 0.01	0.749 0.001	0.758 0.001
jsIL-6R						0.228 ns	-0.495 0.05	0.616 0.01	0.719 0.001	0.374 ns
pTP							-0.439 0.05	0.510 0.05	0.521 0.05	0.590 0.01

pTAO								-0.808 0.001	-0.770 0.001	-0.826 0.001
pOSI									0.714 0.001	0.797 0.001
pIL-6										0.752 0.001

Upper numbers are the Spearman's "r" value and lower numbers are the "P<" value. ns = non-significant. UI = ulcer index; jTP = juice total peroxides; jTAO = juice total antioxidants; jOSI = juice oxidative stress index; jIL-6 = juice IL-6 content; pTP = plasma total peroxides; pTAO = plasma total antioxidants; pOSI = plasma oxidative stress index; pIL-6 = plasma IL-6 content

In the copper glycinate-treated, nicotinate-treated or omeprazole-treated "Shay Rats" (**Tables 3, 4 and 5**), a similar trend of correlations was notice, however, the reduced values for most of the biomarkers studied made several correlations non-significant.

Table 3. Correlation of the investigated parameters in the Glycinate-treated "Shay Rats". See table 2 for nature of the data and abbreveations

Glycinate	**jTP**	**jTAO**	**jOSI**	**jIL-6**	**jsIL-6R**	**pTP**	**pTAO**	**pOSI**	**pIL-6**	**psIL-6R**
UI	0.841 0.001	-0.706 0.001	0.670 0.01	0.717 0.001	0.626 0.01	0.627 0.01	-0.694 0.01	0.693 0.01	0.580 0.01	0.540 0.05
jTP		-0.700 0.01	0.650 0.01	0.683 0.01	0.670 0.01	0.650 0.01	-0.550 0.05	0.704 0.01	0.555 0.05	0.566 0.05
jTAO			-0.650 0.01	-0.586 0.01	-0.387 ns	-0.500 0.05	0.662 0.01	-0.595 0.01	-0.482 0.05	-0.359 ns
jOSI				0.561 0.05	0.535 0.05	0.400 ns	-0.352 ns	0.619 0.01	0.549 0.05	0.597 0.01
jIL-6					0.696 0.01	0.610 0.01	-0.594 0.01	0.563 0.05	0.458 0.05	0.196 ns
jsIL-6R						0.633 0.01	-0.281 ns	0.612 0.01	0.685 0.01	0.680 0.01
pTP							-0.470 0.05	0.401 ns	0.603 0.01	0.530 0.05
pTAO								-0.483 0.05	-0.263 ns	-0.180 ns
pOSI									0.481 0.05	0.453 0.05
pIL-6										0.722 0.001

Table 4. Correlation of the investigated parameters in the Nicotinate-treated "Shay Rats". See table 2 for nature of the data and abbreveations

Nicotinate	jTP	jTAO	jOSI	jIL-6	jsIL-6R	pTP	pTAO	pOSI	pIL-6	psIL-6R
UI	0.845 0.01	-0.605 0.01	0.869 0.001	0.745 0.001	0.764 0.001	0.777 0.001	-0.707 0.001	0.698 0.01	0.788 0.001	0.504 0.05
jTP		-0.600 0.01	0.850 0.001	0.705 0.001	0.757 0.001	0.791 0.001	-0.657 0.01	0.669 0.01	0.711 0.001	0.470 0.05
jTAO			-0.600 0.01	-0.567 0.05	-0.715 0.001	-0.459 0.05	0.551 0.05	-0.462 0.05	-0.712 0.001	-0.548 0.05
jOSI				0.705 0.001	0.739 0.001	0.770 0.001	-0.762 0.001	0.687 0.01	0.835 0.001	0.494 0.05
jIL-6					0.885 0.001	0.733 0.01	-0.713 0.001	0.700 0.01	0.700 0.001	0.545 0.05
jsIL-6R						0.676 0.01	-0.693 0.01	0.503 0.05	0.829 0.001	0.623 0.01
pTP							-0.713 0.001	0.702 0.01	0.713 0.001	0.347 ns
pTAO								-0.786 0.001	-0.765 0.001	-0.559 0.05
pOSI									0.541 0.05	0.428 0.05
pIL-6										0.682 0.01

Table 5. Correlation of the investigated parameters in the Omeprazole-treated "Shay Rats". See table 2 for nature of the data and abbreveations

Omeprazole	jTP	jTAO	jOSI	jIL-6	jsIL-6R	pTP	pTAO	pOSI	pIL-6	psIL-6R
UI	0.701 0.001	-0.744 0.001	0.879 0.001	0.792 0.001	0.747 0.001	0.390 ns	-0.624 0.01	0.717 0.001	0.634 0.01	0.619 0.01
jTP		-0.583 0.01	0.755 0.001	0.594 0.01	0.658 0.01	0.323 ns	-0.659 0.01	0.448 0.05	0.345 ns	0.417 ns
jTAO			-0.770 0.001	-0.718 0.001	-0.558 0.05	-0.392 ns	0.423 ns	-0.555 0.05	-0.367 ns	-0.526 0.05
jOSI				0.836 0.001	0.748 0.001	0.445 0.05	-0.627 0.01	0.758 0.001	0.636 0.01	0.623 0.01
jIL-6					0.817 0.001	0.439 0.05	-0.545 0.05	0.745 0.001	0.619 0.01	0.535 0.05
jsIL-6R						0.480 0.05	-0.545 0.05	0.640 0.01	0.363 ns	0.247 ns
pTP							-0.315 ns	0.255 ns	0.488 0.05	0.394 ns
pTAO								-0.475 0.05	-0.543 0.05	-0.477 0.05
pOSI									0.546 0.05	0.477 0.05
pIL-6										0.858 0.001

Discussion

Ulceration was prevented most potently with copper glycinate complex and copper nicotinate complex then the therapeutic control treatment with omeprazole; without significant difference among them. Gastroprotective effects of copper complexes were previously reported and differentially utilized several mechanisms including; antisecretory, antioxidant, and prostaglandin and cell proliferation modulation (Sorenson, 1989; el-Saadani et al., 1993; Sorenson and Wangila, 2007).

Histopathologically, ulcer lesions showed extensive infiltration with diffuse inflammatory cells, multiple lymphoid follicles through fundic wall layers and congested cappilaries. All of these damage markers were reduced with the treatments used. Inflammatory infiletration causes oxidative stress, DNA damage and mediate microvascular failure leading to induction of peptic ulcer (Jiménez et al., 2004). In addition, the normal stomach does not contain mucosa-associated lymphoid tissue (Jaskiewicz and Kobierska, 2000). Antiulcer agents particularly antioxidants attenuate gastric neutrophilic infiltration and hence myeloperoxidase activity and consequent depletion of antioxidants (Al Moutaery, 2003; Jainu and Mohan, 2008).

A pathogenetic role for oxidative stress in experimental and human natural gastric ulceration is inferred from several avenues; 1) Decrease of individual antioxidant biomarkers and/or increase in individual prooxidant biomarkers with ulceration (Pignatelli et al., 2001), 2) Gastroprotective agents including proton pump inhibitors reverse that picture for the beneifit of antioxidants (Ganguly et al., 2005; Bhattacharya et al., 2006), 3) Antioxidant treatment reduce the ulceration in several models and helped eradicating *H. pylori* infection (Chattopadhyay et al., 2004; Banerjee et al., 2008). However, there was no study that explored the oxidative stress situation globally using total antioxidant capacity and total peroxides to the oxidative stress index like the present study. Depending on the extent of peroxidative injury stressed cells will either survive or succumb via apoptosis or necrosis. Extensive damage beyond both constitutive and inducible repair capacity triggers apoptosis, but gross damage, which preempts any type of programmed metabolic response results in necrosis (Dotan et al., 2004).

The three treatments used almost equipotently reduced total peroxide content as a potential destructive agent to gastric mucosa. The reduction in TAO with ulceration was markedly normalized with copper complexes and omeprazole. Oxidative stress index was greatly equipotently reduced by the three treatments. Copper complexes with several organic ligands including nicotinate and glycinate were antioxidant and improved the individual antioxidant effectors in different tissues (el-Saadani et al., 1993; El-Missiry et al., 2001; Saczewski et al., 2006). The antiulcer effect of omeprazole is also reasoned to its antioxidant activity and its ability to significantly increased TAO activity in gastric mucosa (Banerjee et al 2008). The presence of *H. pylori* caused significant deterioration of stress-induced gastric mucosal lesions through increased oxidative stress and antioxidant treatment by α-tocopherol protects against the gastric injuries (Oh et al., 2005).

Although inflammation and repair mostly occur along a prescribed course, it is disruptable by imbalances in regulatory cytokines. Activation of neutrophils in the context of the wound microenvironment results in enhanced release of proinflammatory cytokines including IL-6. IL-6 recruits mast cells to injury sites to dampen the inflammatory response and initiate repair (Huttunen *et al*., 2000). IL-6 is a multifunctional immunoregulatory cytokine that activates a cell-surface signaling assembly composed of IL-6, transmembrane

80-kD glycoprotein IL-6Rα responsible for ligand specificity, and, the shared cytokine signal transducing receptor gp130. The gp130 is expressed in almost all organs, whereas, IL-6Rα expression is limited to hepatocytes and leukocyte subpopulations (Jones et al., 1998). IL-6Rα has a soluble form (sIL-6Rα) that binds IL-6 with similar affinity and prolongs IL-6 plasma half-life. More important, the sIL-6Rα/IL-6 complex is capable of transsignaling - via interaction with gp130 even on cells that do not express the membrane-bound IL-6Rα. Hence, the sIL-6Rα has the ability to widen the repertoire of cell types that are responsive to IL-6 (Scheller et al., 2006).

IL-6 and its soluble receptor are induced under conditions of cellular metabolic stress to positively modulate inflammation, acute phase responses, and cell proliferation. Thus, IL-6, also, has a protective role by counteracting the manifestation of overwhelming inflammatory responses by suppressing acute neutrophil accumulation and inducing its apoptosis, paralleled by down-regulation of proinflammatory cytokines expression and activity (Kiecolt-Glaser et al., 2003). This enables its level to predict severity and duration of inflammation. Therefore, defective neutrophil clearance with decreased apoptosis was reported in IL-$6^{-/-}$ transgenic mice (Scheller et al., 2006).

In the present study, the ulcerated untreated control rats showed extensive increase in the total IL-6 and its soluble receptor α that were equipotently more than halved after treatment with each of copper nicotinate, copper glycinate and omeprazole. Reportedly, production of IL-6 and sIL-6Rα during inflammation and tissue injury and regeneration are simultaneously induced (Reich et al., 2007).

Induction of IL-6 expression during the natural or experimental induction of gastric ulceration is inferred from several studies. Some studies considered it a reparative event becaused; 1) Indomethacin-induced mucosal injury is accompanied by reduced release of IL-6 (Gislason et al., 1996); 2) Glacial acetic acid-induced stomach ulceration in mutant gp130(7575FF) mice produces more severe gastric ulcers than in wild-type gp130 mice and mice IL-6($^{-/-}$) produced more severe ulceration (Judd et al., 2009); 3) IL-6 gene promoter is repressible by p53 and Rb tumor supressor proteins indicating its mitogenic ability (Santhanam et al., 1991); 4) IL-6 promotes angiogenesis and cellular proliferation and genetic ablation of IL-6 gene in mice, or treatment with IL6-neutralizing antibody retarded H-ras-driven tumorigenesis (Gallucci *et al.,* 2000); 5) IL-6 induces TGF-α expression as a major angiogenic/mitogenic reepithelization effector (Hallbeck et al., 2001), and, TGF-α induces IL-6 transcription (Aragane et al., 1996); 6) Anti-IL-6 receptor antibody reduces VEGF production, and, IL-6-induces VEGF-A expression and angiogenesis and antagonizing the sIL-6Rα by naturally produced S7 peptide suppresses IL-6-induced survival signaling (Nakahara et al., 2003; Su et al., 2005); 8) Antibodies against IL-6 or sIL-6Rα overcome resistance and increase cytotoxicity of chemotherapy in cancer cells (Mizutani et al., 1995); 9) Combined IL-6 and sIL-6Rα accelerates murine liver regeneration (Peters et al., 2000).

However, other studies indicated a damaging role for the induced IL-6/sIL-6Rα production including; 1) Ulcerogenic drugs and *H. pylori* increase gastric mucosal production of IL-6 (Gislason et al., 1996; Mehmet et al., 2004; Jainu and Mohan 2008); 2) Clearance of the infection and gastroprotective treatments significantly decreased IL-6 (Kountouras et al., 2000); 3) Immunoneutralization of endothelin-1 delayed gastric ulcer healing correlating increased release of IL-6 (Nishida et al., 2006); 4) IL-6 reduces gastrin release (Leif et al., 2005); 5) IL-6-mediated decrease in IGF-I production is a major mechanism by which chronic inflammation affects cellular growth (Sawczenko et al., 2005); 6) Risk of mortality

score correlated significantly with level of IL-6 in severe septic shock and severe injury (Schluter et al., 1991; de Groof et al., 2002); 7) Chronic stressors accelerate aging of the immune response through induction of IL-6 production (Kiecolt-Glaser et al., 2003); 8) Inflammatory infiltration recruitd by IL-6 induces damaging oxidative stress (Crabtree et al., 1991); 9) There is marked reduction in the expression of the sIL-6Rα during apoptotic atresia of the ovarian follicles (Maeda et al., 2007) and IL-6Rα blockade after trauma-hemorrhage down-regulates cardiac IL-6 and improves cardiac functions (Yang et al., 2007).

The use of knockout models for IL-6 and/or IL-6Rα as experimental animals for gastric ulcer induction, prevention and treatment would favor one of these hypotheses. However, most probably its role is reparative and regenerative as inferred from stem cell biology. Moreover, specific genetic polymorphisms of IL-6 are associated with the development of H. pylori-associated gastroduodenal disease (Kim et al., 2008). Specific ethnic genotypic SNPs variations particularly occurring at the proteolytic cleavage site are significantly associated with increased circulating sIL-6Rα and IL-6 levels (Reich et al., 2007).

Mucosal level of IL-6 was significantly decreased after the treatment of *H. pylori*, especially so in the lansoprazole + amoxicillin + rebamipide treated patients. The addition of an antioxidative drug to the regimen further decreased cytokine levels generated (Hahm et al., 1998). Water immersion and restrain stress or acetic acid induced gastric ulceration were reduced by omeprazole treatment correalting reduced plasma level of proinflammatory cytokines and reduced severity of inflammation (Warzecha et al., 2001). The immunosuppressive prostaglandin E_2 (PGE_2) down-regulates both the IL-6Rα protein and mRNA expression and suppresses the potent myeloid mitogenic effect of IL-6 (de Silva et al., 2003). In reality abrogation of PGE_2 production or PGE_2 receptor blockade improves survival and normalizes myeloid commitment that may explain ulcer recurrence after the stoppage of prostaglandin treatment (Shoup et al., 1998; Santangelo et al., 2000).

In conclusion: the current study revealed for the first time a possible mechanistic connection between histopathological damage, oxidative stress, and increased IL-6 and sIL-6Rα in the Shay Rat gastric ulceration model; and, their modulation copper glycinate and nicotinate as possible commonplace gastroprotective mechanisms of their action. Omeprazole used similar approach.

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HISTOLOGICAL ASPECTS OF THE OVARY IN QUAIL'S (*COTURNIX COTURNIX JAPONICA*) EMBRYO

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Abstract
This study reveals histological aspect of the ovary in quail's embryo, in different stages of embryo development. Embryos were sacrificed from the 5th day of hatching until the 17th day of hatching. HEA, PAS and van Gieson stains were carried out to obtain histological images. The results showed that on the 5th day of incubation, the gonad of the embryo began to be formed and exhibited the feature of ovary or testis. On the 7th day of incubation, the right ovary began to degenerate. On the 10th day of incubation there were many oogonia in the ovary, some wich were surrounded by some other cells distributed like circles. On the 11th day of incubation, there were more oogonia, the cortex and become thicker while the medulla was thinner. On the 13th day, the division between cortex and medulla was obvious. On the 17th day of hatching the shape ovary tended to be mature, also the ovum was clear and the medulla more vascularized.

Keywords: quail, ovary, histology, embryo

The process of blood vessel formation is crucial to vertebrate development, because embryos cannot develop without a source of oxygen and nutrients. Thus, blood vessels begin to form very early in embryo development, along with the heart and primitive blood cells. The relatively small size of the adults, short time to sexual maturity, short period of incubation (16 days) and highly egg production of the Japanese quail make it a suitable experimental animal model.(1, 2)

During early embryonic development, the gonads are sexually indifferent and can differentiate into a testis or an ovary depending on the genotypic sex of the embryo. The period of indifferent sexual development until 5 day of incubation in the quail, but morphological differentiation occurs on the 5-7th day of incubation and gonads differentiate completely on the 8-10th day of incubation. (3, 4)

This study reveals the development of quail embryo gonads with the following objectives: the 1st day of gonads identification, the development of gonads and morphological characteristics.

Materials and method

Fertilized eggs in this experiment were obtained from Coturnix coturnix japonica quail. Fertilizzed eggs were incubated at 37,5 °C and a relative humidity of 58%. Total of 67 embryos were collected from day 5 of post-incubation to hatch day. Finally, the embryo and gonads were fixed into 10% formaldehyde and Bouin's solution overnight. The fragments were embedded with paraffin.

Sections 5 µm were cut with a microtome and mounted on glass slides, then the slices were stained with HEA, PAS and van Gieson. Finally, they were observed under the optical microscope and photographed.

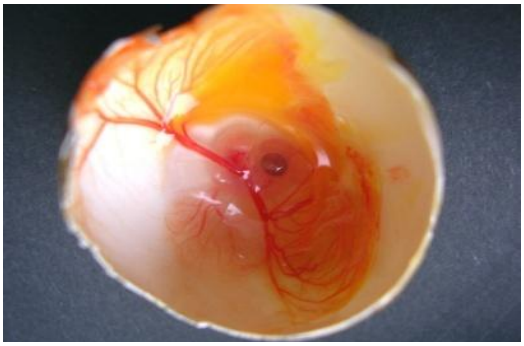

Fig. 1. Image showing quail's embryo vascularisation in the 5th day of incubation

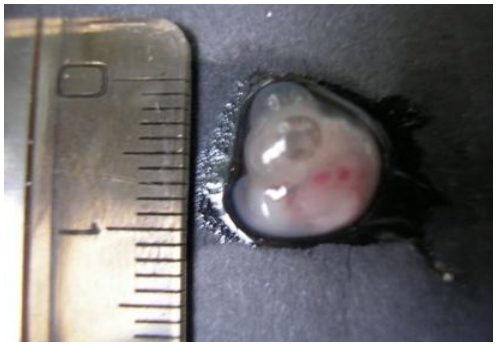

Fig. 2. Embryo length in the 5th day of incubation

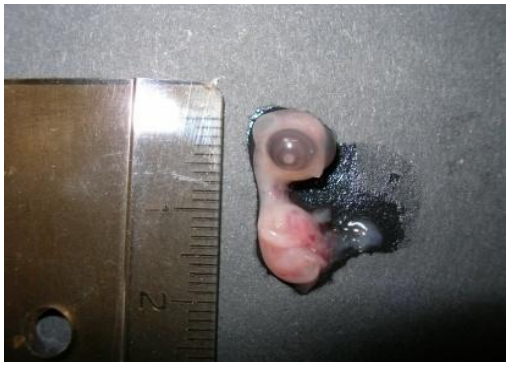

Fig. 3. Embryo size at 7 days of incubation

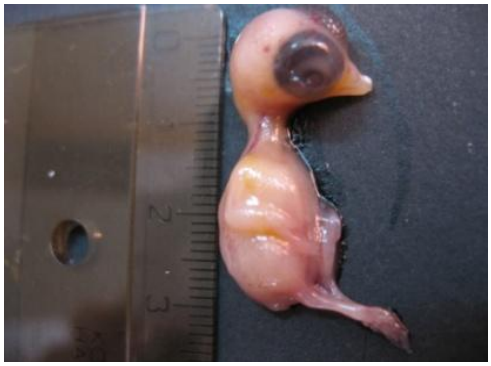

Fig. 4. Quail embryo at 9 days of incubation

Fig. 5. Retraction of blood vesels at 13 days of incubation

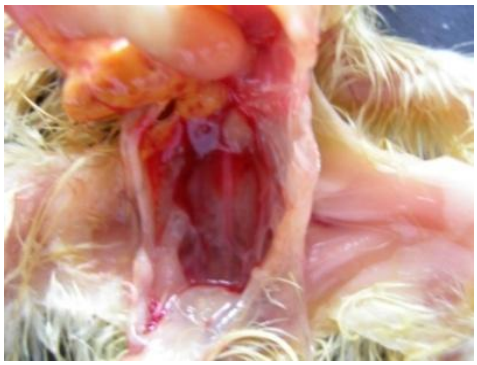

Fig. 6. Presence of quail's ovary in 1st day hatching out

Results and discussions

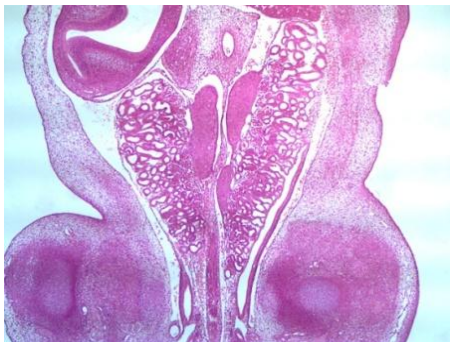

Fig. 7. Region of gonad development on the 5th day of embryonic life HEA stain; x 100

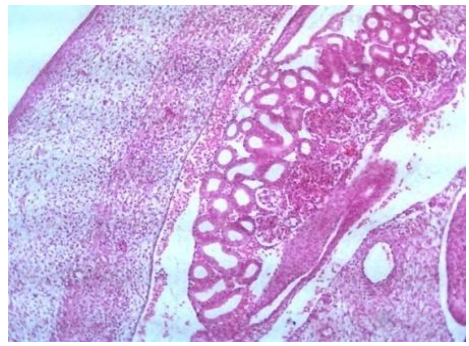

Fig. 8. Gonad on the 5th embryonic day, the morphological characteristics of ovarian development , HEA stain; x 100

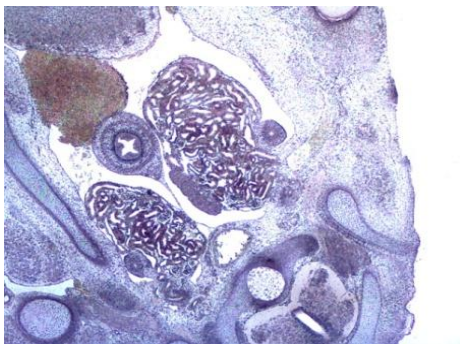

Fig. 9. Longitudinal Section. The left ovary longer than the right ovary. van Gieson stain; x 40

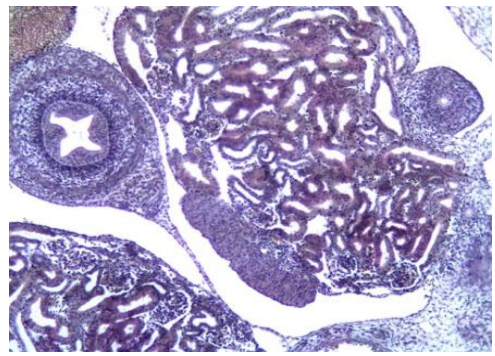

Fig. 10. The left ovary is more obvious. Metanephros began to form. van Gieson stain; x 100

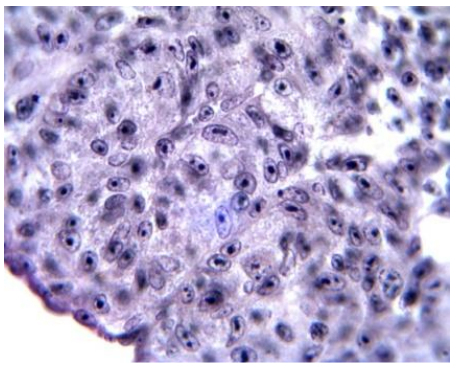

Fig. 11. Histological image showing large cells with emerged nuclei. van Gieson stain; x 1000

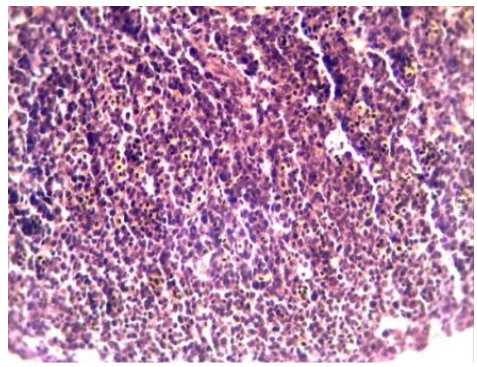

Fig. 12. Ovary was divided into cortex and medulla. van Gieson stain; x 400

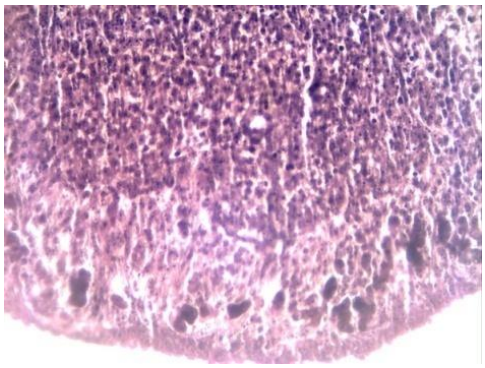

Fig. 13. Cortical area becomes wider. van Gieson stain; x 400

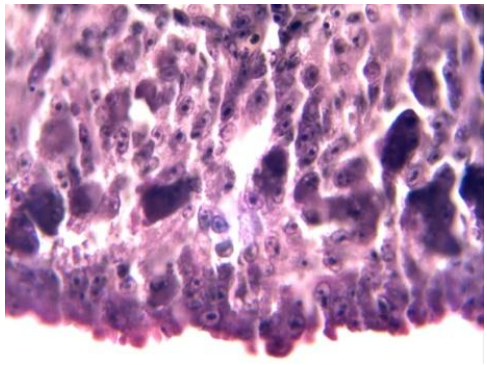

Fig. 14. Primordial follicles start to appear in cortical area. van Gieson stain; x 1000

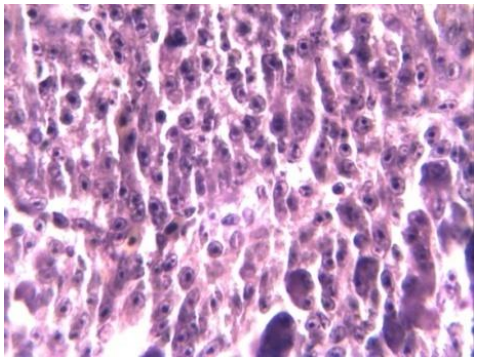

Fig. 15. The cortex of the ovary is clearly separated from medulla. van Gieson stain; x 1000

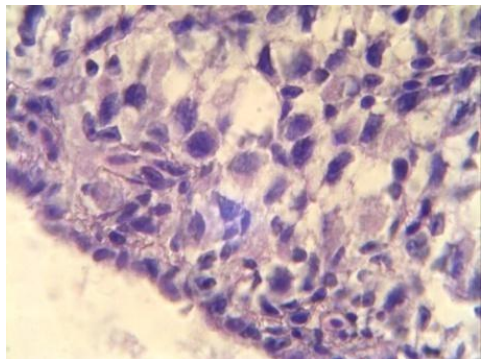

Fig. 16. Quail's ovary on the 1st day of hatching out. PAS stain; x 1000

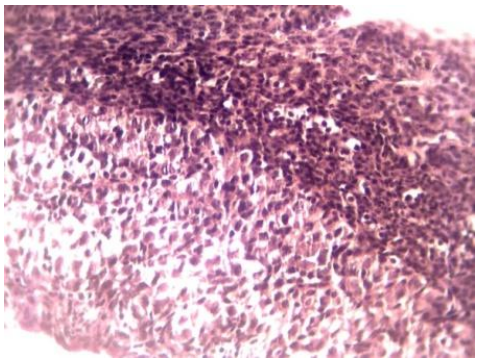

Fig. 17. The ovary cortex is wider. van Gieson stain; x 400

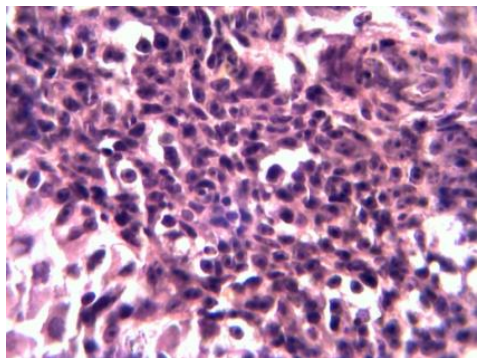

Fig. 18. The development of foliclles could be seen in the cortex. van Gieson stain; x 1000

This histological study revealed that from the 5th day untill the 10th day of incubation, the gonads began to differentiate and had an emerge of the characteristics of the testis or ovary , on the 7th untill the 10th day the sex differences were very obvious, and the right ovary degraded naturally while the left developed naturally. Quail embryonic development was slightely faster than in chicken according to the literature.

Conclusions

1. In the 5th day of incubation the differentiation between ovary and testis could be seen.
2. On the 7th day no morphological differences could be seen between cortex and medulla of ovary.
3. In the 10th day differences between cortex and medulla appeared.
4. In the 13th day the cortical area is clearly separated from the medulla, the cortical area becomes wider and primordial follicles start to appear in cortical area.
5. 1st day of hatching out the cortex was wider and primordial follicles development could be seen in the cortical area.

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HISTOLOGICAL ASPECTS OF THE TESTIS IN QUAIL'S (*COTURNIX COTURNIX JAPONICA*) EMBRYO

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Abstract
This study surprised the histological aspects of the testis in quail's embryo between the 5th day of incubation until the 17th day of incubation. The embryos were collected and PAS ,van Gieson and Mason stains protocols were conducted. On the 5th day of incubation, gonad began to differentiate. On the 7th day of incubation and later, the gonads could be differentiated, aiming at different characteristics of testis structure at various developmental stages. In the end of incubation the testis were almost mature with mesenchymal cells, connective tissue, vascular and seminiferous tubules.
Keywords: quail, testis, histology, embryo

Gonads in poultry are consisted in paired testis in males and usually a single ovary in females. Embryo development of the gonads is a part of the development of reproductive system and ultimately forms the testis in males and ovaries in females. The testis is developed in much the same way as the ovary, originating from mesonephros. This study had the objectives to reveal the main variations of gonads structure in embryonic life. Knowing this facts help us understand better, quail gonad structure and their evolution to a testis or ovary. The procent of males and females at hatching it's still unknown.(1, 2, 3, 4, 5).

Materials and method

Fertilized eggs in this experiment were obtained from Coturnix coturnix japonica quail. Fertilizzed eggs were incubated at 37,5 °C and a relative humidity of 58%. Total of 67 embryos were collected from day 5 of post-incubation to hatch. day. Finally, the embryo and gonads was fixed into 10% formaldehyde and Bouin's solution overnight. The fragments were embedded with paraffin.

Fig. 1. Vascularization embryo at 5 days

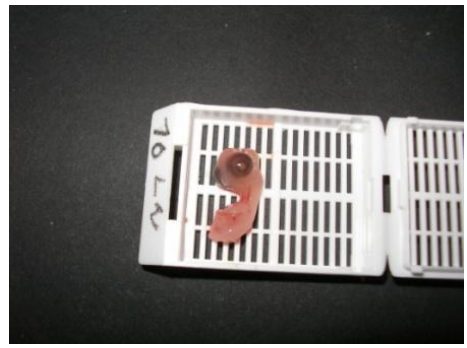

Fig. 2. The way of positioning into the including casette

Sections 5 µm were cut with a microtome and mounted on glass slides, then the slices were stained with PAS, van Gieson and Masson. Finally, they were observed under the optical microscope and photographed.

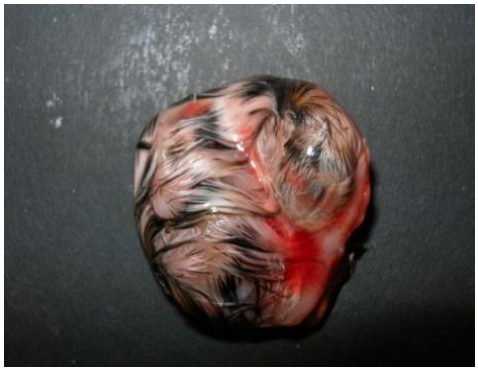

Fig. 3. Quail's embryo on 14 days of incubation

Fig. 4. Embryo weight in 5 days

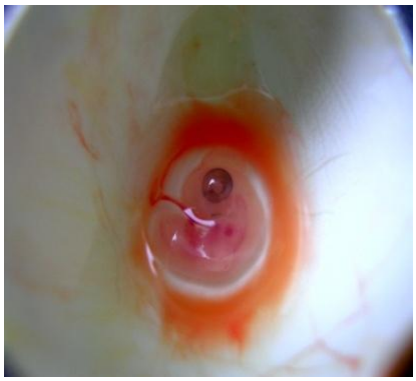

Fig.5. Embryonic fetal position

Fig.6. Testis in 1st day of hatching out

Results and discussions

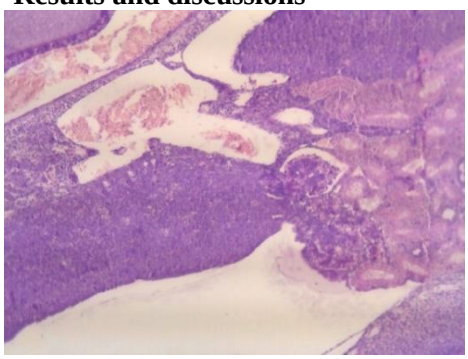

Fig. 7. Undifferenciated gonads. PAS stain; x 100

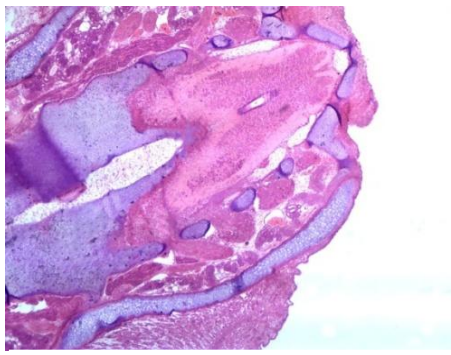

Fig. 8. Genital area and kidney they are not completely separated. Masson stain: x 40

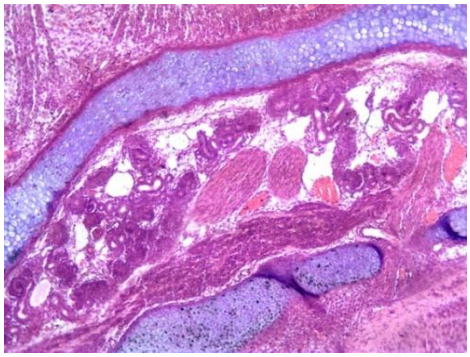

Fig. 9. Genital area and kidney they are not completely separated. Masson stain; x100

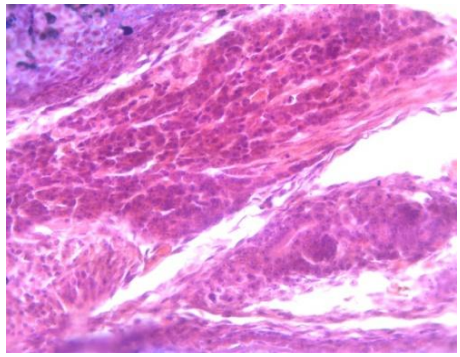

Fig. 10. Distributed cells without distinction of ovary –like cortex and medulla. Masson stain; x 400

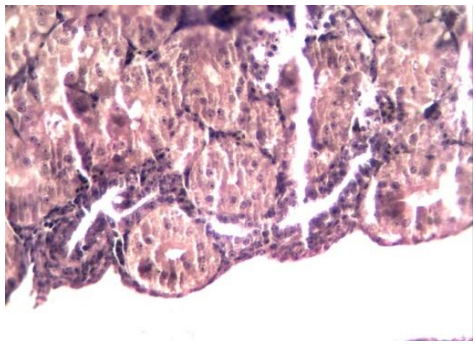

Fig. 11. Typical seminiferous tubules at 14 days of incubationVan Gieson stain; x 400

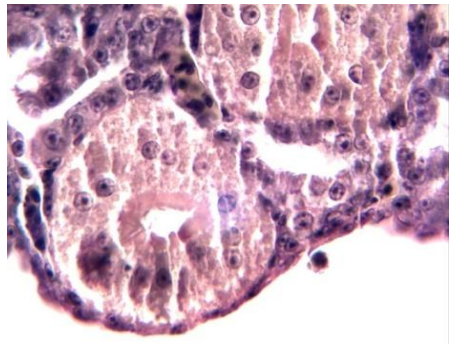

Fig. 12. Typical seminiferous tubules at 14 days of incubation, with evident Sertoli cells Van Gieson stain; x 1000

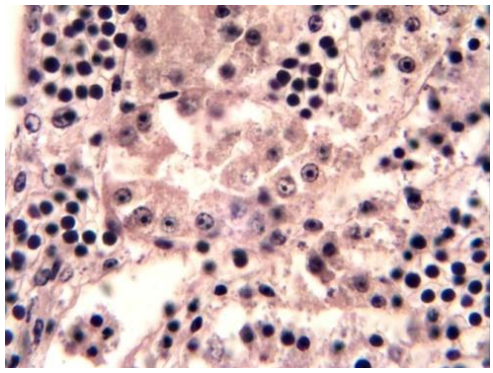

Fig. 13. Sertoli cells start ovalising Van Gieson stain; x 1000

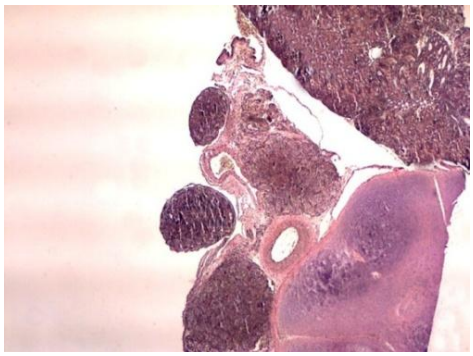

Fig. 14. The local observation of testis Van Gieson stain; x 40

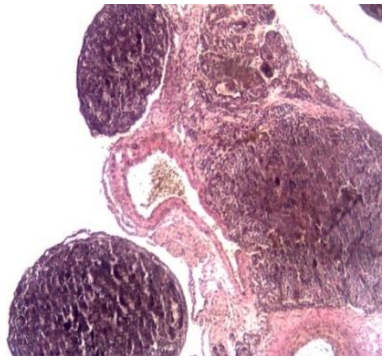

Fig. 15.Evidentiation of quail testis became more evident, the testis are longer and bigger Van Gieson stain; x 100

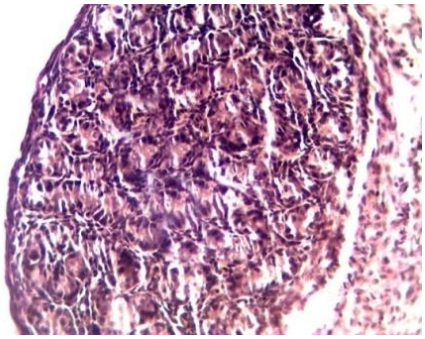

Fig. 16. Elastic fibers, blood vesels and lymphatic tubs among the seminiferous tubulesVan Gieson stain; x 400

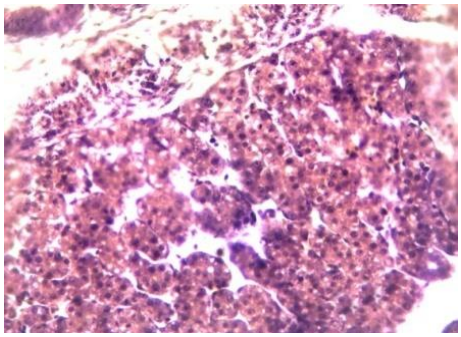

Fig. 17. The seminiferous tubules were more mature and evident Van Gieson stain; x 400

On the 5 th day the gonad differentiated into testis or ovary, but the morphology of testis began to be more obvious in the 7th to 9th day of hatching. Gonadal development depends on the development of cortical and medulla parts wich differentiated from mesoderm at early embryonic stage.

In general testicular cells are consisted two areas wich functional different : testicular cords and mesenchymal cells.

Conclusions

1. Between 5^{th} and 7^{th} day of incubation genital area and kidney they are not completely separated.
2. Distributed cells without distinction of ovary –like cortex and medulla could be seen.
3. From 7^{th} day the real differenciation begins, typical seminiferous tubules can

be observed at 14 days of incubation, with evident Sertoli cells.

4. In 1st day of hatching the seminiferous tubules were more mature and evident, elastic fibers, blood vesels and lymphatic tubs among them could be seen.
5. Quails reach sexual maturity very fast and so they are a good breed for reproduction.

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ORGANOGENESIS OF THE OVARY IN THE CHICK EMBRYO AFTER SEXUAL DIFFERENTIATION

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Abstract
The female genital system forms from the intermediate mesoderm. By day 3 of incubation the nephrogenous mesenchyme forms the urogenital ridge. Although an inspection of the chromosomes can disclose the sex of the embryo, it is not possible in the early stages of the gonad development to determine from its morphology or histology whether it will become a testis or an ovary. This is called the indifferent stage. Starting with incubation day 7, the genital systems begin to develop differently. The ovaries are different from the testicles and from each other. The right ovary develops only on behalf of the medullar zone, the cortical zone being undeveloped. Starting with the 10th incubation day the gonad undergoes an involution process. By the time of hatching this ovary appears as a rudiment, very small in comparison with the left one, with only the medullar zone that consists of elongated primary sex cords. The left ovary (that will become functional) develops into two distinct zones: the medullar zone and the cortical zone. The medullar area consists of primary sex cords that develop, lengthen and become vacuolised in day 13. At hatching, the medullar area presents connective tissue, blood vessels and mesenchymal cells. The cortical zone is structured by secondary sex cords and primordial germ cells. During the embryonic development the primordial germ cells proliferate and develop into oocytes (day 12). The oocytes gather follicular cells nearby and develop into primordial follicles (day 17). During the last days of incubation the medullar and cortical zones are very distinct in structure, and about the same size. For the present study we used 70 Lohmann Brown embryos. Five embryos were sacrificed daily, starting with the 7th embryonic day. The samples were fixed in 4% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained H.E.A., PAS, Novelli, van Gieson, Masson and Gomori.

Keywords: chick embryo, histology, ovary, organogenesis, morphology

Introduction

The objective of this paper was to study the ovary development of the Lohmann Brown embryos after sexual differentiation. This subject has been chosen due to the lack of data in the literature concerning the development of the female genital system of this hybrid specialized in egg production. The Lohmann Brown hybrid has been chosen due to its high standards in egg production.

Material and method

The research for the present study was conducted on 70 Lohmann Brown embryos. The embryos were obtained from fertilized Lohmann Brown parents eggs from Avicola Brasov farm. The eggs were incubated in an automatic incubator at 37,7 °C (± 0,2°C) and 60% humidity (± 10%). The eggs were turned automatic, once every two hours, starting with the 4th day and finishing with the 17th day of incubation. 5 embryos were sacrificed daily, staring with the 7th day of incubation until the chicks hatched. The first (smaller) embryos were embedded in paraffin as a whole, either in a vertical or dorsal position (fig. 1, 2). From the older embryos we took samples from the abdominal cavity or just the organs we have taken into study (fig. 3, 4).

The embryos and samples were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at 5 µm and stained using the H.E.A., P.A.S., Novelli and Gomori protocols.

The slides obtained were studied using a "B series" Motic optic microscope and the most important and relevant histological images were captured using the "Moticam 1000" microscope camera.

Results and discussions

The female genital system forms from the intermediate mesoderm. By day 3 of incubation the nephrogenous mesenchyme forms the urogenital ridge (1, 2, 4).

Although an inspection of the chromosomes can disclose the sex of the embryo, it is not possible in the early stages of the gonad development to determine from its morphology or histology whether it will become a testis or an ovary. This is called the indifferent stage (3, 5, 8 12).

Starting with incubation day 7, the gonads begin to differentiate into testicles (for the ZZ embryos) or ovaries (for the ZW embryos) (6, 7, 10). At this time, the left gonad is larger than the right one in both male and female embryos (6, 9, 11). The histological structure of the gonads consists of a germinal epithelium with somatic and germinal cells that form the secondary sex cords, and primary sex cords with mesenchymal cells (fig 5, 6).

On the 8^{th} embryonic day, the ovaries present two distinct areas: the cortex and the medulla. The medulla consists of primary sex cords and the cortex of secondary sex cords (fig 7, 8).

In the 9^{th} day of development we can see that the cortex of the left ovary develops on behalf of the proliferation of the primordial germ cells (PGC). The primary sex cords of the medulla begin to lengthen (fig 9, 10).

Starting with the 10^{th} incubation day the right gonad undergoes an involution process. By the time of hatching this ovary appears as a rudiment, very small in comparison with the left one, with only the medullar zone that consists of elongated primary sex cords (fig. 27).

On the 11^{th} embryonic day, the cortex is larger, on behalf of the primordial germ cells, and the medulla has lengthened on behalf of the primary sex cords (fig 11, 12).

In the 12^{th} day of development, the primordial germ cells of the left ovary proliferate and develop into oocytes (fig 13, 14).

On day 13 of development, the medulla of the left ovary becomes vacuolised. The primary sex cords of the medulla present lacunae and are very lengthened (fig 15, 16).

Starting with the 14^{th} day of development, the cortical area begins to enlarge compared with the medullar zone (fig 17, 18).

In the 15^{th} day of development, using the Gomori stain we can observe the interstitial space that is still very developed in comparison with the cell mass of the medulla and the cortex (fig 19, 20).

The histological structure of the medulla in the 16^{th} embryonic day consists of connective tissue, large blood vessels, mesenchymal cells and lacunae. In the cortex, the oocytes and the somatic cells proliferate (fig 21, 22).

In the cortex of the left ovary, in the 17^{th} day of development, the oocytes gather small follicular cells around them and form the first primordial follicles (fig 23, 24).

In the left ovary of the 18^{th} day embryos, the cortex is very well developed with numerous oocytes and primordial follicles. The medulla is very fragmented and between the kidney and the medulla there are numerous large blood vessels in the hilum (fig 25, 26).

Two days before hatching, the left ovary is clearly different from the right one. The right ovary appears as a rudiment, with only the medulla that consists of elongated primary

sex cords, without the lacunae. The left ovary has the two areas (the cortex and the medulla) very well defined (fig 27, 28).

Prior to hatching, the cortex of the left ovary appears thickened with primordial follicles that gather nearby follicular cells and develop into primary follicles, and the medulla with numerous lacunae defined by connective tissue (fig 29, 30).

Fig. 1. Lohmann Brown embryo. 8 embrvonic davs

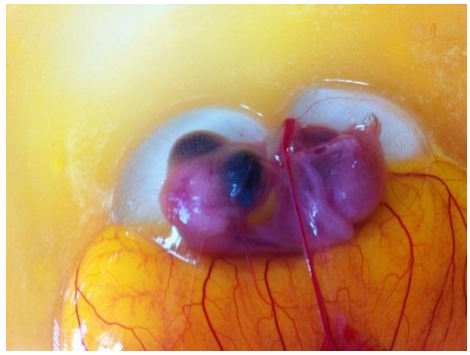

Fig. 2. Lohmann Brown embryo. 12 embryonic days

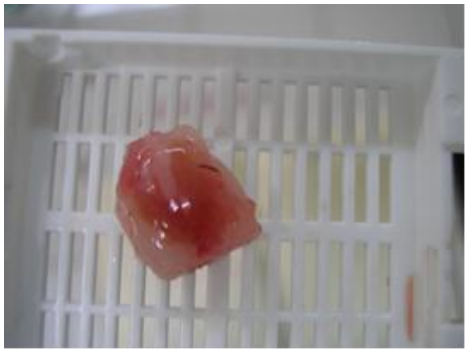

Fig. 3. Lohmann Brown embryo. 17th day of embryonic development. The two ovaries

Fig. 4. Lohmann Brown embryo. 18th day of incubation.

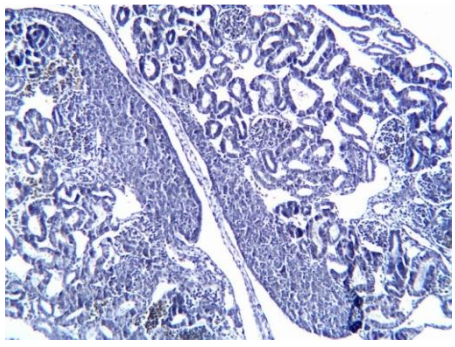

Fig. 5. Female L.B. embryo- 7 days: The two ovaries with different size; Van Gieson stain; x 100

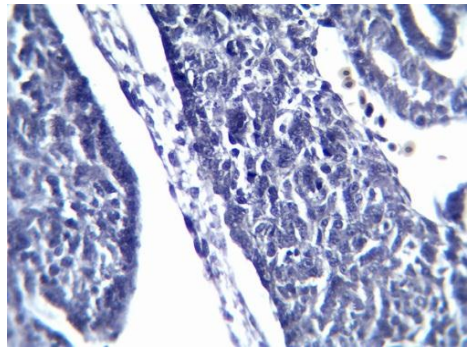

Fig. 6. Female L.B. embryo- 7 days: The difference between the two ovaries; Van Gieson stain; x 400

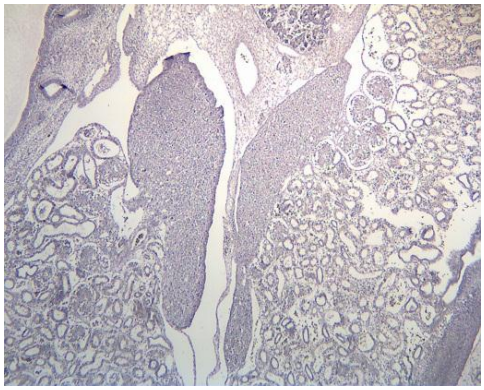

Fig. 7. Female L.B. embryo- 8 days: The development of the two ovaries; Van Gieson stain; x 40

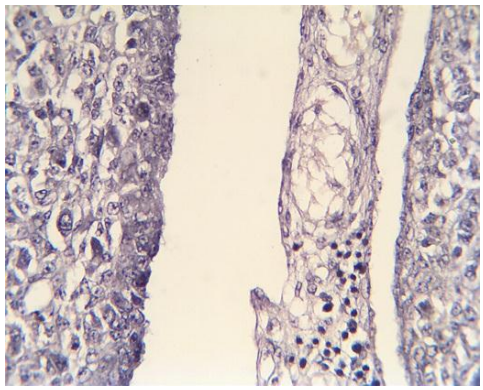

Fig. 8. Female L.B. embryo- 8 days: The cortex of the left ovary and the right ovary; Van Gieson stain; x 400

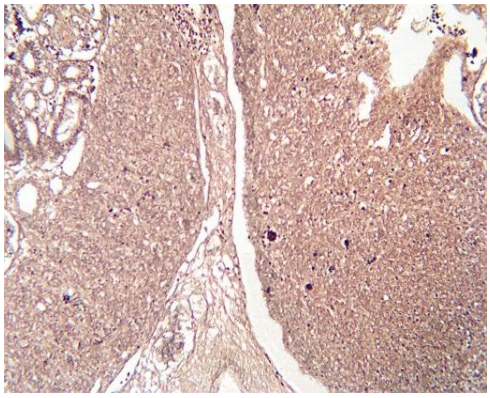

Fig. 9. Female L.B. embryo- 9 days: The cortex and the medulla of the left ovary and the righ gonad; Gomori stain; x 100

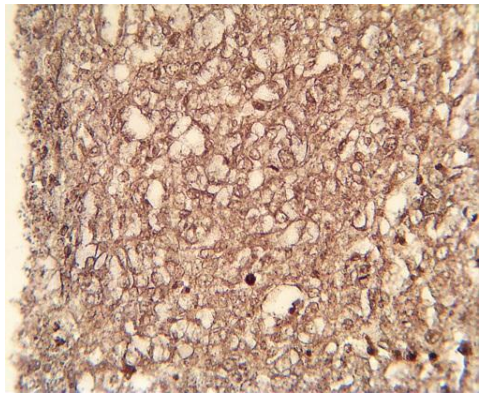

Fig. 10. Female L.B. embryo- 9 days: The cortex and the medulla of the left ovary; Gomori stain; x 400

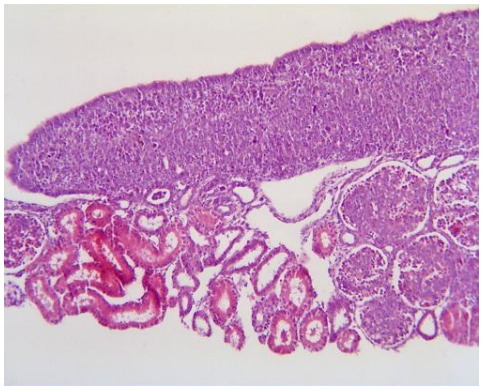

Fig. 11. Female L.B. embryo- 11 days: The proliferation of the left ovary cortex; HE stain; x 100

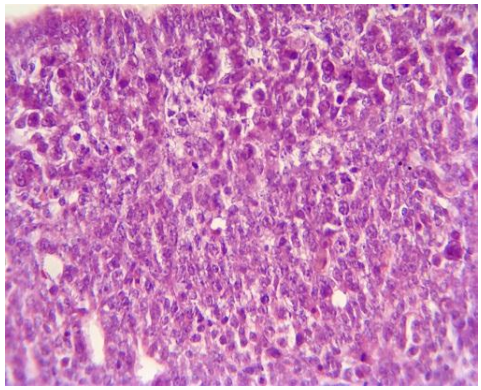

Fig. 12. Female L.B. embryo- 11 days: The intense proliferation of the cortex; HE stain; x 400

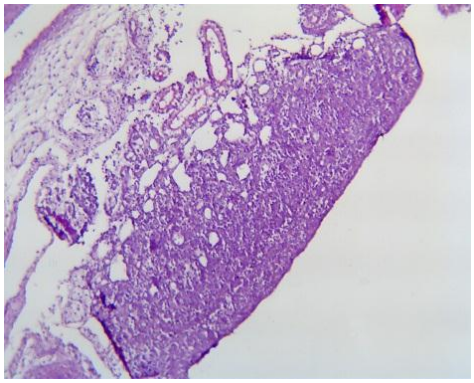

Fig. 13. Female L.B. embryo- 12 days: The medulla of the left ovary with lengthen primary sex cords and the cortex; PAS stain; x 100

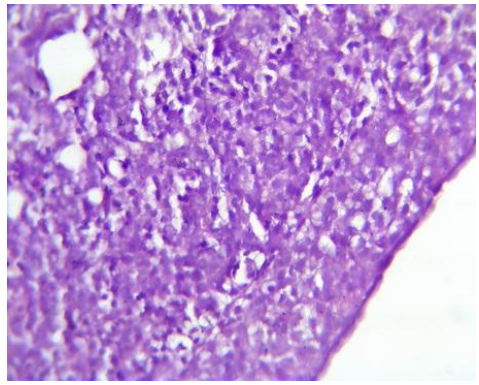

Fig. 14. Female L.B. embryo- 12 days: The cortex of the left ovary with oocytes; PAS stain; x 400

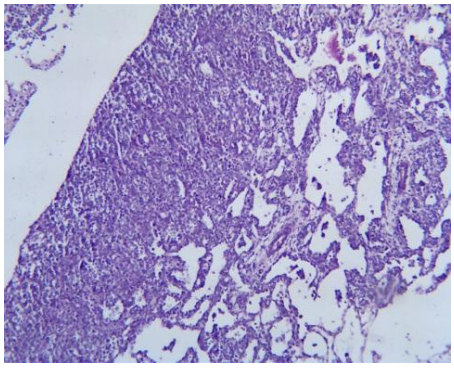

Fig. 15. Female L.B. embryo- 13 days: The medulla of the left ovary with lacunae; PAS stain; x 100

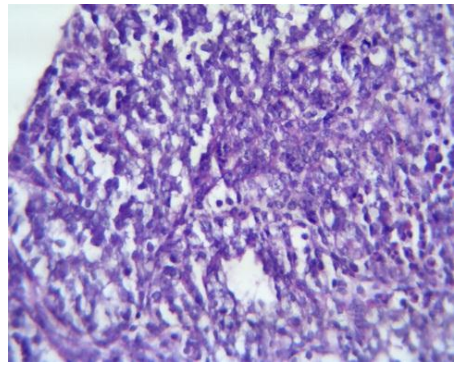

Fig. 16. Female L.B. embryo- 13 days: The left ovary-cortex and medulla with lacunae; PAS stain; x 400

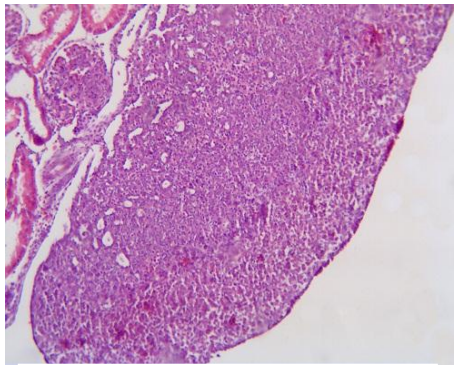

Fig. 17. Female L.B. embryo- 14 days: The development of the cortex; HE stain; x 100

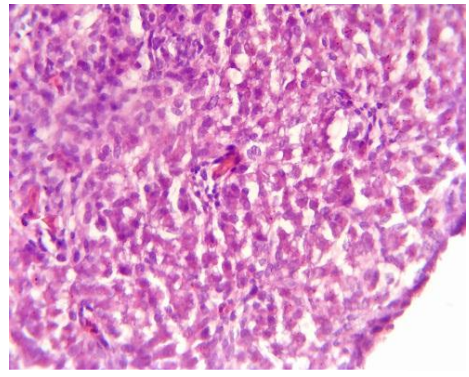

Fig. 18. Female L.B. embryo- 14 days: The development of the cortex; HE stain; x 400

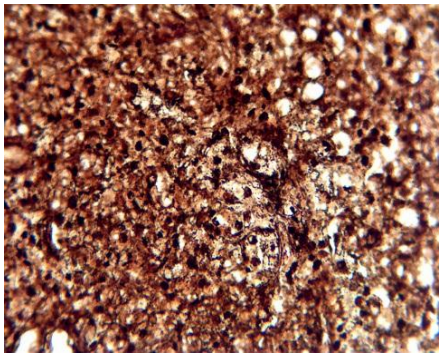

Fig. 19. Female L.B. embryo- 15 days: The interstitial space of the left ovary cortex; Gomori stain; x 400

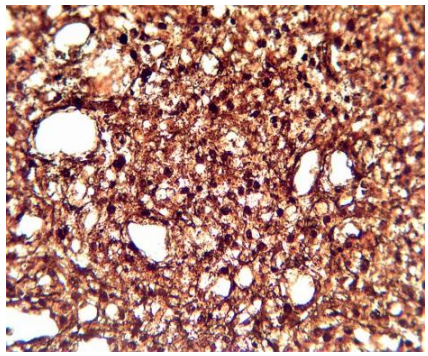

Fig. 20. Female L.B. embryo- 15 days: The interstitial space of the left ovary medulla: Van Gieson stain: x 400

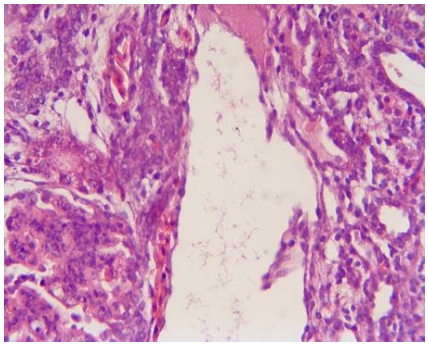

Fig. 21. Female L.B. embryo- 16 days: The left ovary hilum with large blood vessels; HE stain; x 400

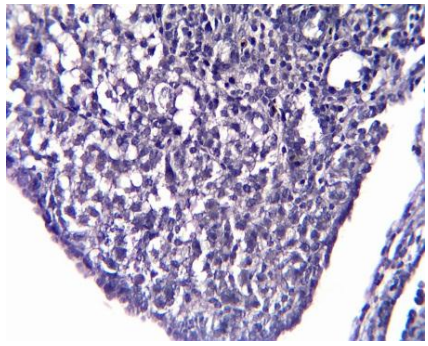

Fig. 22. Female L.B. embryo- 16 days: The intense proliferation of the oocytes in the cortex; Van Gieson stain; x 400

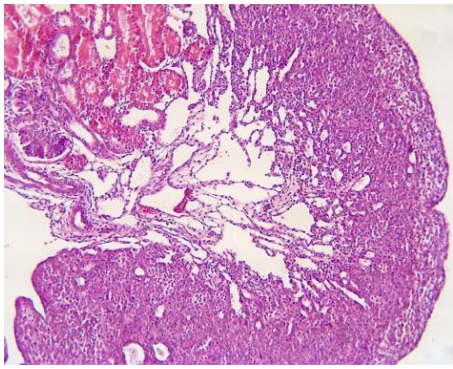

Fig. 23. Female L.B. embryo- 17 days: The left ovary with the fragmented medulla and lacunae; HE stain; x 100

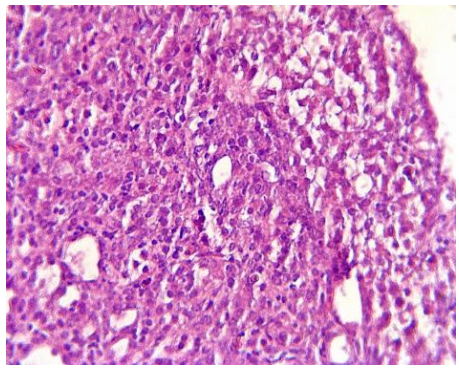

Fig. 24. Female L.B. embryo- 17 days: The primordial follicles in the cortex of the left ovary; HE stain; x 400

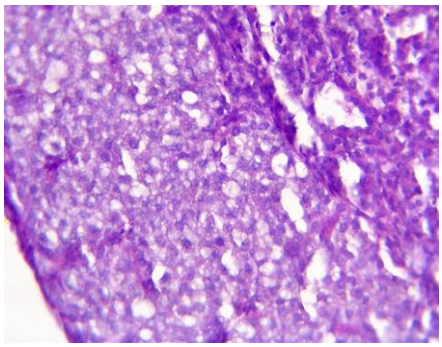

Fig. 25. Female L.B. embryo- 18 days: The cortex with numerous oocytes and primordial follicles; PAS stain; x 400

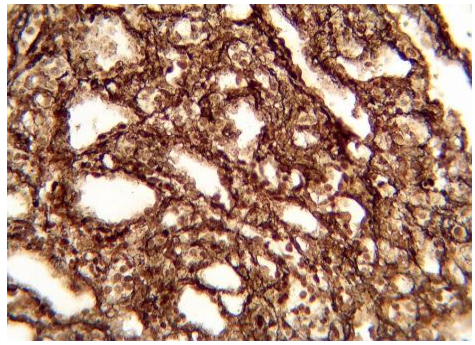

Fig. 26. Female L.B. embryo- 18 days: The medulla of the left ovary with lacunae and blood vessels; Gomori stain; x 400

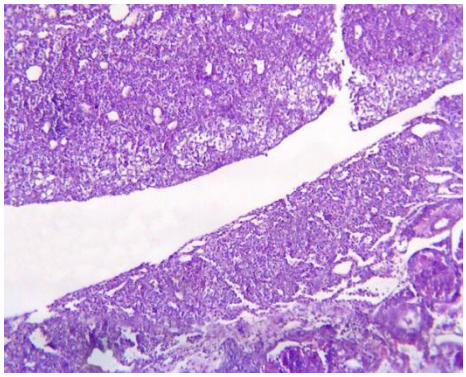

Fig. 27. Female L.B. embryo- 19 days: The difference between the two ovaries; PAS stain; x100

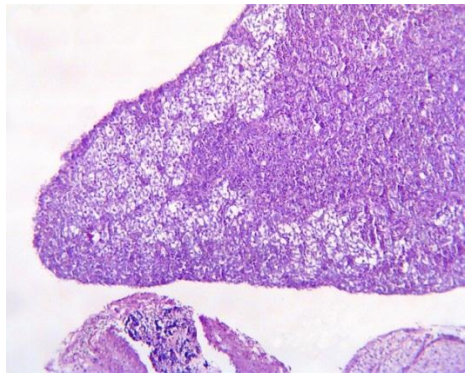

Fig. 28. Female L.B. embryo- 19 days: The left ovary cortex and medulla; PAS stain; x 100

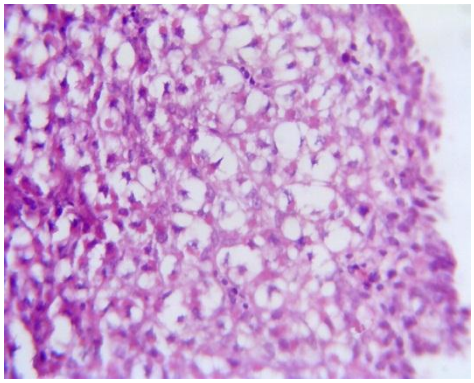

Fig. 29. Female L.B. embryo- 20 days: The cortex of the left ovary with primary follicles; Novelli stain; x 400

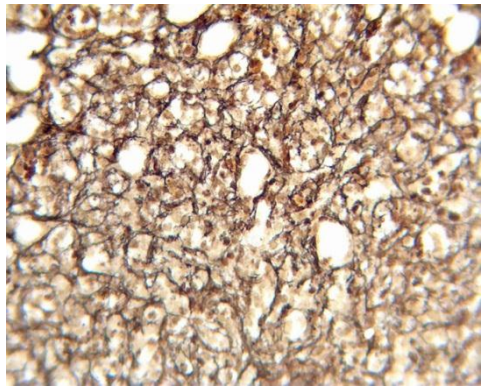

Fig. 30. Female L.B. embryo- 20 days: The medulla of the left ovary with connective tissue, mesenchymal cells and lacunae; Gomori stain; x 400

Conclussions

1. The female genital system forms from the intermediate mesoderm;
2. Starting with incubation day 7, the genital systems begin to develop differently;
3. In the 10th day of incubation the right gonad undergoes an involution process. By the time of hatching this ovary appears as a rudiment;
4. The left ovary develops into two distinct zones: the medulla and the cortex;
5. The medullar area consists of primary sex cords that develop, lengthen and become vacuolised in day 13.
6. Prior to hatching, the medulla presents connective tissue, blood vessels and mesenchymal cells.
7. The cortex of the left ovary is structured by secondary sex cords and primordial germ cells.
8. Starting with the 12th embryonic day the primordial germ cells proliferate and develop into oocytes;
9. The oocytes gather follicular cells nearby and develop into primordial follicles on day 17, and primary follicles prior to hatching.

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ORGANOGENESIS OF THE OVIDUCT IN THE CHICK EMBRYO AFTER SEXUAL DIFFERENTIATION

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Abstract
The Müllerian ducts form on the lateral side of the mesonephros, from a thickened epithelium called the Müllerian ridge. Until the eighth embryonic day, the development of the right and left, female and male duct is identical. In the male embryo, both Müllerian ducts, and in the female embryo the right Müllerian duct, undergo regression starting with the eighth day of development. The morphological features of the right Müllerian duct regression consist of a dense mesenchyme that surrounds the duct which has a narrowed lumen and a decreased diameter. Apoptotic bodies appear within the epithelium. In the last days of incubation only a small portion (the caudal part) of the right Müllerian duct is visible, the rest has been replaced by a dense mesenchyme. The left Müllerian duct develops near the left metanephros, parallel with the Wolffian duct. On the seventh day of development the structure of the left duct consists of a simple cuboidal epithelium surrounded by a dense mesenchyme. During the next days of development the Müllerian epithelium thickens and becomes pseudostratified. This epithelium is highly proliferative as indicated by the numerous mitotic figures. Prior to hatching (starting with day 18) the epithelium becomes columnar nonciliated and presents primary folds. The lumen is wide and the diameter is larger. The future shell gland (uterus) can be distinguished as a dilatation of the tract near its distal end. For the present study we used 70 Lohmann Brown embryos. Five embryos were sacrificed daily, starting with the 7th embryonic day. The samples were fixed in 4% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained H.E.A., PAS, Novelli, van Gieson, Masson and Gomori.

Keywords: chick embryo, histology, organogenesis, oviduct, Müller

Introduction

The objective of this paper was to study the development of the oviduct (left Müllerian duct) of the Lohmann Brown embryos after sexual differentiation. We chose the Lohmann Brown hybrid for these studies mainly because it has a high egg production that is reached earlier than most of the ordinary chicken breeds. We chose to undergo this research because of the lack of research on this subject in breeds specialized in egg production such as Lohmann Brown.

Material and method

The research for the present study was conducted on 70 Lohmann Brown embryos. The embryos were obtained from fertilized Lohmann Brown parents eggs from Avicola Brasov farm. The eggs were incubated in an automatic incubator at 37,7 °C (± 0,2°C) and 60% humidity (± 10%). The eggs were turned automatic, once every two hours, starting with the 4th day and finishing with the 17th day of incubation. 5 embryos were sacrificed daily, staring with the 7th day of incubation until the chicks hatched. The first (smaller) embryos were embedded in paraffin as a whole, either in a vertical or dorsal position (fig. 1, 2). From the older embryos we took samples from the abdominal cavity or just the organs we have taken into study (fig. 3, 4).

The embryos and samples were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at 5 µm and stained using the H.E.A., P.A.S., Novelli and Gomori protocols.

The slides obtained were studied using a "B series" Motic optic microscope and the most important and relevant histological images were captured using the "Moticam 1000" microscope camera.

Results and discussions

The Müllerian ducts form on the lateral side of the mesonephros, from a thickened epithelium called the Müllerian ridge (6, 10). Until the eighth embryonic day, the development of the right and left, female and male duct is identical (3, 5) (fig. 5).

In the male embryo, both Müllerian ducts, and in the female embryo the right Müllerian duct, undergo regression starting with the eighth day of development (4, 8, 9). The process of regression of the right Müller duct is signaled by the anti-Müllerian hormone (AMH) also named Müllerian-inhibiting substance (MIS) (1, 2). The morphological features that indicate the Müller duct regression consist of the loss of the basal lamina and then a condensation of the mesenchyme. Compared with the left Müller duct, the right one has a narrowed lumen and a decreased diameter. In the decreasing epithelium there are numerous apoptotic bodies. Soon after, the epithelium will disappear, and instead we find a dense mesenchyme. In the last days of incubation only a small portion (the caudal part) of the right Müllerian duct is visible.

The left Müllerian duct develops near the left metanephros, parallel with the Wolffian duct (5, 7).

On the 7^{th} day of development the structure of the left duct consists of a simple cuboidal epithelium surrounded by a dense mesenchyme (fig 6, 7, 8). During the next days of development the Müllerian epithelium proliferates, thickens, and the surrounding mesenchyme becomes thinner (fig. 10, 11). There are numerous mitotic figures in the epithelium which prove that it is highly proliferative (fig. 9). The lumen becomes wider and the diameter larger (fig. 12).

During the 17^{th} day of incubation, the epithelium becomes pseudostratified (fig. 12, 13).

Prior to hatching (starting with day 18) the epithelium becomes columnar, nonciliated, and presents primary folds (fig. 15, 16). The future shell gland (uterus) can be distinguished as a dilatation of the tract near its distal end. The lumen is wide and the diameter is larger (fig. 14).

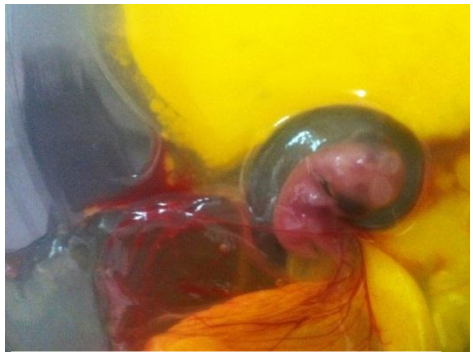

Fig. 1. Lohmann Brown embryo.
7 embryonic days

Fig. 2. Lohmann Brown embryo.
13 embryonic days

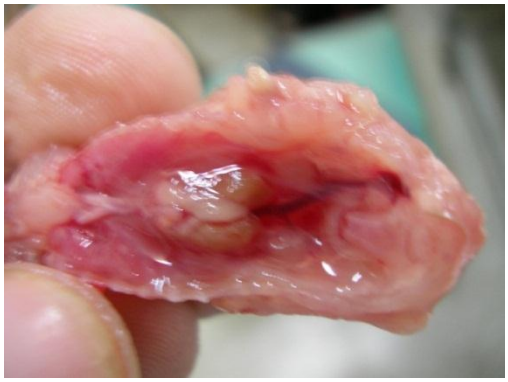

Fig. 3. Lohmann Brown embryo. 18 embryonic days. The ovary and Müller duct

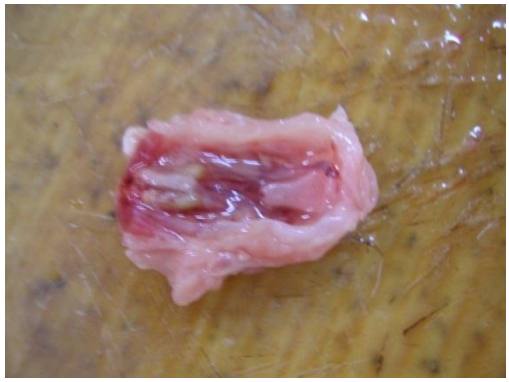

Fig. 4. Lohmann Brown embryo. 20 embryonic days. The ovary and Müller duct

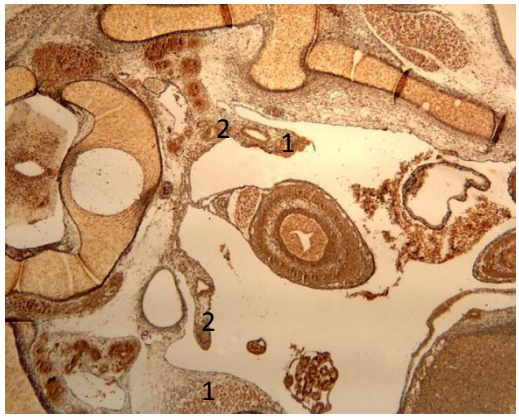

Fig. 5. Female L.B. embryo- 7 days: The Müller ducts (1) and the Wolff ducts (2); Gomori stain; x 40

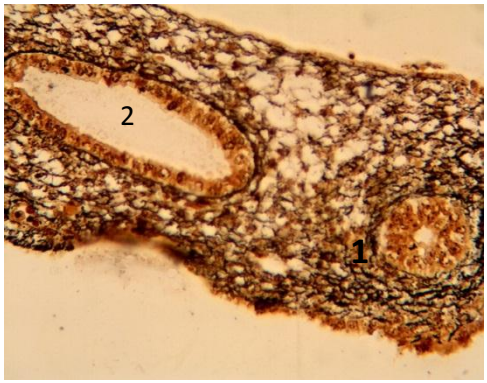

Fig. 6. Female L.B. embryo- 8 days: The left Müller duct (1) and the Wolff duct (2); Gomori stain; x 400

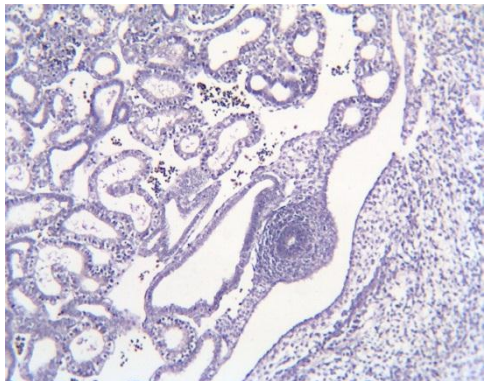

Fig. 7. Female L.B. embryo- 9 days: The left Müller duct with cuboidal epithelium surrounded by a dense mesenchyme; Van Gieson stain; x 100

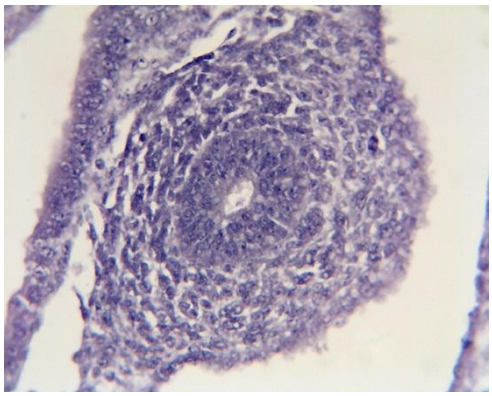

Fig. 8. Female L.B. embryo- 10 days: The left Müller duct with cuboidal epithelium surrounded by a dense mesenchyme; Van Gieson stain; x 400

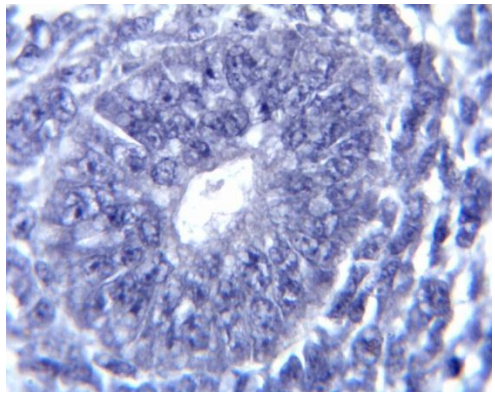

Fig. 9. Female L.B. embryo- 9 days: The left Müllerian epithelium with numerous mitotic figures; Van Gieson stain; x 1000

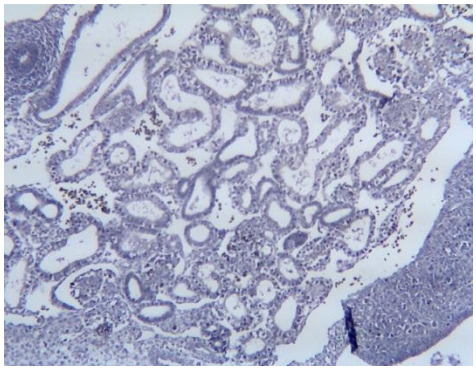

Fig. 10. Female L.B. embryo- 11 days: The location of the left Müller duct, kidney and left ovary; Van Gieson stain; x 40

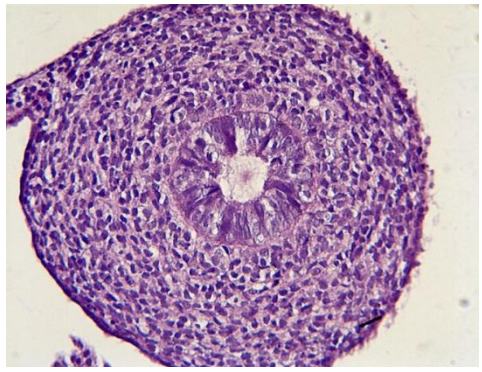

Fig. 11. Female L.B. embryo- 12 days: The left Müller duct with cuboidal epithelium surrounded by a dense mesenchyme; PAS stain; x 400

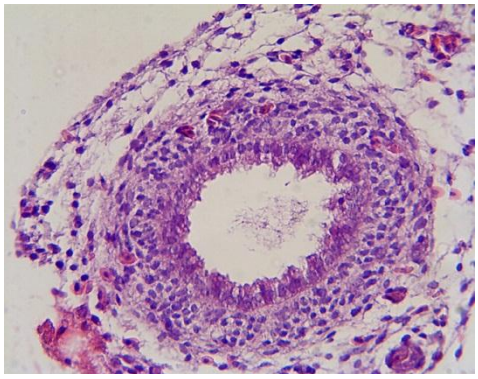

Fig. 12. Female L.B. embryo- 17 days: The left Müller duct with pseudostratified epithelium surrounded by a thin mesenchyme; HE stain; x 100

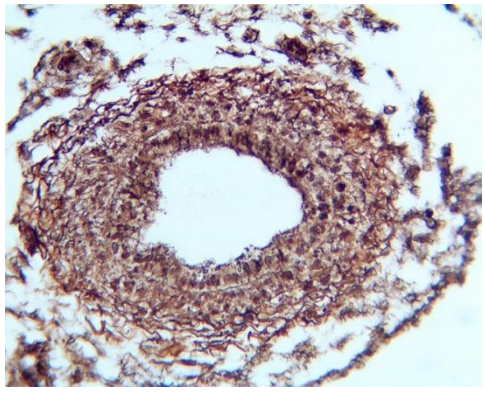

Fig. 13. Female L.B. embryo- 17 days: The left Müller duct with pseudostratified epithelium surrounded by a thin mesenchyme; Gomori stain; x 100

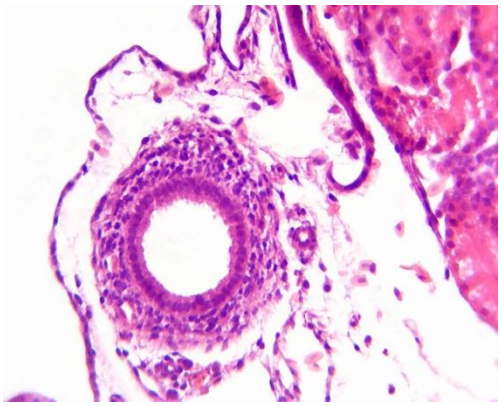

Fig. 14. Female L.B. embryo- 18 days: The left Müller duct with columnar epithelium surrounded by a thin mesenchyme; PAS stain; x 100

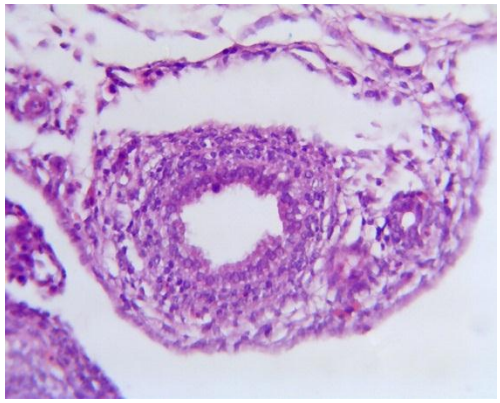

Fig. 15. Female L.B. embryo- 20 days: The left Müller duct with columnar epithelium and primary folds; PAS stain; x 400

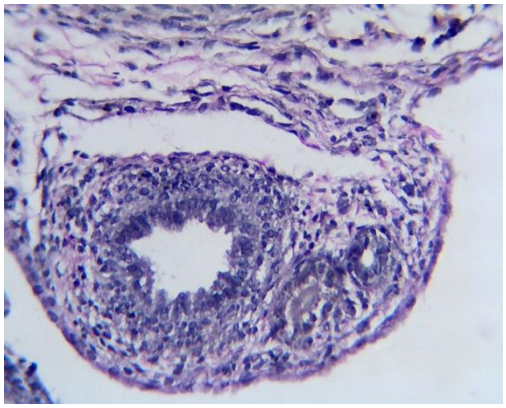

Fig. 16. Female L.B. embryo- 20 days: The left Müller duct with columnar epithelium and primary folds; Van Gieson stain; x 400

Conclusions

1. Until the eighth embryonic day, the development of the right and left, female and male Müller duct is identical;
2. In the female embryo the right Müllerian duct undergoes regression starting with the eighth day of development;
3. The left Müllerian duct develops near the left metanephros, parallel with the Wolffian duct;
4. On the seventh day of development the structure of the left duct consists of a simple cuboidal epithelium surrounded by a dense mesenchyme;
5. During the next days of development the Müllerian epithelium thickens and becomes pseudostratified;

6. The epithelium is highly proliferative as indicated by the numerous mitotic figures;
7. Prior to hatching (starting with day 18) the epithelium becomes columnar nonciliated and presents primary folds. The lumen is wide and the diameter is larger;
8. The future shell gland (uterus) can be distinguished as a dilatation of the tract near its distal end.

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THE KARYOTYPE OF INTERSEX SWINES

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Abstract
Intersexuality - is an abnormality characterized by the presence, in the same individual, of gonads of both sexes and the genital tract is of opposite sex. Intersexuality in domestic animals plays an important role in the pathology of genital tract and is also one of the most common forms of manifestation of sterility defects. The phenomenon of sexual differentiation is the result of genetic information present in a zygote. This information has an impact on development directions of the structures and functions of each body, including sexual. The research activity was carried out in the disciplines of Cell Biology, Histology and Embryology of the Faculty of Veterinary Medicine Iasi and The Laboratory of Immunology and Genetics in the "St. Spiridon " Hospital, Iasi. The purpose of this paper was to determine the genetic sex of individuals presenting different clinical genital abnormalities. The research pursued, was focused the most actual investigations, so that the results give a fair view of these forms of infertility. Compared with X chromatin test, performing the karyotype is more expensive, harder to performed and depends heavily on how the blood was collected and the health of the animal, and is harder to interpret.
Keywords: swine, intersexuality, karyotype, chromosomes.

Introduction

The process of sexualization is a very complex phenomenon of mechanisms, hard to decode, which is and will remain a clear pattern of genetic development. Each stage of the sexual determinism and differentiation can be disrupted, causing various disturbances of the entire process - sex chromosome abnormalities, abnormal formation of the gonadal sex, abnormal sexual differentiation. The causes of such problems are multiple, from environmental factors such as administration of drugs during pregnancy with virilizing effect, chromosomal and monogenic mutations or multiple causes.

In the *Suidae* family, chromosome number varies. This was shown by Melander and Hansen - Melander Eve in 1980 when were made cytogenetic studies on African wild pig compared to domestic pigs. So, "giant forest hog" (*Hylochoerus meinertzhageni*) has 2n = 32 with secondary constriction of pairs 7 and 11 near the centromeric area. *Phacochoerus aethiopicus* - "savannah pig" - has 2n = 34, the X chromosome is large, metacentric while the Y chromosome is a small metacentric chromosome, pairs 6 and11 have primary constriction near the centromeric area. *Potamochoerus larvatus* - "bush pig" - has 2n = 34. The European wild pig, the total number of chromosomes is 2n = 36 due to a translocation of two pairs of acrocentric chromosomes into a submetacentric pair of chromosomes, while the domestic pig (*Sus scrofa domestica*) has 2n = 38, the same chromosomal constitution as of the pig Thai (*Sus scrofa jubatus*) (Tanomtong et al., 2006).

The first banded techniques of chromosomes appeared in 1972 (Berger, 1972, Gustavsson et al., 1972). Since then, different methods of techniques have been tried to increase the possibility of individualization and characterization of each chromosome from pigs karyotype. Since the introduction of different methods of treatment (by dry air and low temperature - Hansen-Melander et al., 1974) and the banded techniques of chromosomes -

Giemsa (Hageltorn and Gustavsson, 1973; Hansen-Melander et al., 1974) the arrangement of chromosomes has undergone various changes.

Among the first attempts to sort and to describe the chromosomes, stands the work of Hansen-Melander and Melander, 1974 which is based on morphological criteria, the length of chromosomes and centromere position. In less than a year of this attempt, using the "G" staining techniques, Pace et al. 1975 described each chromosome, description which is closest to the standard karyotype of 1988.

In 1988, the ***Committee for the Standardized Karyotype of the Domestic Pig*** led by Gustavsson, standardized normal karyotype of domestic pigs, after many attempts in history to stain and classification. Then, the chromosomes were classified into 4 groups of autosomes chromosomes and a group with sex chromosomes (fig.1,2):

- group A includes 5 pairs of metacentric chromosomes, the first being the largest;
- group B includes 2 pairs, the largest submetacentric chromosomes and if this classification would be from morphological point of view it would also include the sex chromosomes;
- group C includes the next 5 pairs of metacentric chromosomes;
- group D contains 6 pairs of acrocentric chromosomes;
- sex chromosome group – X chromosome is medium metacentric and Y chromosome is the smallest metacentric.

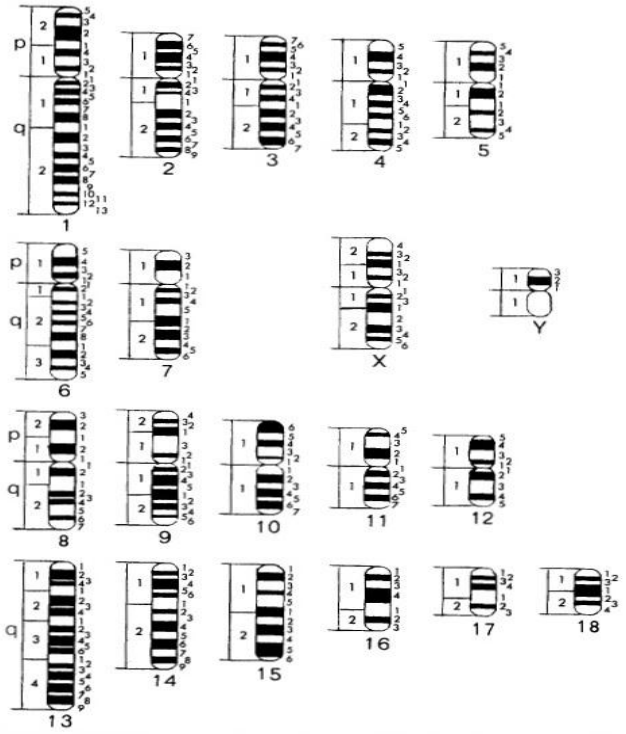

Fig. 1. Swine karyotype "G" banded chromosomes
(***Committee for the Standardized Karyotype of the Domestic Pig***)

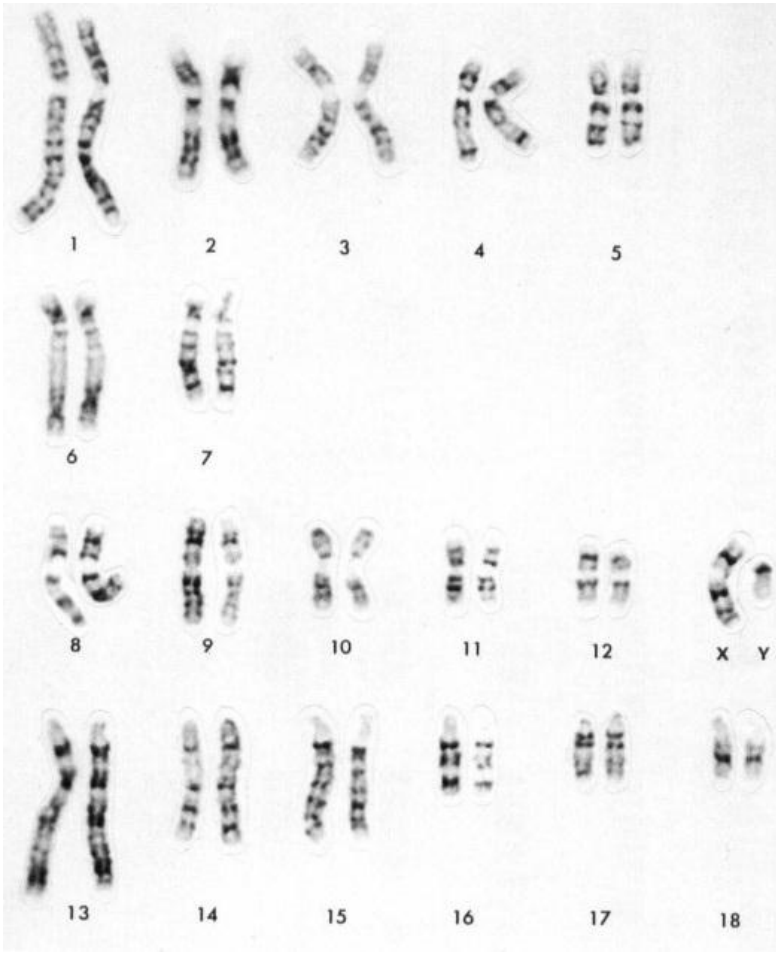

Fig. 2. Swine karyotype "G" banded chromosomes
(***Committee for the Standardized Karyotype of the Domestic Pig***)

Matherial and methods

The investigations were performed on 4 cases of intersex swines, on them realizing ultrasound exams, histologycal tests, cytogenetical studies – Barr body determination.
The method used to perform karyotype is the culture of peripheral blood leukocytes.

Results and discussions

The first case was represented by a 70 kg gilt with female phenotype and the presence of two prominently testicular bags (fig.3). Because of this anomaly, the animal has been hypothesized that would be intersex. To establish the exact type of intersexuality, the gilt was examined from anatomical and ultrasound point of views, in which were noticed two structures resembling with normal sow ovaries. Regarding the genital tract, in ultrasound

exam there were noticed two ducts that appeared to be the uterin horns (Ciornei Cristina and Pavli, 2009). At slaughter, it was noticed that the genital tract consists of two structures similar which supposed to be the gonads. In fact, the structures appeared larger than the normal ovaries and were attached to the genital tract through two long and thins ducts which were expected to be the uterine horns and uterus. But histologically these two ducts were the deferent ducts. The vagina was about 4cm long and inside there was the urinary opening (fig.4) (Ciornei Cristina et al.., 2010a).

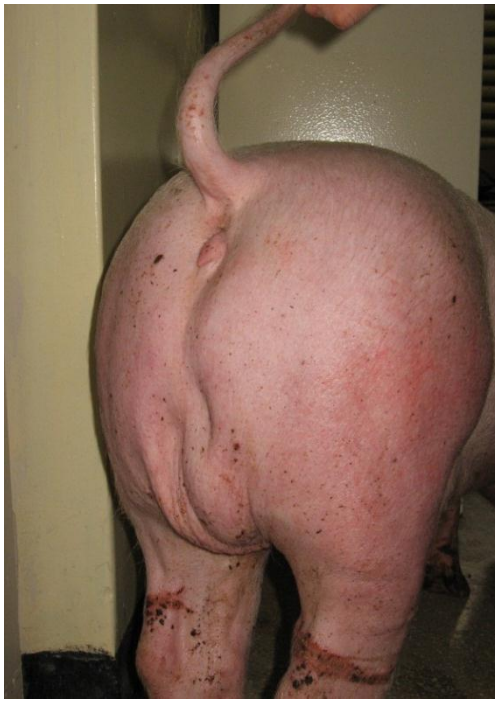

Fig. 3. Macroscopical appearance of the animal - vulva openning and scrotum

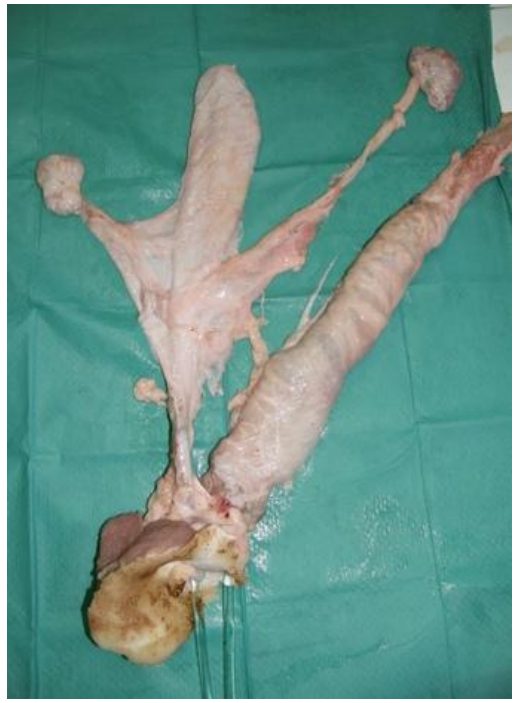

Fig. 4. Macroscopical appearance of the genital tract – gonads, deferent ducts, short vagina, urinary bladder and rectum

The karyotype was performed from peripheral blood, from ear veins and standard method of staining of chromosomes (fig. 5, 6) but also the "G" banded method were made (fig. 7, 8). The genetic tests as in karyotype but also the presence of Barr body in the oral mucosa and vaginal cells showed that the genetic sex is female, 38XX, morphologically, this case being a true bilateral hermaphrodite.

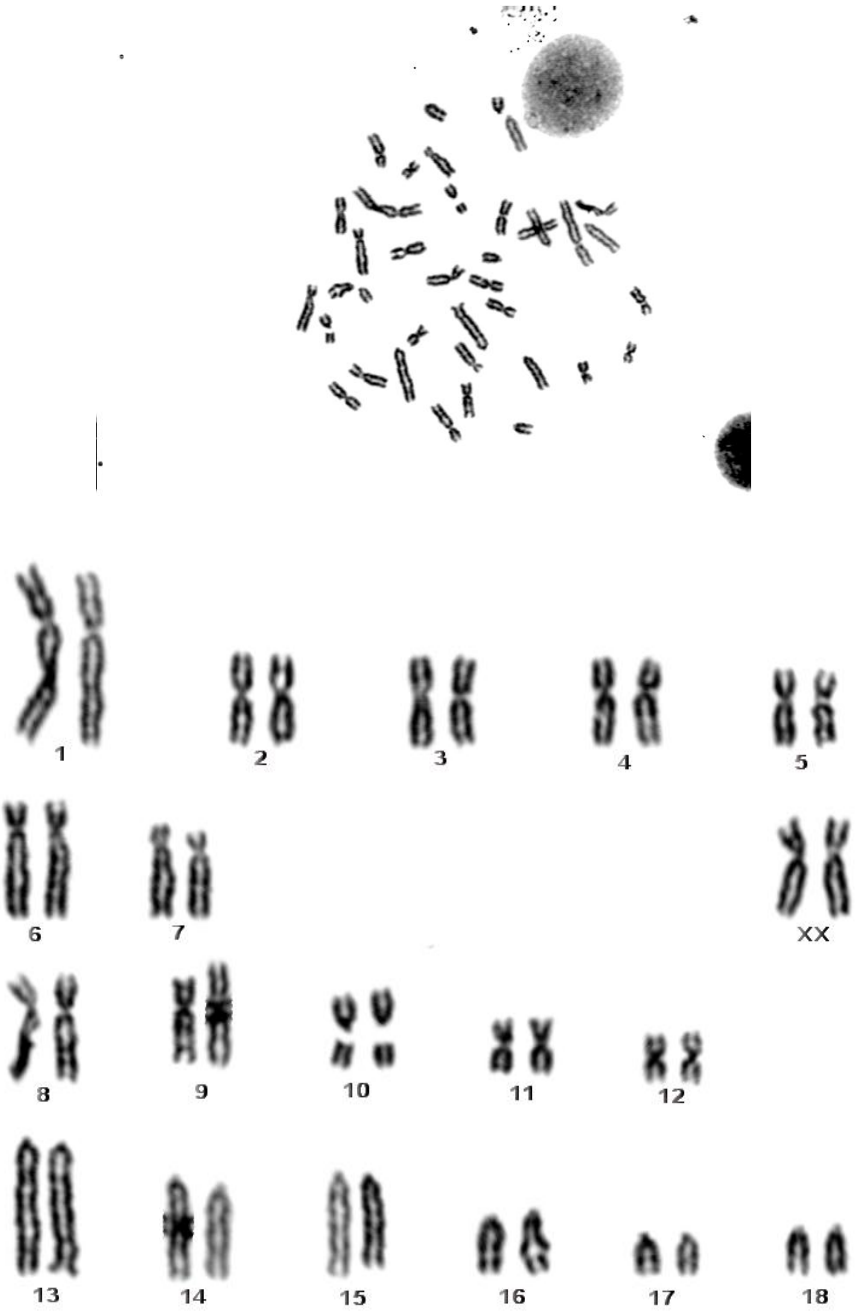

Fig. 5 and 6. Metaphase and karyotype, 38XX
Standard staining method

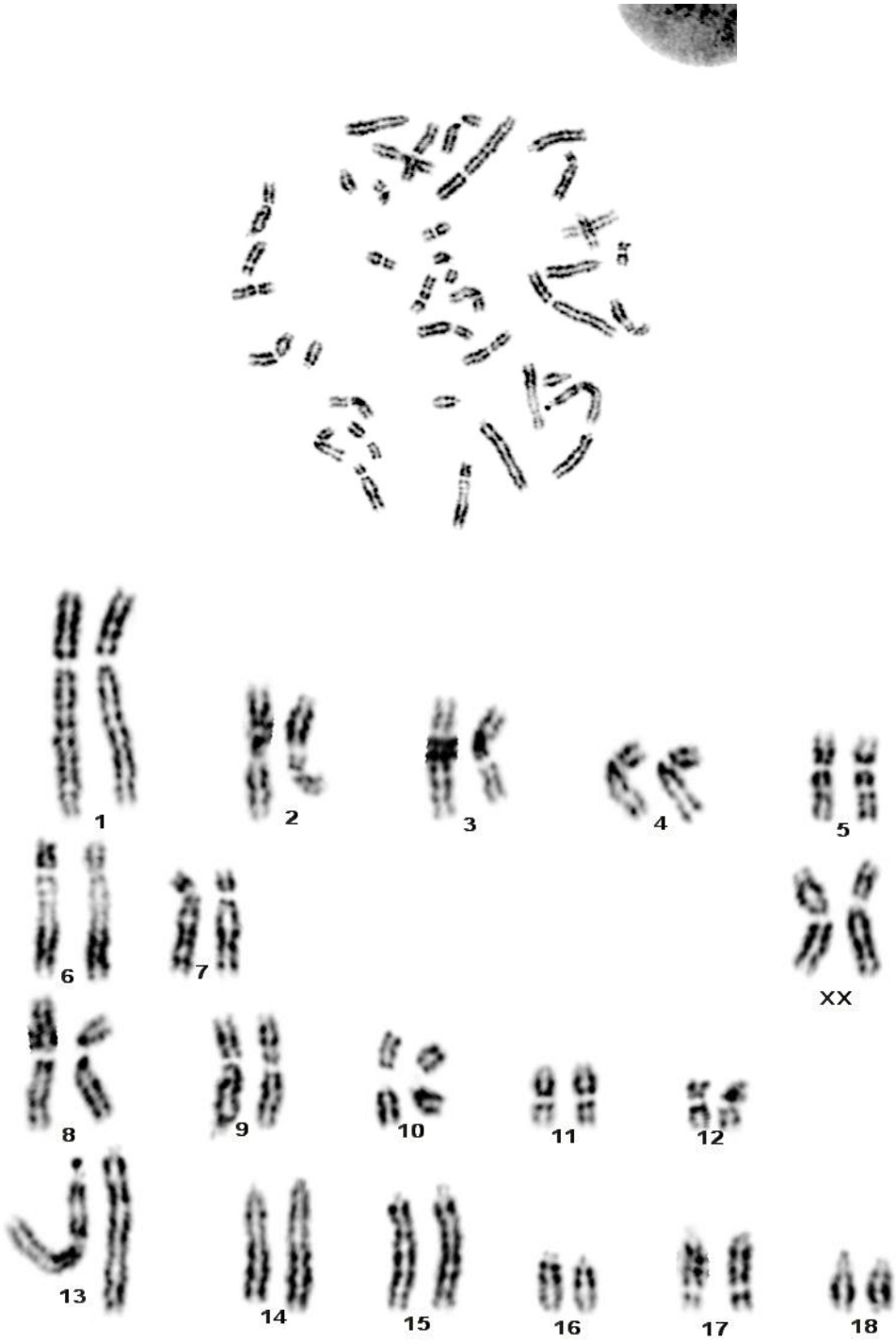

Fig. 7 and 8. Metaphase and karyotype, 38XX
"G " banded chromosomes

The second case had two testicular bags which at palpation revealed two structures similar to the testes and the epididymis. The scrotum was place in normal position for a boar. Also macroscopically there was present the vulva orifice with a hypertrophied clitoris (fig. 9).

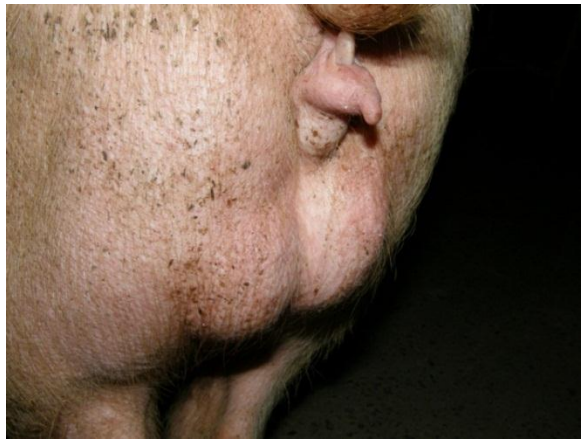

Fig. 9. Macroscopical appearance of the animal - vulva openning and scrotum

At slaughtered the gonads, weighing 108g the left gonad and 110g the right gonad were found to be testes. Externally the testes were covered by a thick pale pink albuginea that keeps the testicular parenchyma under tension. Left uterine horn had a length of 58 cm and the right one had 54 cm - both not looking sinuous characteristic for pigs (fig. 10) (Ciornei Cristina et al., 2010b).

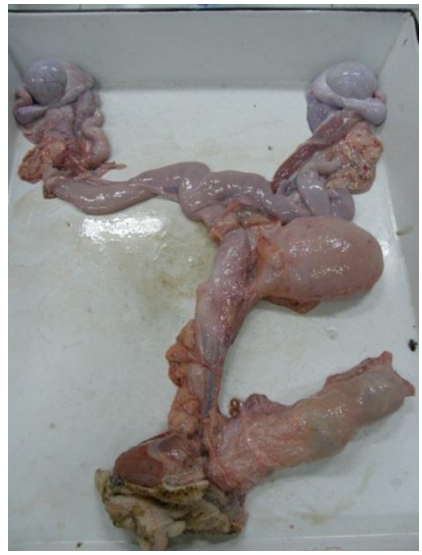

Fig. 10. Macroscopic appearance of the genitalia - presence of testes, internal female genital tract and uterus, rectum and bladder

Karyotype was performed from peripheral blood collected from ear veins and was made the standard staining method (fig. 11, 12) and the "G" banded method for chromosomes (fig. 13,14) - the resultant showing that the genetic sex of this case was also female, and from morphological point of view was a male pseudohermaphrodite.

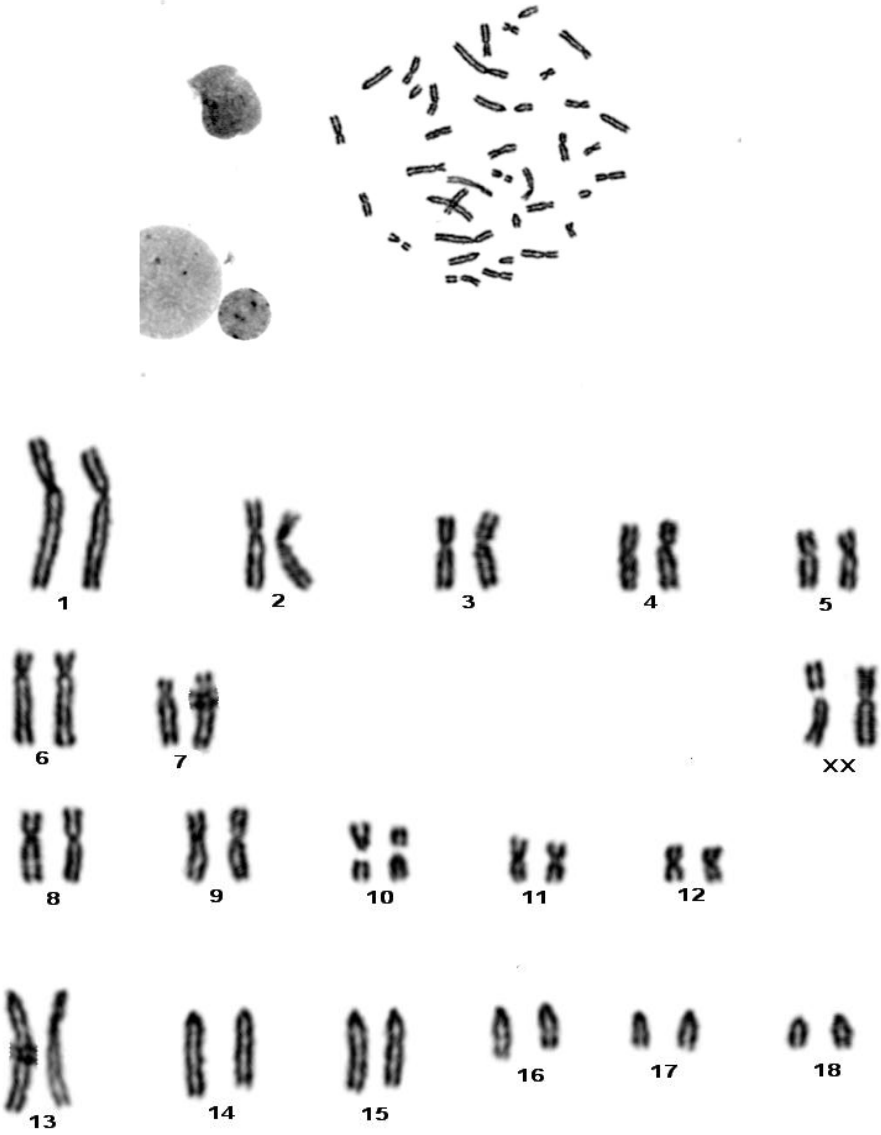

Fig. 11 and 12. Metaphase and karyotype, 38XX
Standard staining method

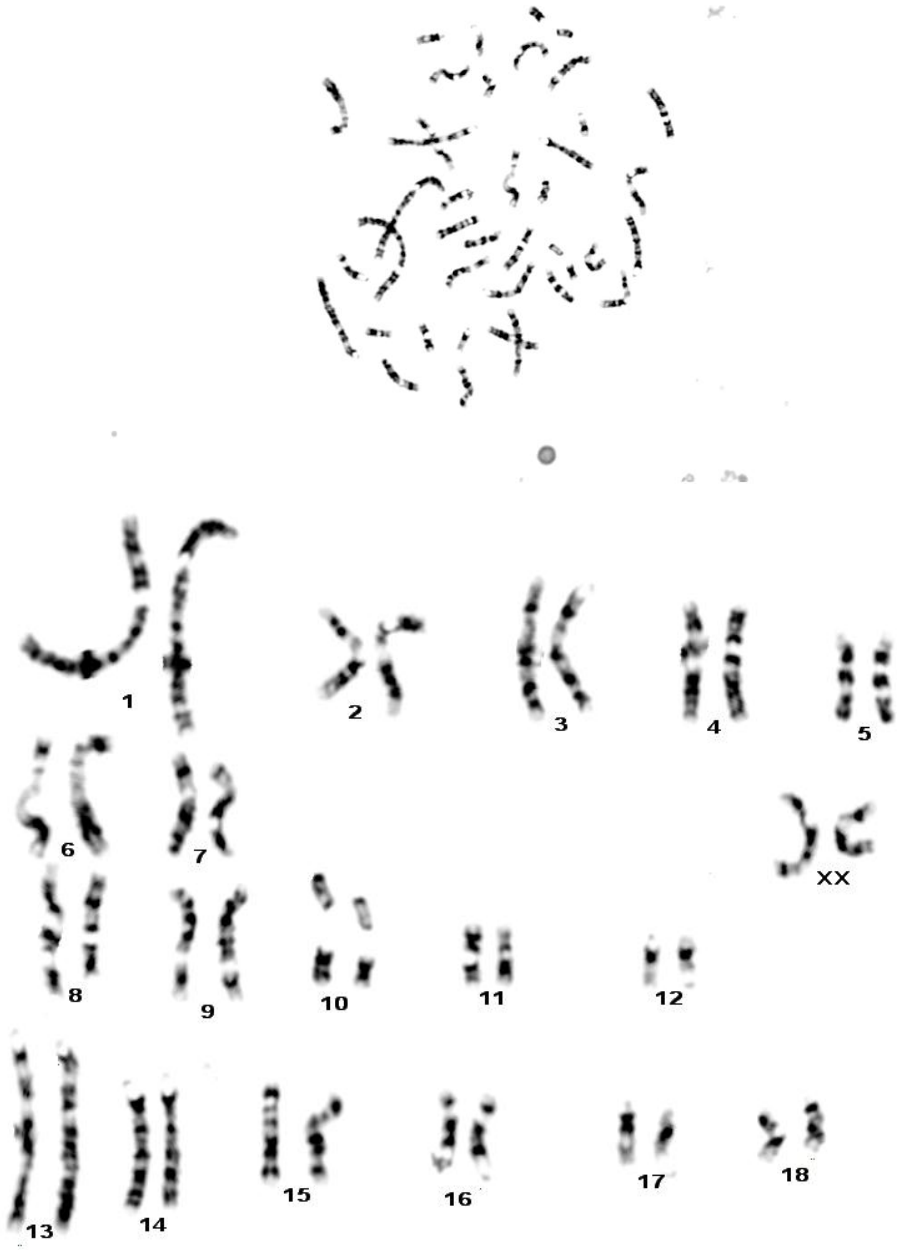

Fig. 13 and 14. Metaphase and karyotype, 38XX

The third case of swine intersex examined in the Faculty of Veterinary Medicine, showed in the normal position a scrotum very well developed. Above the scrotum, under the

anal orifice it was shown the vulva and vaginal opening with a clitoris pointing dorsal and hypertrophied (fig. 15). On palpation and ultrasound of the scrotal area, it was observed the presence of testicular looking formations covered by a structure similar to an epididymis (fig.16) (Ciornei Cristina et al., 2011).

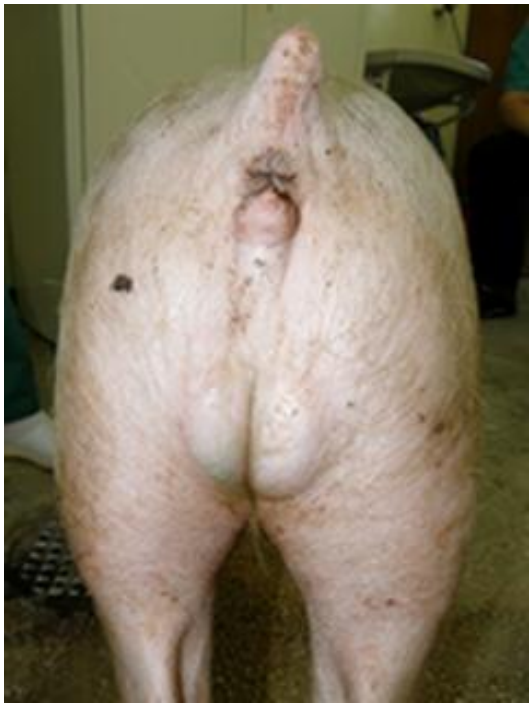

Fig. 15. Macroscopical appearance of the animal - vulva openning with clitoris and scrotum

Fig. 16. Macroscopic appearance of the genitalia - presence of testes, deferent ducts, a short vagina and the prostate gland

After slaughtering the animal was observed that the reproductive tract of this case consisted of two small testes of 28 and 35 grams. They were covered by the epididymis, highly developed in comparison with testicular tissue. From the two testes started two deferent ducts, which decrease in diameter, and even as they approached the urinary vestibule tended to merge. In the region of the opening of the bladder in vaginal vestibule, there was a glandular structure in the form of a "heart" as noted histologically - rudiment of the prostate gland (fig. 16). Internal female genital tract were represented only by vaginal vestibule, which in its histological structure were observed prostatic acini.

The vaginal mucosa and oral analysis by staining with acid fuchsin showed the presence of Barr corpuscles. The karyotype (fig. 17-20) demonstrates that the genetic sex is female although morphological this case was also male pseudohermaphrodite.

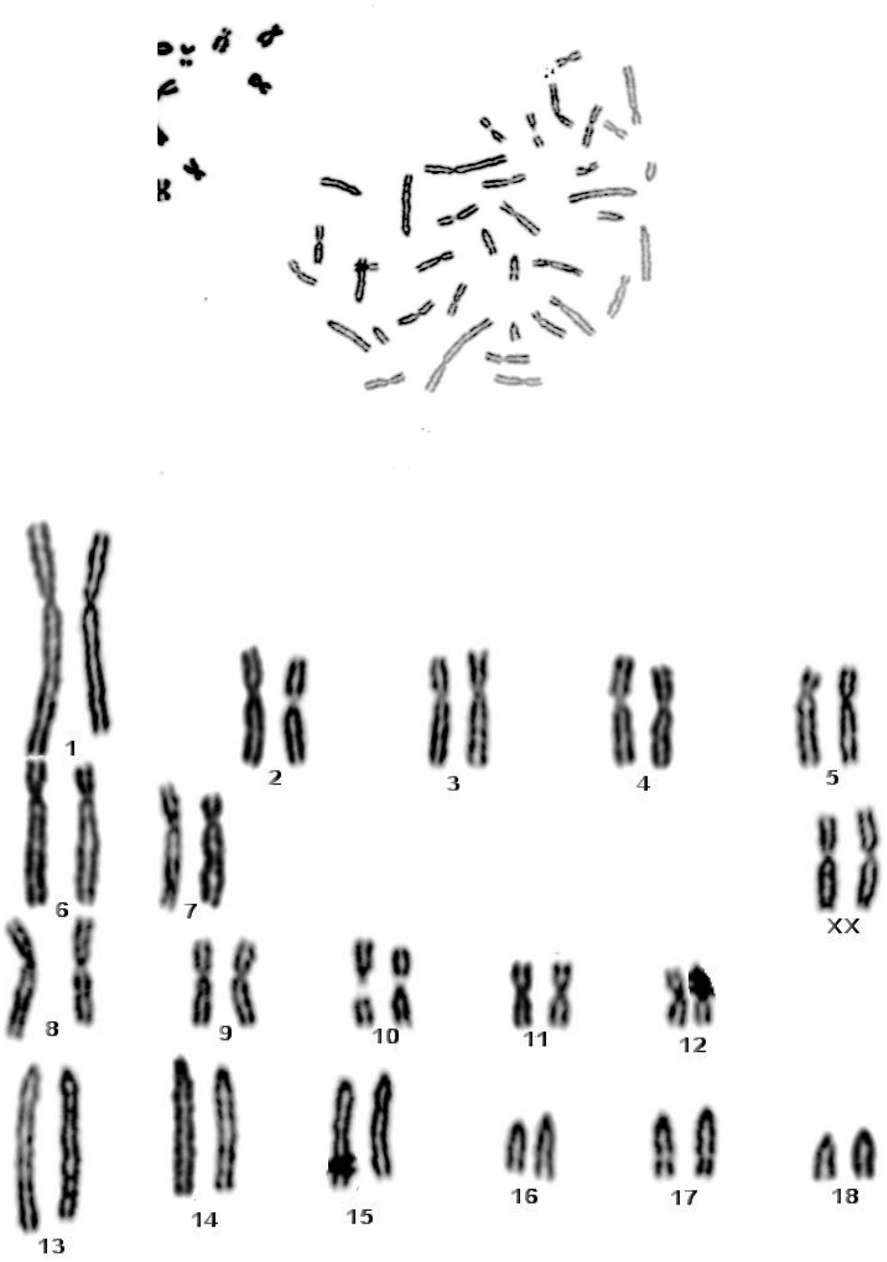

Fig. 17 and 18. Metaphase and karyotype, 38XX
Standard staining method

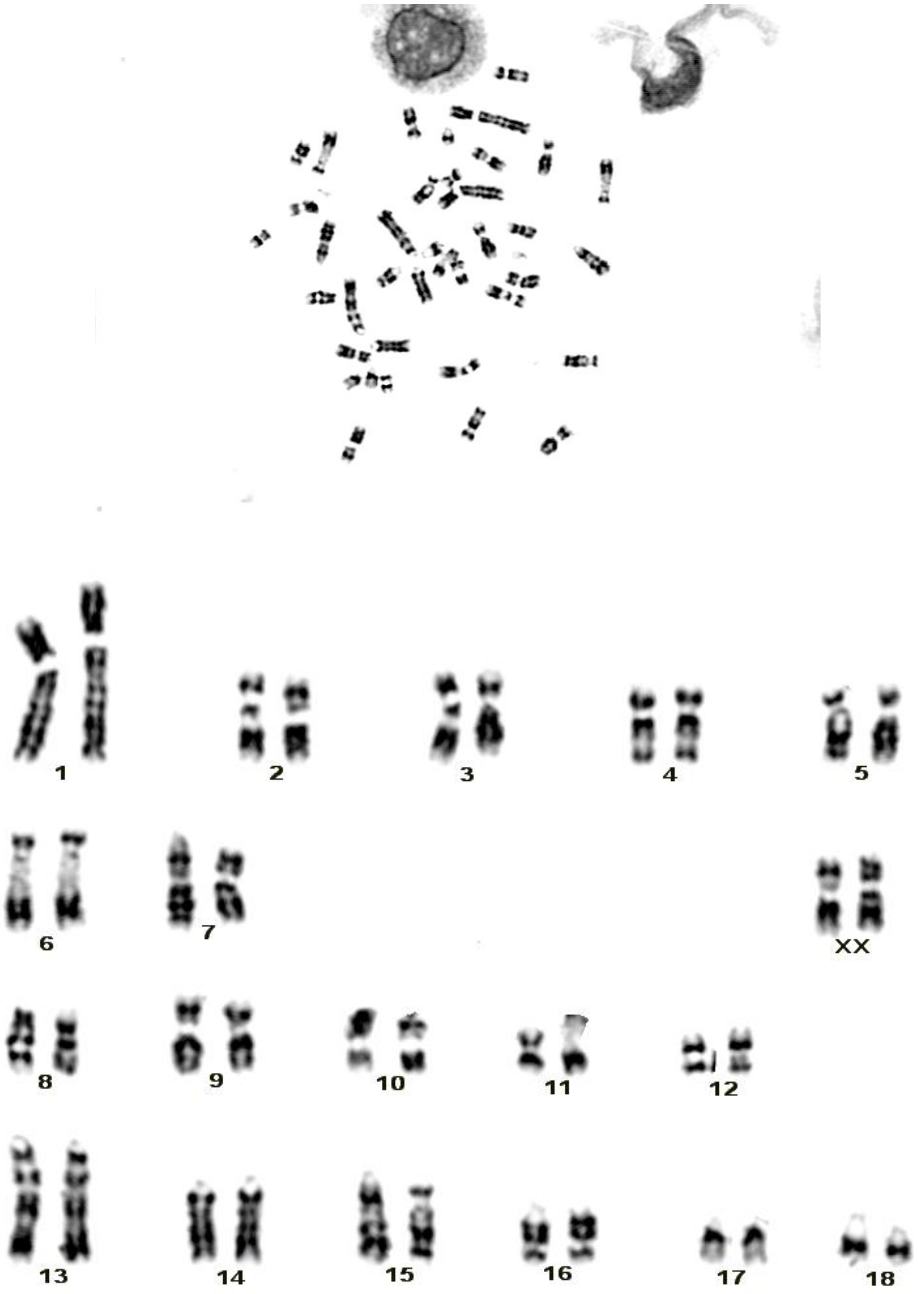

Fig. 19 and 20. Metaphase and karyotype, 38XX
"G " banded chromosomes

The fourth case analyzed in the FVM Iasi was represented by a pig – male pseudohermaphrodit from a private household of Iași County. This pig presented on the outside, as the other cases, a scrotum in the normal position, well developed. Between the two testes, the scrotum showed on the center line a wrinkled vulva with soft skin and the externalized clitoris was absent (Fig. 21).

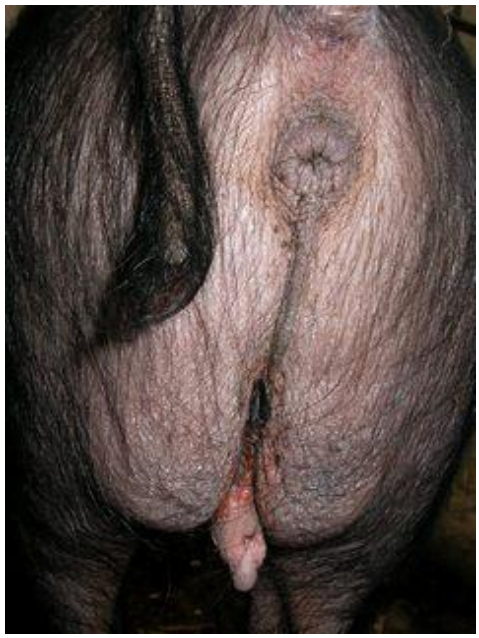

Fig. 21. Macroscopical appearance of the animal - vulva openning on the medial lone o fthe scrotum

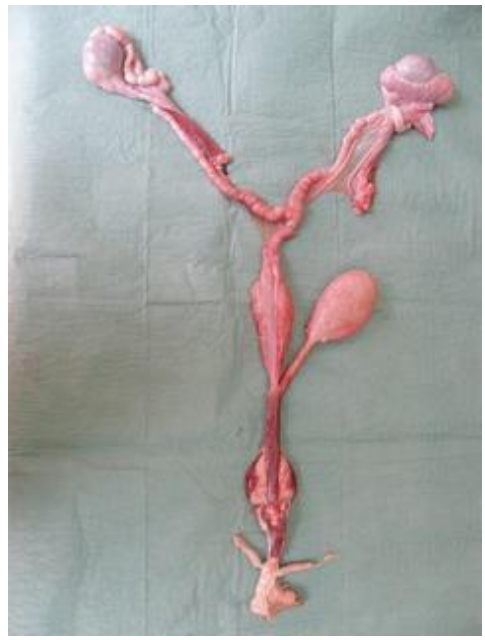

Fig. 22. Macroscopic appearance of the genitalia - presence of testes, internal female genital tract and uterus, rectum and bladder

At slaughter, it was found that the internal genitalia consisted of two testes as gonads, covered the epididymis - one of the testes being covered by an epididymis with cerebriform appearance. Uterine horns were slightly coiled and they had parallel paths with the deferent duct. Regarding the genial tract the cervix was missing, unable to distinguish clearly between the uterus, cervix, and vagina. Macroscopically along the vaginal vestibule and vagina were observed two male accessory gland-like structures. These will be confirmed by morphopathologic diagnosis (fig.22).

Cytogenetic and karyotype tests carried out showed that in this case of male pseudohermaphroditism is female (fig. 23-26).

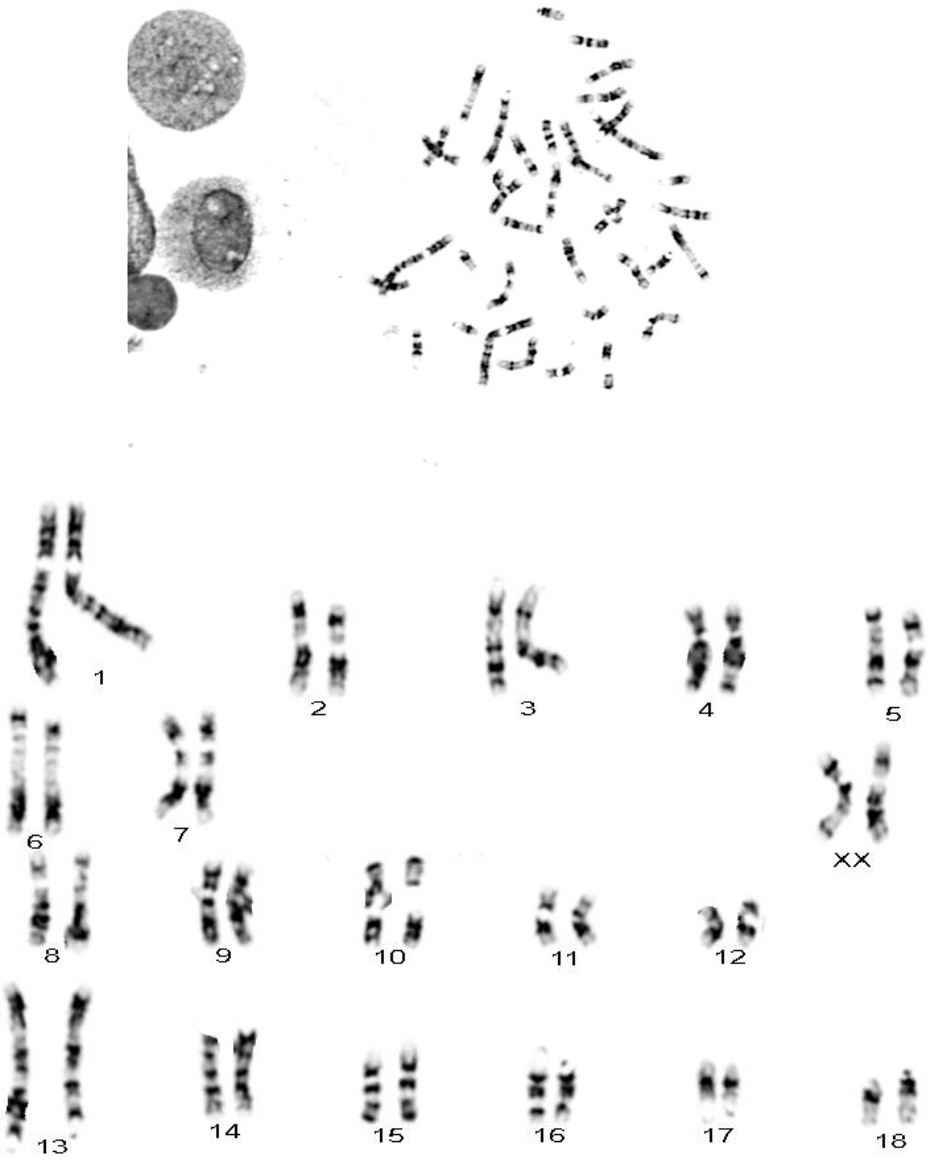

Fig. 23 and 24. Metaphase and karyotype, 38XX
"G " banded chromosomes

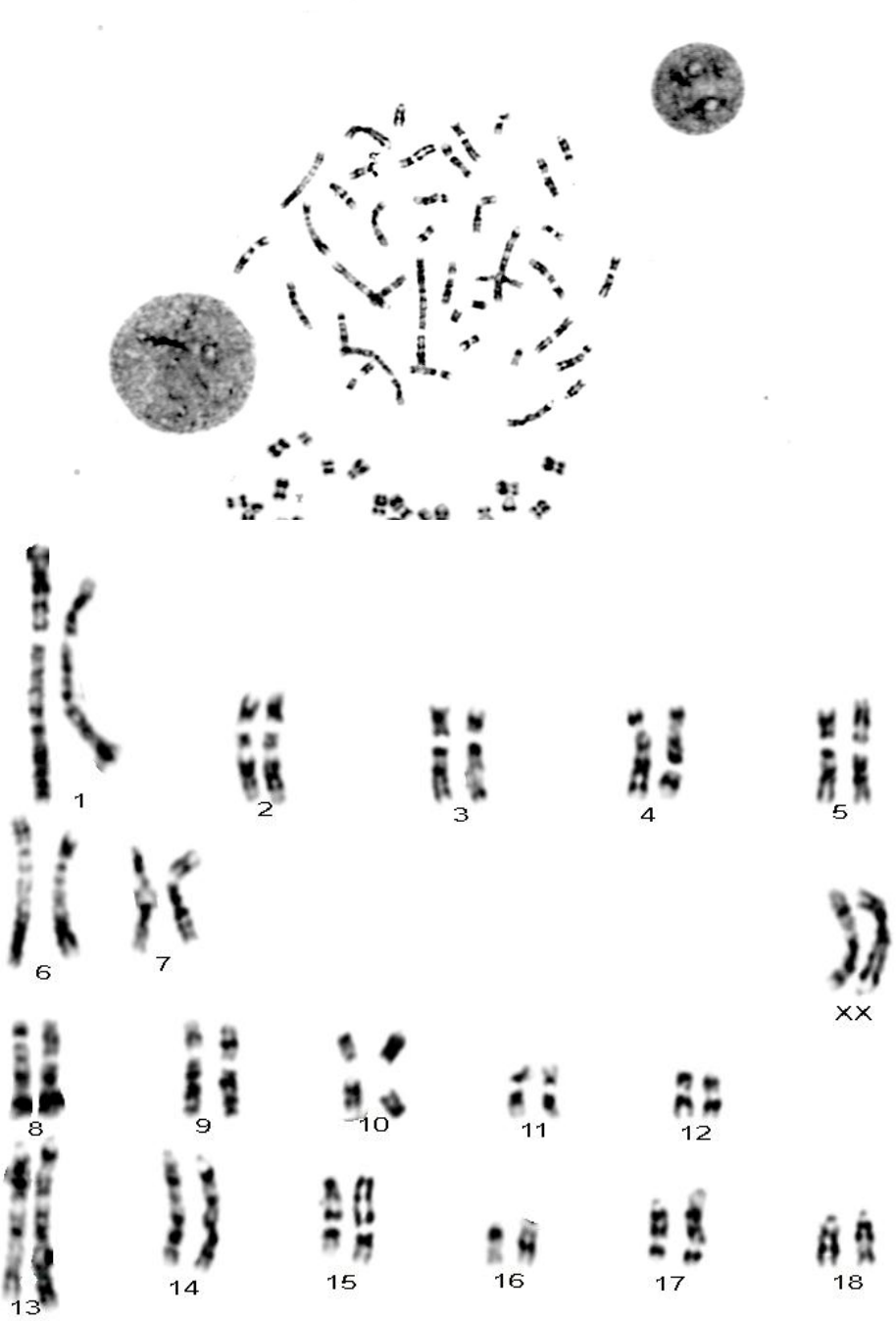

Fig. 25 and 26. Metaphase and karyotype, 38XX
"G " banded chromosomes

Conclusions

1. X chromatin test is a rapid test, easy to make giving clear information on the existence of X chromosome in cells of individuals with sexual abnormalities.
2. Of the three stains tested for evidence of chromatin best, from a personal point of view, the one with cresyl violet is better because the chromatin appears intensely colored on inner face of the nuclear membrane, and 1% acetic orcein method, performed under pressure flattens the nuclei so the is chromatin was also flattened, slightly larger in size than the other two stains.
3. Compared to chromatin test, making the karyotype is harder to performed, depends very much on how the blood was collected and the health of the animal, and it is more expensive and harder to interpret.
4. In all cases in which X chromatin test was performed and came out positive suggests the existence of the presence of two chromosomes but does not exclude the presence of the Y chromosome.
5. Correlation between the chromatin test and karyotype established that in all cases of swine intersexes the genetic sex is female although from morphological point of view the phenomen of intersexuality it varies.

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MORPHOLOGIC PARTICULARITIES OF THE BROWN BEAR (*URSUS ARCTOS*) LIVER

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Abstract
The brown bear liver can be framed within a quadrilateral shape. Liver lobulation consists of six lobes: caudate lobe, lateral right lobe, medial right lobe, quadrate lobe, medial left lobe and lateral left lobe. Incisures are deep, without reaching the hilum. Right lobes are of equal proportions, left lobes are proportionally more developed than the right ones and unequal. The caudate lobe is divided in two processes: the caudate process itself, oriented towards right and the doubly incised papillary process oriented towards left. There is no common hepatic duct, and the common bile duct opens in the anterior portion of the descending duodenum commonly with the main pancreatic duct (Wirsung), at the level of the major duodenal papilla. The gall bladder presents hepato-cystic channels, and its bottom is situated lower than the ventral edge of the liver.
Keywords: liver, anatomy, *Ursus arctos*

Materials and methods

The adult bear was a female bear, from the zoo who die from complication do to the infestation of internal parasites, and the 4 month cub die from unknown cause.

The time between the death of the bears and collection and fixation of tissues was of 2–12 h. Histological sections was 5 μm thick, the stained use were Van Gieson and HEA for anatomic microarchitecture.

Results and discussions

The liver (*Hepar*) is the largest digestive gland, the first organ placed retrodiafragmatic the intrathoracic portion of the abdominal cavity with the large axis oriented almost transversely from right to left, without exceeding ventral costal arch. The liver reached to be placed on both sides about until the last ribs (fig.1, fig. 2, fig. 3).

The liver has two faces, one diaphragmatic (*Facies diaphragmatica*) and a visceral (*Facies visceralis*), with four edge: dorsal, ventral, right and left. His form has an irregular shape, but may fall into an oval.

Diaphragmatic face (*Facies diaphragmatica*) is convex and smooth, molded into the diaphragm. On the back of the diaphragm face is observed *Sulcus vena cava*, very deep (fig. 5).

Visceral face (*Facies visceralis*) is slightly concave and irregular. In the center of this side is placed hepatic hilum (*Porta hepatis*) with extrahepatic biliary ducts and the impression made on the surface by the portal vein. Emerging from the hilum is the lesser omentum (*Omentum minus*) at the bottom of the vessels and nerves (fig. 6, fig. 7).

The visceral face is bearing the impression of the organs in contact near by: gastric impression (*Impressio gastrica*), [the left side of stomach body], oesophageal impression (*Impressio esophagea*) on the back [to the caudate lobe] and duodenal impression (*Impressio duodenalis*) given by the cranial portion of the duodenum [in the lateral lobe] .

Dividing the lobes are the to main notch, right (*Incisurae interlobares dexter*) and left (*Incisura interlobarest sinistrum*) paced on the underside of the liver. On the visceral face,

right notch corresponds to gallbladder fossa (*Fossa vesicae felleae*), being placed next to it. Main left notch is round ligament fissure (*Fissura lig. teretis*), vestige of umbilical vein.

Besides the main notch, liver suffers second notch accessories: right and left. Interlobular notch are deep, but not extend to the hilum hepatic. They delimit six lobes well individualized: caudate, right lateral, right medial, square, left lateral and medial left (fig. 3).

Caudate lobe (*Lobus caudatus*) is reduced in size, not exceeding the lateral edge of lateral right lobe. The caudate lobe is divided in two processes: the caudate process itself, oriented towards right and the doubly incised papillary process oriented towards left.

Right lateral lobe (*Lobus hepatis dexter lateralis*) is thicker than the left lateral lobe, but smaller, compared with it, with rounded edges.

Right medial lobe (*Lobus hepatis dexter medialis*) is bigger than its counterpart on the left, about twice, and has a triangular appearance, whose free ventral edge portion does not exceed the square lobe.

Square lobe (*Lobus quadratus*) is of intermediate size, and has a deep fissure on the visceral and parietal face. Ventral extremity does not exceed the free edge of the liver.

The left medial lobe (*Lobus hepatis sinister medialis*) is smaller, triangular, ventral extremity does not exceed the free edge of the liver. It is partly covered by the left lateral lobe and square lobe on the visceral face of the liver.

Left lateral lobe (*Lobus hepatis sinister lateralis*) is the most developed lobe of the liver, with an oval shape, showing at the ventral edge a fissure not very deep.

Gall bladder (*Vesica fella*) is present in the bile fossa (*Fossa vesicae fellesa*), located in the main right notch on the visceral face, between the right medial lobe and square lobe. It has a capacity of about 100 ml, is visible on both the visceral and diaphragmatic face, whose bottom exceeding the ventral edge of the liver (fig. 5, fig. 6)

There is no common hepatic duct (*Ductus hepaticus communis*), fact observed at dogs to. Hepatic duct coming from right lateral lobe is formed dorsal to the cystic duct (*Ductus cysticus*) and the one from the right medial lobe connects with the base of the cystic duct. The other ducts approach from the ventral and left side before the the origin of cystic duct.

Cystic duct (*Ductus cysticus*) continues dorsal the bilarebladder neck, being located between the right lobe and medial lobe square, on the visceral face of liver. It adheres to the liver parenchyma and measured 2 cm. . Mucosa lining the cystic duct have holes which are hepato-cystic ducts (fig.6, fig. 7).

Bile duct (*Ductus choledochus*) is detached from the hepatic hilum, placed between hepato-duodenal ligament (right portion of the small omentum), opening in the anterior portion of the descending duodenum, together with the main pancreatic duct (Wirsung), on the surface of the major duodenal papilla (Papilla duodeni major) (fig.6, fig. 7, fig. 8).

The attachment means of the liver are multiple. The maintenance of normal anatomical position is possible because of the abdominal press, compression of other viscera and special reports with the two large veins, the portal vein and caudal *vena cava*.

On the other hand the liver is attached to the diaphragm and surrounding organs by ligaments: coronary, falciform, right triangular, triangular left, hepato-renal and lesser omentum.

Coronary ligament (*Lig. coronarium hepatis*) is short, strong, formed by passing of the serous from the diaphragm to the liver, where its foils insert on one side of the caudal vena cava ditch , and join ventral to continue with falciform ligament.

Falciform ligament (*Lig. falciforum hepatis*) is very strong and has two parts: a proximal, disposed between the diaphragm and diaphragmatic face of the liver; a distal part, disposed between the floor of the abdominal cavity and round ligament of the liver. Parietal insertion of falciform ligament begins at the diaphragm orifice of vena cava , and continues down to the stern and floor of the abdominal cavity to the umbilical scar. Insertion of the liver continues the insertion coronar ligament , in the ventral direction until the main left notch. Round ligament paced at the base of the falciform ligament, is divided into three branches that are placed between square lobe and left medial lobe, at the fissure that bears his name.

Right triangular ligament (*Lig. triangulare dextrum*) is shorter and thicker than the left, being structured by passing of the serosa from the diaphragm on the dorso-lateral side of the right lateral lobe, and extending with the coronary ligament.

Left triangular ligament (*Lig. triangulare sinistrum*), consists of passage of serosa from the diaphragm on dorso-medial side of the left lateral lobe; is finer and as wide as its counterpart (fig. 3).

Lesser omentum (*Omentum minus*) is formed by passing the serosa around the hilum of the liver to the lesser curvature of stomach. It is form in two distinct parts : *Lig hepatogastricum* and *Lig hepatoduodenale* (fig. 4).

Histological structure of the liver was characterized by the existence of hepatic lobules lacking interlobular fibrous septae. The capsule of *Glisson* was relatively thin. The liver lobules are polyhedral pyramid form similar to the domestic mammals (fig. 9, fig. 10, fig. 11).

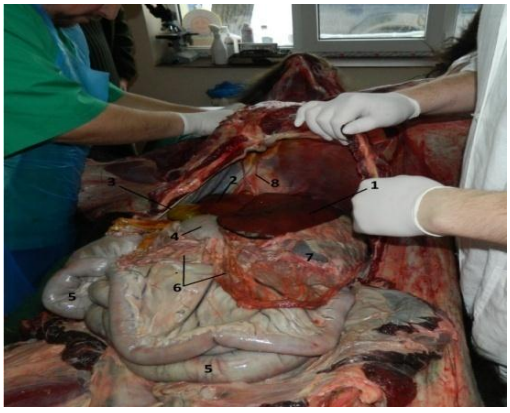

Fig. 1. Amplasarea ficatului in raport cu organelle din cavitatea abdominala: 1-lobus hepatis sinister lateralis;2- lobus hepatis dexter medialis; 3- vesica fellea; 4- gaster; 5- jejunum; 6- omentum majus; 7 lien

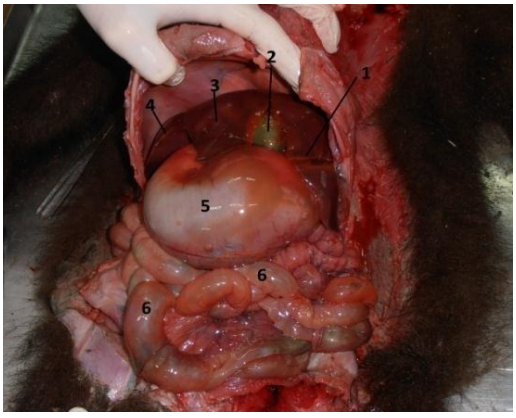

Fig. 2. Ficatul la pui de 4 luni: 1-lobus hepatis sinister lateralis; 2- vesica fellea; 3- lobus hepatis dexter medialis; 4- lobu hepatis dexter lateralis; 5- gaster; 6- jejunum.

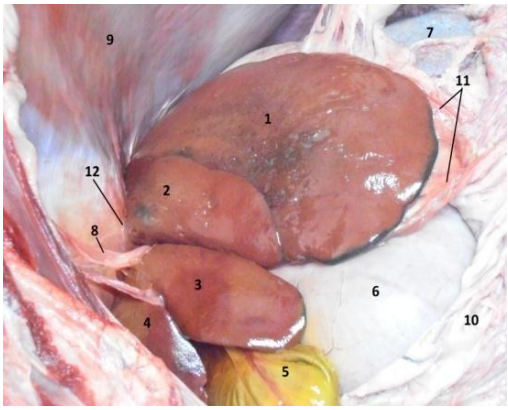

Fig. 3. Ficatul pe fata diafragmatica: 1- lobus hepatis sinister lateralis; 2- lobus hepatis sinister medialis; 3- lobus quadrates; 4- lobus hepatis dexter medialis; 5- vesica fellea; 6- gaster; 7- lien; 8- lig. falciforme hepatis; 9- diaphragm; 10- omentum majus; 11- lig. triangulare sinistrum; 12- lig. coronarium hepatis

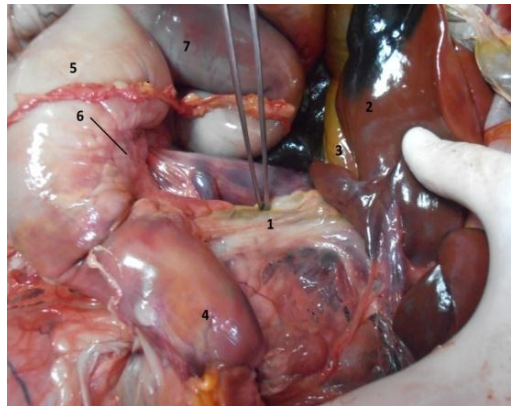

Fig. 4. Hilul ficatului: 1- lig. hepatoduodenale; 2- hepar; 3- vesica fellea; 4- duodenum, pars cranialis; 5- duodenum, pars descendens; 6- pancreas; 7- jejunum.

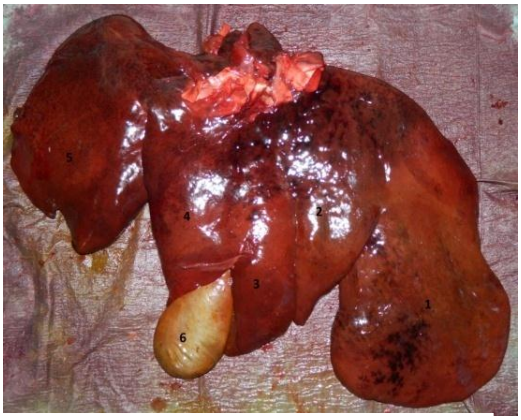

Fig. 5. Diaphragmatic face of the liver: 1- lobus hepatis sinister lateralis; 2- lobus hepatis sinister, medialis; 3- lobus quatratus; 4- lobus hepatis dexter medialis; 5- lobus hepatis dexter lateralis; 6- vesica fellea.

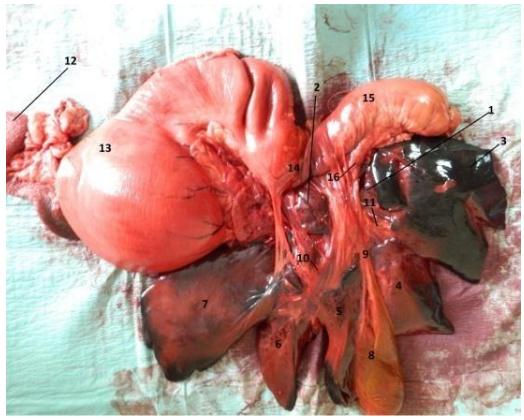

Fig. 6. Visceral face of the liver and the stomach : 1- lobus caudatus, processus caudatus; 2- lobus caudatus, processus papillaris; 3- lobus hepatis dexter lateralis; 4- lobus hepatis dexter medialis; 5- lobus quadratus; 6- lobus hepatis sinister medialis; 7- lobus hepatis sinister lateralis; 8- vesica fellea; 9- ductus cysticus; 10- ductus hepaticus dexter; 11- ductus hepaticus sinister; 13- gaster; 14- duodenum, pars cranialis; 15- duodenum, pars descendens;

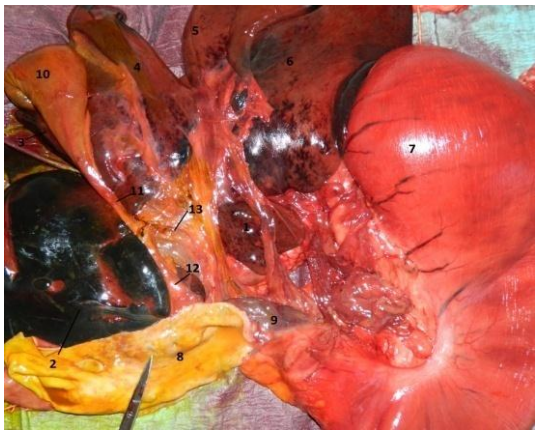

Fig. 7. The bile duct opening: 1- lobus caudatus, procesus papillaris; 2- lobus hepatis dexter lateralis; 3- lobus hepatis dexter medialis; 4 lobus quadratum; 5- lobus heaptis sinister medialis; 6- lobus hepatis sinister lateralis; 7- gaster; 8- duodenum, pars descendens; 9- duodenum, pars cranialis; 10- vesica fellea; 11- ductus cysticus; 12- ductus choledochus; 13- ductus hepaticus sinister.

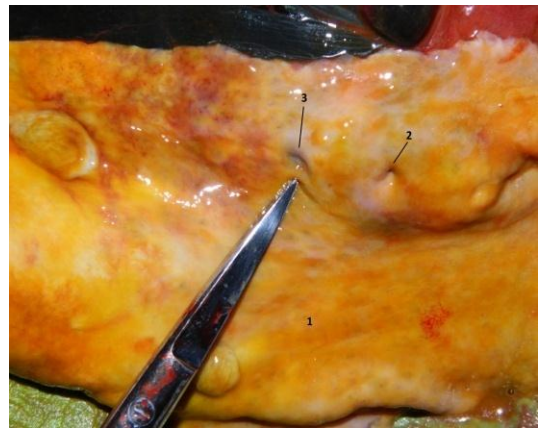

Fig. 8. Duodenal mucosa appearance : 1- duodenum , pars descendens; 2- papilla duodeni major; 3- papilla duodeni minor.

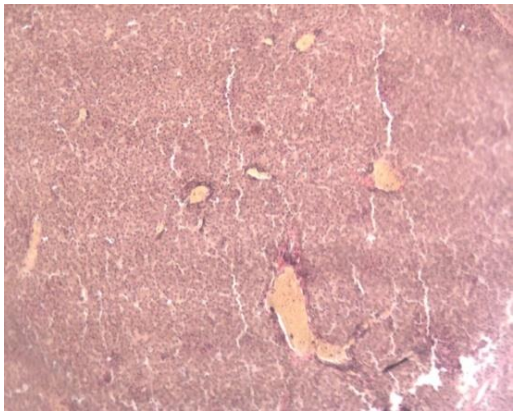

Fig. 9. Portal tract .Van Gieson stain; x 100

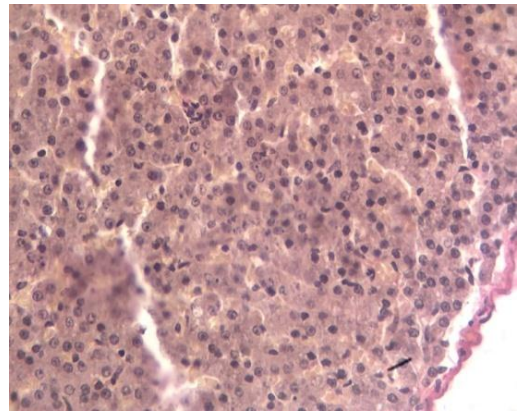

Fig. 10. Hepatic lobules and the capsule of Glisson . Van, Gieson

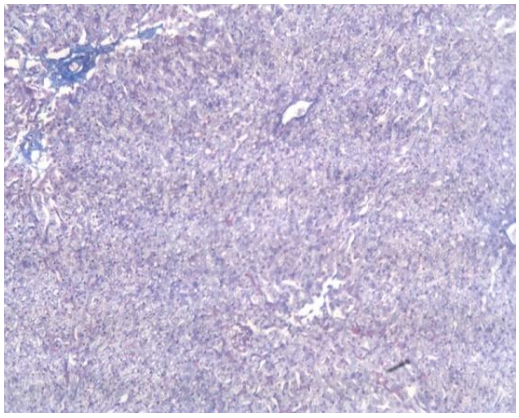

Fig.11. Hepatic lobules. HEA stain; x 100

Conclusions

1. Interlobular notch are deep, but not extend to the hilum hepatic. They delimit six lobes well individualized: caudate, right lateral, right medial, square, left lateral and medial left.
2. Right lobes are of equal proportions, left lobes are proportionally more developed than the right ones and unequal. The caudate lobe is divided in two processes: the caudate process itself, oriented towards right and the doubly incised papillary process oriented towards left.
3. There is no common hepatic duct, and the common bile duct opens in the anterior portion of the descending duodenum commonly with the main pancreatic duct (Wirsung), at the level of the major duodenal papilla.
4. The gall bladder is present. The gall bladder presents hepato-cystic channels.

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MORPHOLOGIC PARTICULARITIES OF THE PANCREAS OF THE BROWN BEAR (*URSUS ARCTOS*)

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Abstract
The pancreas is a mixed (amphicrine) gland, with exocrine and endocrine secretion. It is highly developed, almost 50 cm long, 2-3 cm wide and 1 cm thick. The body is flattened dorsoventrally and placed caudally to the pyloric region of the stomach, next to the second lumbar vertebra. The right lobe is found in contact with the descending portion of the duodenum, until its caudal curve, at the level of the fifth lumbar vertebra. The right lobe is very long and it curves itself at the level of the transverse portion of the duodenum, following its path. The left lobe forms a round edge caudally to the bottom of the stomach, placing itself between it, the left kidney and the base of the spleen.

Keywords: pancreas, structure, topography, *Ursus arctos*

Materials and methods

The adult bear was a female bear, from the zoo who die from complication do to the infestation of internal parasites, and the 4 month cub die from unknown cause.

The time between the death of the bears and collection and fixation of tissues was of 2–12 h. Histological sections was 5 μm thick, the stained use were HEA and PAS for anatomic microarchitecture.

Results and discussions

Like the liver the pancreas has both an exocrine and endocrine function. The pancreas is located into the dorsal part of the abdominal cavity in close relationship to the proximal part of the duodenum.

It is highly developed, almost 50 cm long, 2-3 cm wide and 1 cm thick at the adult bear. Body appearance is similar to the main salivary glands. Can be distinguished: a body, two lobes (left and right), two edges (cranial and caudal) and two sides (dorsal and ventral) (fig.1, fig. 2, fig.3)

The body (*Corpus pancreatis*) is flattened dorsoventrally and placed caudally to the pyloric region of the stomach, next to the second lumbar vertebra.

The right lobe (*Lobus dexter*) is found in contact with the descending portion of the duodenum, until its caudal curve (*Flexura duodeni caudalis*), at the level of the fifth lumbar vertebra. . The right lobe is very long and it curves itself at the level of the transverse portion of the duodenum (*Duodenum, pars transversa*), following its path. It is longer than the left lobe of the pancreas and is palced between the foils of the ligament who suspend the duodenum.

The left lobe (*Lobus sinister*), shorter but wider, is placed between the foils of great omentum (*Omentum majus*). Accompanying the greater curvature of the stomach (*Curvatura ventriculi major*) the left lobe forms a round edge caudally to the bottom of the stomach, placing itself between it, the left kidney and the base of the spleen.

Cranial edge (*Margo cranialis*) is convex, in contact with the small curvature of the duodenum and greater curvature of the stomach.

Caudal edge (*Margo caudalis*) is concave and embrace cranial mesenteric artery origin, and forming pancreatic notch (*Incisura pancreatis*) which is deep by passing of the portal vein.

Dorsal face (*Facies dorsalis*) of the pancreas is the most adherent to the abdominal cavity ceiling and through high level of tissue, to the big vessels, comes into contact with: right kidney, right lateral lobe of the liver, portal vein, caudal vena cava , aorta, diaphragm, left kidney and the spleen.

Ventral face (*Facies ventralis*) of the pancreas comes in contact with the stomach (the left lobe), the duodenum and transverse colon.

Pancreatic ducts, namely the main pancreatic duct (*Ductus pancreaticus*), *Wirsung*, and accessory pancreatic duct (*Ductus pancreaticus accesorius*), *Sartorini*, are resulting from the confluence of interlobular ducts. The main pancreatic duct opens mutually with the common bile duct at the level of the major papilla (*Papilla duodeni major*), in the anterior portion of the descending duodenum (*Duodenum, pars desdendens*), and the accessory pancreatic duct opens at the level of the minor papilla (*Papilla doudeni minor*) situated 2 cm posterior to the major papilla (fig. 4, fig. 5)

The histological structure is similar to those of domestic carnivore (fig. 6, fig. 7, fig. 8, fig. 9, fig 10).

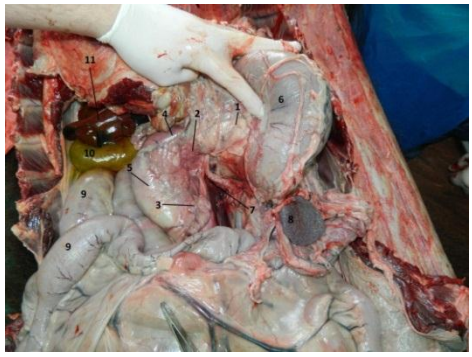

Fig. 1. The pancreas : 1- lobus pancreatis sinister; 2- corpus pancreatis; 3- lobus pancreatis dexter; 4- duodenum, pars cranialis; 5- duodenum, pars descendens; 6- gaster; 7- incisura pancreatis; 8- lien; 9- jejunum; 10- vesica fellea; 11 – gaster

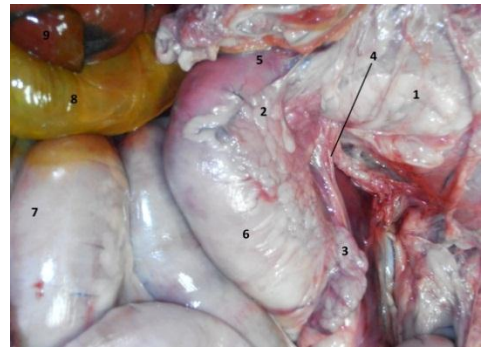

Fig. 2. The pancreas : 1- lobus pancreatis sinister; 2- corpus pancreatis; 3- lobus pancreatis dexter; 4- incisusura pancreatis; 5- duodenum, pars cranialis; 6- duodenum, pars descendens; 7- jejunum; 8- vesica fellea; 9- hepar

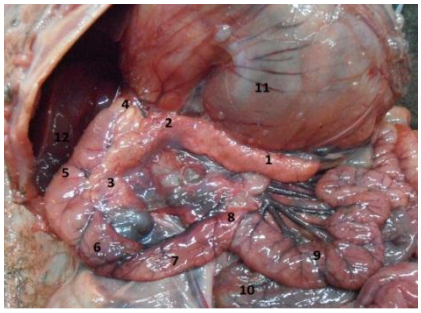

Fig. 3. The pancreas at bear cub, 4 months old: 1- lobus pancreatis sinister; 2- corpus pancreatis; 3- lobus pancreatis dexter; 4 – duodenum, pars cranialis; 5- duodenum pars descendens; 6- duodenum, pars caudalis; 7 –duodenum, pars ascendens; 8- flexura duodenosejunalis; 9- jejunum; 10- colon; 11- gaster; 12- hepar

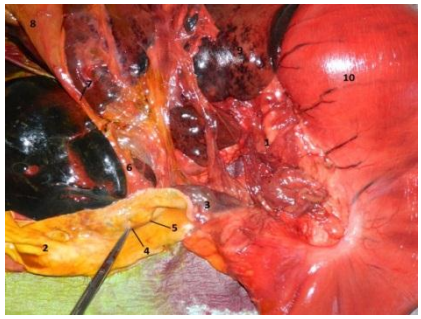

Fig. 4 Pancreatic ducts opening :1- pancreas; 2- duodenum pars descendens; 3- duodenum, pars cranialis; 4- papilla duodeni major; 5- papilla duodeni minor; 6- ductus choledochus; 7- ductus cysticus; 8- vesica fellea; 9- hepar; 10- gaster

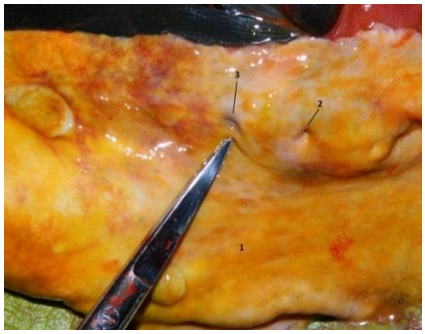

Fig. 5. Duodenal mucosa : 1- duodenum, pars descendens; 2- papilla duodeni major; 3- papilla duodeni minor

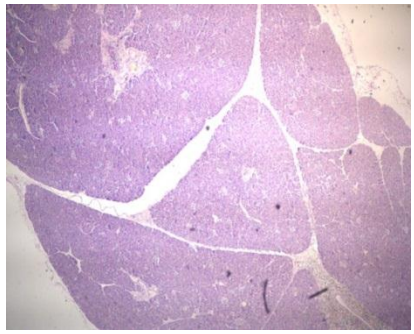

Fig. 6. Pancreatic lobes. PAS stain; X40

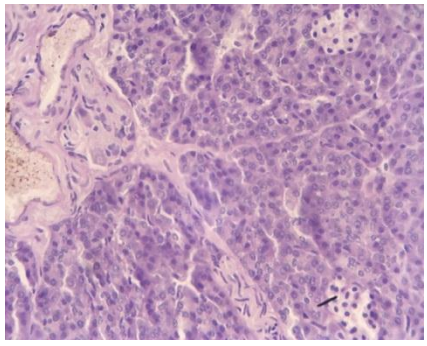

Fig. 7. Pancreatic lobes. PAS stain; X400

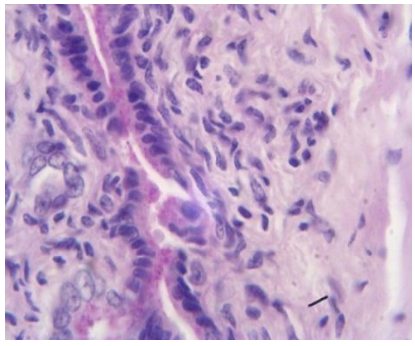

Fig. 8. Intralobular duct. PAS stain; x 1000

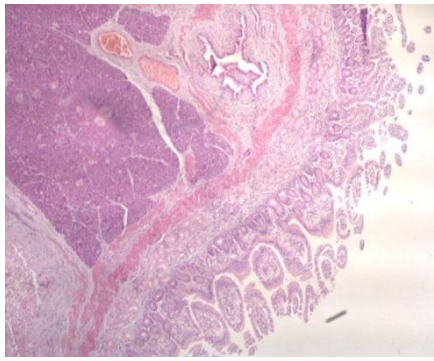

Fig. 9. Wirsung's duct. HEA stain; x 40

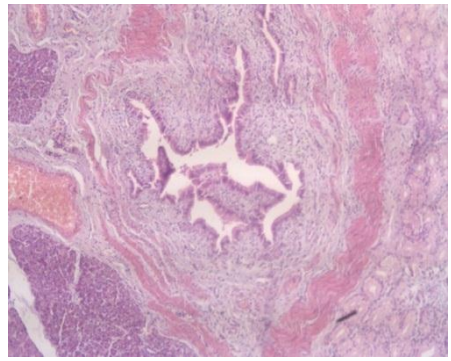

Fig. 10. Wirsung.'s duct. HEA stain; x 100

Conclusions

The pancreas has the same shape and aspect as in domestic carnivore:

- the 'V' shaped with a notch (*Incisaura pancreatic*);
- the topography with two lobes similar to those at the dog;
- the present of the two pancreatic duts, *Wirsun* and *Santorini*
- the main pancreatic duct opens mutually with the common bile duct at the level of the major papilla

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MORPHOLOGIC PARTICULARITIES OF THE STOMACH OF THE BROWN BEAR (*URSUS ARCTOS*)

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Abstract
The brown bear (Ursus arctos) belongs to the Carnivora order, being adapted to an omnivorous diet. The stomach consists of a single cavity, with an external conformation similar to that of the dog stomach, pear-like shape, prominent bottom, a moderately profound cardiac incisure and deep angular incisure. As for its conformation on the inside, the stomach has a rather small surface of cardiac mucosa, the remaining surface consisting of fundic and pyloric mucosa (glandular mucosa). Its musculature at cardiac level is less developed than the one in the zone of the pyloric orifice, which also presents a pyloric torus.
Keywords: stomach, anatomy, histology, *Ursus arctos*

Materials and methods

The adult bear was a female bear, from the zoo who die from complication do to the infestation of internal parasites, and the 4 month cub die from unknown cause.

The time between the death of the bears and collection and fixation of tissues was of 2–12 h. Histological sections was 5 μm thick, stained used were Van Gieson for anatomic microarchitecture.

Results and discussions

The brown bear stomach (*Gaster-Ventriculus*) is a single cavity type. Its shape can variate depending by the depending on the degree of fullness as well as the domestic carnivores. On the outside appears white to yellowish and relatively small size compared to the body weight. He looks like a bag curved, "C"- shaped, slightly flattened anteroposterior and presents for description two faces, two curves and two openings (fig. 3, fig. 4).

Both sides, parietal (*Facies parietalis*) and visceral (*Facies visceralis*), are smooth, convex, covered by blood vessels flexuase.

Lesser curvature, (*Cuvatura ventriculi minor*),is concave, positioned dorsal and to the right. It begins and ends at the cardia to pylorus. Angular notch (*Incisura angularis*) that limits the pyloric portion (*Pars pylorica*) is profound. From the lesser curvature detaches hepato-gastric ligament (*Lig. hepatogastricum*), part of small omentum.

Greater curvature (*Curvatura ventriculi major*) is convex, more elongated oriented ventral and left, joining the left edge of the cardic region with the ventral part of the pyloric region. Pyloric region is narrow and dorsally oriented, and because of the lesser curvature of concave shape causes the close-up to the cardiac region.

The bottom sac of the stomach (*Saccus cecus ventriculi*), is highly developed and clearly in a state of fullness with relatively thin walls (fig. 4).

Cardiac notch (*Incisura cardiaca*) is shallow and marks the opening of the esophagus into the stomach. The sizes for small and large curvature are 30 and 70 cm in a state of wholeness at the adult bear.

From the greater curvature detaches the great omentum (*Omentum majus*), which consists of two strips, highly developed, loaded with fat, especially in the period preceding hibernation (fig.2).

In terms of macroscopical, the type of the stomach mucosa, can be distinguish in two areas. Main area of gray, is occupied by fundic glands and covers about two thirds of the inner surface. The other portion, occupying a third, lighter and closer to the pylorus, contains pyloric glands. Not distinguish the macroscopical cardiei (Pars heart). The cardiac region (*Pars cardiaca*) cannot be distinguished (fig.5).

Pyloric muscle is well developed and form a strong locking system, evident in wall thickness in this area. At the end of the pyloric canal (*Canalis pyloricus*), the pyloric opening (*Ostium pyloricum*) provide a torus pyloric (*Torus pyloricus*), a mucous plug pedicle, currently less developed than the one present at the pigs (fig. 6, fig. 7).

Topographic, the stomach was observed in the state of fullness, distended, in contact with the ventral abdominal wall, just caudal from hypochondrium and xiphoid cartilage. We conclude that without content, the stomach is projected only intrathoracic portion of the abdominal cavity.

Lesser omentum (*Omentum minus*) is detached from the lesser curvature, of which consists of hepato-duodenal ligament (*Lig. hepatoduodenale*) and hepato-gastric (*Lig. hepatogastricum*) (fig.3).

The greater omentum (*Omentum majus*) detached from the greater curvature of the stomach, stretching from near the bottom sac of the stomach to the pyloric region and continue up to the duodenum. One sheet of the grear omentum forms the gastro- splenic ligament (*Lig. gastrolienale*), extending to the splenic hilum.

The greater omentum is partially covering the cranial portion of the stomach and intestinal mass, inserting on the transverse colon (*Colon transversum*). *Jejunum* is in contact with the caudal flanks walls and ventral wall of the caudal half of the abdominal cavity (fig.1, fig. 2, fig. 3, fig. 4).

Gastro-phrenic ligament (*Lig. gastrophrenicum*) is very short, thick and strong. It includes among its foils the abdominal portion of the esophagus, and its being attached on the bottom sac of the stomach, that correspond to it.

Mucosa has many folds that are oriented longitudinally in the fundic region (fig.5). On examination under the magnifying glass are observed numerous gastric pits resulting from clogging of the surface epithelium. In the bottom of these crypts opens fundic glands that are present in the *lamina propria.*

The esophageal region is nonglandular portion of the stomach lined by squamous epithelium, light keratinezed (fig. 8, fig. 9).

The cardiac glands are branched, tubular an coiled.The neck of each is nearest the gastric pit opening.The nech and the upper portion of the body are lined by mucus-secreting cuboidal cells. The reamining cells are columnar and mucus-secreting. There are parietal cells in this region (fig. 10).

The fundic gland region is similar to the cardiac gland region.The gland are proper gastric glands- branched, tubular and longer that cardiac region glands. Fundic gland lenght tickens the *lamina propria mucosae*.

A fundic gland is divisible into isthmus, neck, body and base.The isthmus continues into the constricted neck. The body continues from the neck and terminates as a dilated and bent adenomere, the base.The same three types of cells were present in the structure : mucous neck, chief and parietal.

Mucous neck cells are cuboidal with a pale-stained cytoplasm.Chief cells are pyramid –shaped with a basally positioned round nucleus. Parietal cells are large cells, their spheroidal or pyramidal shape with round nucleus (fig.12, fig. 13).

Pyloric gland region is similar to that od the cardiac region, the pits are deeper that other region of the stomach, but the pyloric glands are similar to the cardiac glans, shorter, simple. A well-developed inner circular lamina of the tunica muscularis is a striking feature; it forms the pyloric sphincter at the gastroduodenal junction, very developed (fig.14, fig. 15, fig. 16, fig. 17).

The *tela submucosa*, *tela muscularis* and *serosa* are typical.

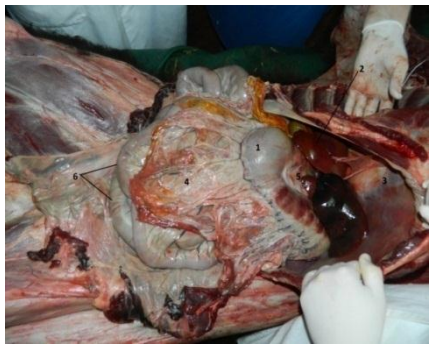

Fig. 1. The stomach ” C”-shaped, behind the diaphragm and liver: 1- gaster; 2- hepar; 3- diaphragma; 4- omentum majus 5- omentum minus; 6- jejunum

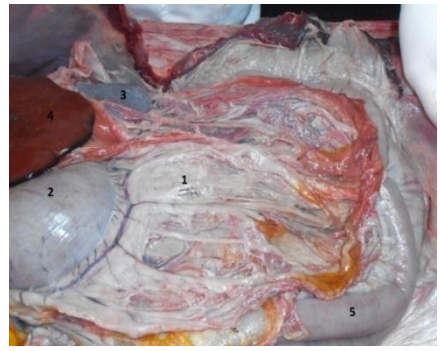

Fig. 2. Greater omentum : 1- omentum majus; 2- gaster; 3- lien; 4- hepar; 5- jejunum

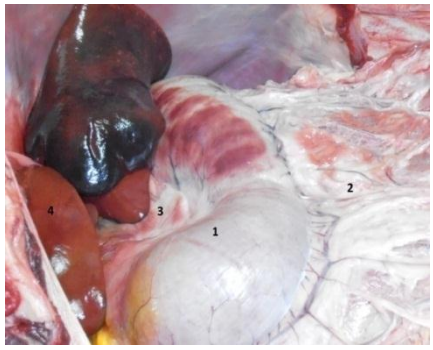

Fig. 3. The stomach on the parietal surface : 1-gaster; 2- omentum majus; 3- omenutum minus; 4- hepar

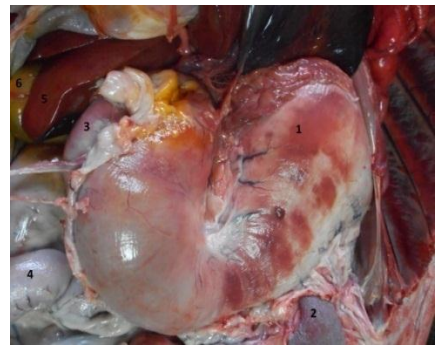

Fig. 4. The stomach “C”-shaped : 1-gaster; 2- lien; 3- duodenum, pars descendens; 4- jejunum; 5- hepar; 6- vesica fellea

Fig. 5. The mucosa of the stomach : 1-esophagus; 2- ostium cardiacum; 3- corpus ventriculi; 4-antrum pyloricum; 5-ostium pyloricum; 6-duodenum, pars cranialis

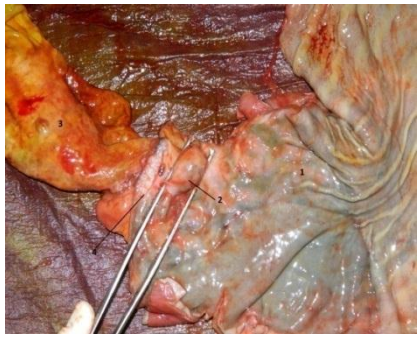

Fig. 6. Pyloric region: 1-antrum pyloricum; 2-torus pyloricus; 3- duodenum, pars cranialis; 4-ostium pyloricum

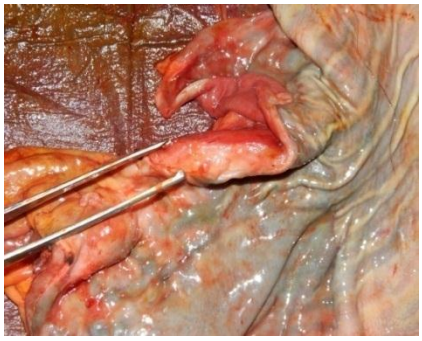

Fig. 7. The pyloric wall

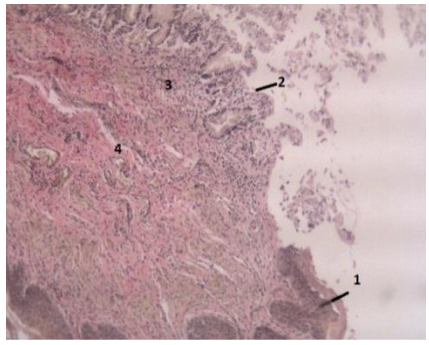

Fig. 8. Jonction,esophagus and cardiac gland region: 1- satratified squamous, epithelium keratinized, esophagus; 2- faveolae gastricae ; 3- lamina propria; 4-muscularis mucosae; Van Giesson stain; x 40

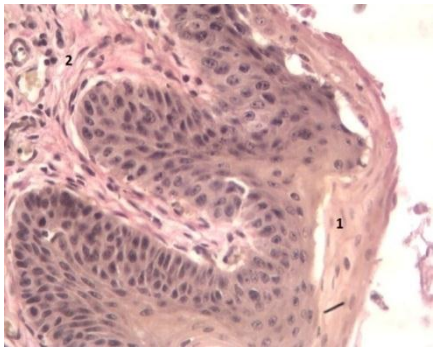

Fig. 9. Stratified squamous epithelium, light keratinized, in the cardiac region:1- stratified squamous, epithelium keratinized esophagus; 2-lamina propria; Van Gieson stain; x 400

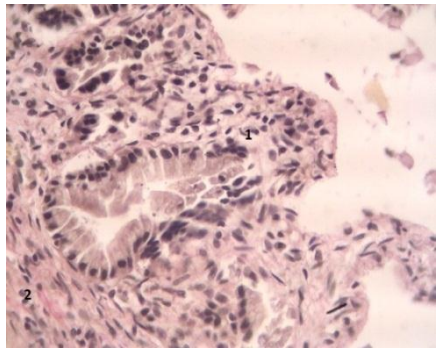

Fig. 10. Columnar epithelium covers the surface of the mucous membranes in the cardiac region: 1- faveolae gastricae; 2- lamina propria. Van Gieson stain; x 400

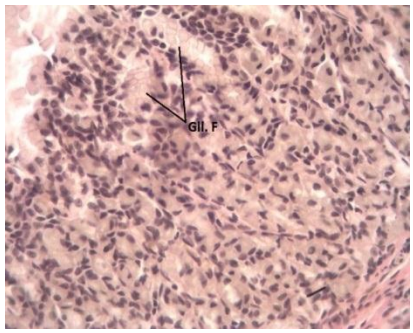

Fig. 11. Fundic glands . Van Gieson stain; x 400

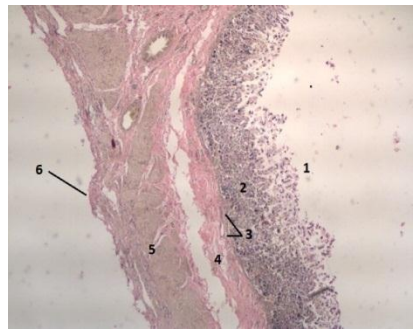

Fig. 12. Fundic region of the stomach: 1-gastric pit; 2- lamina propria; 3- muscularis mucosae; 4- tela submucosa; 5- tela muscularis ; 6- serosa. Van Gieson stain; x 40

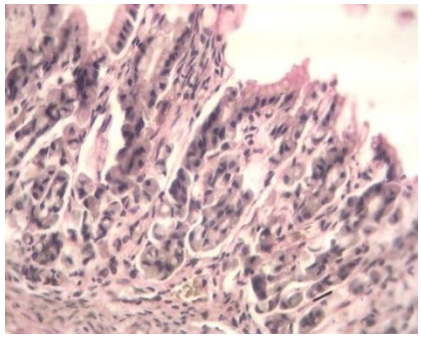

Fig.13. Gatric pits of the fundic region. Van Gieson stain; x 400

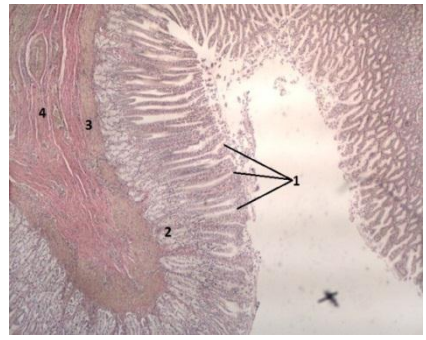

Fig. 14. The mucous coat in the pyloric region: 1-gastric pit; 2- lamina propria with pyloric glands; 3- muscularis mucosae; 4- tela submucosa. Van Gieson stain; x 40

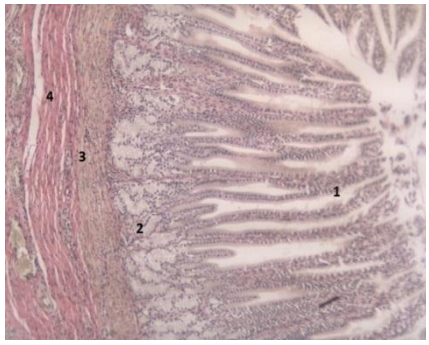

Fig. 15. Gastric pits in the pyloric region: 1-gastric pit; 2- lamina propria with pyloric glands; 3- muscularis mucosae; 4- tela submucosa. Van Gieson stain; x 100

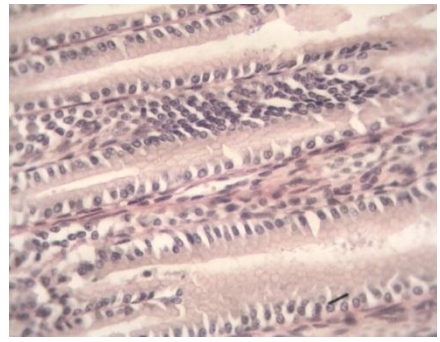

Fig. 16. Gastric pits in the pyloric region. Van Gieson stain; x 400

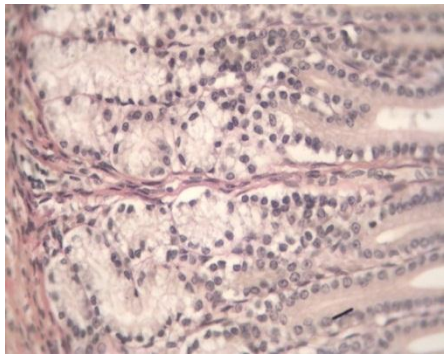

Fig. 17. Pyloric glands. Van Gieson stain; x 400

Conclusions

1. The stomach shape can variate depending by the depending on the degree of fullness as well as the domestic carnivores.
2. He looks like a bag curved, "C"- shaped.
3. In terms of macroscopical, the type of the stomach mucosa, can be distinguish in two areas, fundic and pyloric.
4. Pyloric muscle is well developed and form a strong locking system, evident in wall thickness in this area. At the end of the pyloric canal (*Canalis pyloricus*), the pyloric opening (*Ostium pyloricum*) provide a torus pyloric (*Torus pyloricus*).
5. The cardiac glands are branched, tubular an coiled.
6. A well-developed inner circular lamina of the tunica muscularis is a striking feature; it forms the pyloric sphincter at the gastroduodenal junction, very developed.

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MORPHOLOGIC PARTICULARITIES OF THE POST-DIAPHRAGMATIC DIGESTIVE TRACT OF THE BROWN BEAR (*URSUS ARCTOS*)

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Abstract
The brown bear (Ursus arctos) belongs to the Carnivora order, being adapted to an omnivorous diet. The duodenum presents the same topography as found in dog. In the proximal portion of the descending duodenum the minor and major papilla are placed, where the common bile duct and main pancreatic duct open on the surface of the major papilla, and the accessory pancreatic duct opens at the level of the minor papilla. The jejunum is the longest segment of the digestive tract, covering almost the entire bottom of the abdominal cavity, suspended by the developed great mesentery, which grants it increased mobility. The absence of the cecum from the digestive tract is a characteristic more akin to a carnivore. The colon is structured in three parts: ascending, transverse and descending, its topography being similar to the one found in dog; its shape is that of an U, and the opening is placed caudally. The digestive tract of the brown bear presents the same topography as found in dog, while being proportionally longer.
Keywords: digestive tract, anatomy, topography, *Ursus arctos*

Materials and methods

The adult bear was a female bear, from the zoo who die from complication do to the infestation of internal parasites, and the 4 month cub die from unknown cause.

The time between the death of the bears and collection and fixation of tissues was of 2–12 h. Histological sections was 5 μm thick, theused were PAS and HEA for anatomic microarchitecture.

Results and discussions

Stomach

The brown bear stomach (*Gaster-Ventriculus*) is a single cavity type. Its shape can variate depending by the depending on the degree of fullness as well as the domestic carnivores. On the outside appears white to yellowish and relatively small size compared to the body weight. He looks like a bag curved, "C"- shaped, slightly flattened anteroposterior (fig.1, fig. 2).

Both sides, parietal (*Facies parietalis*) and visceral fat (*Facies visceralis*), are smooth, convex, covered by blood vessels flexuase.

Lesser curvature (*Cuvatura ventriculi minor*) is concave, positioned dorsal and to the right. It begins and ends at the cardia to pylorus. Angular notch (*Incisura angularis*) that limits the pyloric portion (*Pars pylorica*) is profound. From the lesser curvature detaches hepato-gastric ligament (*Lig. hepatogastricum*), part of small omentum.

Greater curvature (*Curvatura ventriculi major*) is convex, more elongated oriented ventral and left, joining the left edge of the cardic region with the ventral part of the pyloric region. Pyloric region is narrow and dorsally oriented, and because of the lesser curvature of concave shape causes the close-up to the cardiac region.

The bottom sac of the stomach (*Saccus cecus ventriculi*), is highly developed and clearly in a state of fullness with relatively thin walls.

Cardiac notch (*Incisura cardiaca*) is shallow and marks the opening of the esophagus into the stomach. The sizes for small and large curvature are 30 and 70 cm in a state of wholeness at the adult bear.

In terms of macroscopical, the type of the stomach mucosa, can be distinguish in two areas. Main area of gray, is occupied by fundic glands and covers about two thirds of the inner surface. The other portion, occupying a third, lighter and closer to the pylorus, contains pyloric glands. Not distinguish the macroscopical cardiei (Pars heart). The cardiac region (*Pars cardiaca*) cannot be distinguished (fig. 3, fig.4).

Pyloric muscle is well developed and form a strong locking system, evident in wall thickness in this area. At the end of the pyloric canal (*Canalis pyloricus*), the pyloric opening (*Ostium pyloricum*) provide a torus pyloric (*Torus pyloricus*), a mucous plug pedicle, currently less developed than the one present at the pigs (fig. 4, fig. 5).

Topographic, the stomach was observed in the state of fullness, distended, in contact with the ventral abdominal wall, just caudal from hypochondrium and xiphoid cartilage. We conclude that without content, the stomach is projected only intrathoracic portion of the abdominal cavity.

Mucosa has many folds that are oriented longitudinally in the fundic region. On examination under the magnifying glass are observed numerous gastric pits resulting from clogging of the surface epithelium. In the bottom of these crypts opens fundic glands that are present in the *lamina propria* (fig.3, fig. 4).

Intestinum

Duodenum is the first segment of the small intestine (*Intestinum tenuate*) and measures about 60-70 cm long, starting from the pyloric orifice to the duodeno-jejunal curvature, where it continues with *jejunum*. It is divided into four parts: cranial, descending, transverse and ascending.

Cranial portion (*Pars cranialis*) starts with an expansion,(*Ampulla duodeni*), in contact with the visceral surface of the liver. Has a length of about 10 cm until the cranial curvature of the *duonenum (Flexura duodeni cranialis)* (fig. 6).

Descendent portion (*Pars descendens*) is oriented downward on the right side of the abdominal cavity, reaching the fifth lumbar vertebra. Is related to the right lobe of the pancreas, caudal to the right lobe of the liver, between the right flank wall and the great omentum, which separates him from the ascending colon and jejunum. In this portion , the mucosa structure the large duodenal papilla (*Papilla duodeni major*) and small duodenal papilla (*Papilla duodeni minor*). The major papilla marks the opening of the bile (*Ductus choledochus*) and prime pancreatic ducts (*Ductus pancreaticus), Wirsung*'s duct. The small duodenal papilla marks the opening of accessory pancreatic duct (*Ductus pancreaticus accessorius*), *Santorini*'s duct (fig. 6, fig. 7, fig. 8, fig. 9).

Transvers portion (*Pars transversa s. Pars caudalis*) corresponds to the caudal curvature and measure approximately 15 cm and is oriented from right to left, without passing the median plane, at the same lavel with the right kidney (fig. 9).

Ascending portion (*Pars ascendens*) is oriented parallel to the descending portion of the *duodenum*, till the duodeno-jejunal curvature (*Flexura duodenojejunalis*), but it is shorter that this portion, in contact with the the medial side of the descending colon (fig. 9).

Jejunum and *ileum* are described as one segment, due to the impossibility of defining the exact length of the two segments because, the *cecum* is absent. This segment has a length of 8-10 m; is suspended by the great mesentery and separated from the ventral abdominal wall by interposing of the great omentum, only in the cranial portion of the abdominal cavity (fig.1, fig. 2, fig. 7, fig. 11)

The small intestine has a thick mucosa, smooth and gray red colored. Along the lenght, there are longitudinal folds that disappear at the distension of the organ.The mucosa strucures the large and small papilla, in the descending portion of the duodenum also in the jejunum, its strucured circular folds- callded cornivete valves (fig. 21).

The lamina epithelialis mucosae consist of lining cells, goblet cells, gastrointestinal endocrine cells and M cells. The lining cells are typical columnar epithelial cells whose apical borders have many microvilli arranged in orderly fashion(fig. 12. fig.13, fig. 14).

Epithelium lining in the small intestine is simple, prismatic, with digitiform appearance because of intestinal villous (fig. 12, fig 13, fig. 20) .

Intestinal crypts open at the base of each villus as simple, branched, tubular invagination. The epithelium consists of columnar lining, goblet, gastrointestinal endocrine and acidophilic granule (Paneth) cells (fig. 15).

The tela submucosa is typical. Submucosal glands are simple, branched, tubuloacinar glands opening into crypts (Brünner's glands) (fig 16, fig. 17).

The tunica muscularis with myenteric plexus is typical. This smooth muscle contraction pdoduces peristalsis (fig 18, fig.19).

Cecum is absent and all that it is notable is a change of calibre at the transition from the small intestine to the large intestine.

Colon is reduced to approximately 1.2 to 1.5 m long (fig. 11)

It is structured in three parts: ascending, transverse and descending.

Colon ascendens is routed to the pylorus, bordering with the medial descending duodenum, from who it is separately by the great omentum. Is in contact with the right lobe of the pancreas, and dorsal with the right kidney.

Transverse colon (*Colon transversum*) is short, directed from right to left, passing the great root of the mesentery and offers surface of insertion for the greater omentum .

Descending colon (*Colon descendens*) is the longest segment and has a straight trajectory. Spleen is in contact with the cranial portion and the medial portion is in contact with ascending part of duodenum (fig.10).

Rectum has a length of 15 cm and a diameter of 3 cm. Has numerous transverse folds (fig. 11).

Anal canal (*Canalis analis*) is short, only 2 cm long. Around the anus there are two perianal sinuses with anal glands.

Histological, the large intestine is characterized by: the absence of intestinal villi; the presence of a large number of goblet cells; intestinal crypts, which are elongated and straight, open to the surface at the luminal margin; acidophilic granule cells are absent (fig. 22, fig. 23, fig. 24, fig. 25, fig. 26).

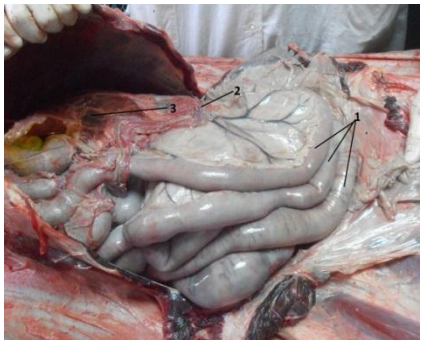

Fig. 1. Abdominal cavity: 1- jejunum; 2- omentum majus; 3- hepar

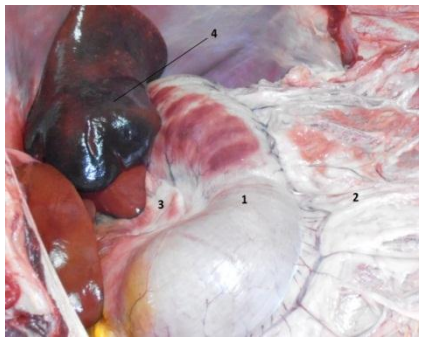

Fig. 2. The stomach on the parietal surface : 1- gaster; 2- omentum majus; 3- omentum minus; 4-

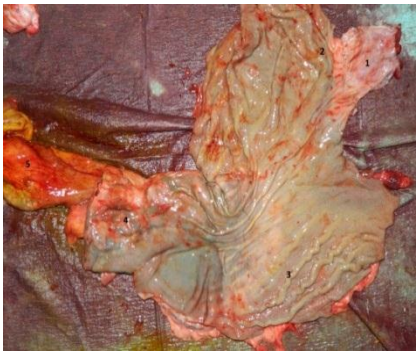

Fig. 3. The mocosa of the stomach: 1- esophagus; 2 –pars cardiaca; 3- corpus ventriculi; 4- pars pylorica; 5- duodenum, par cranialis

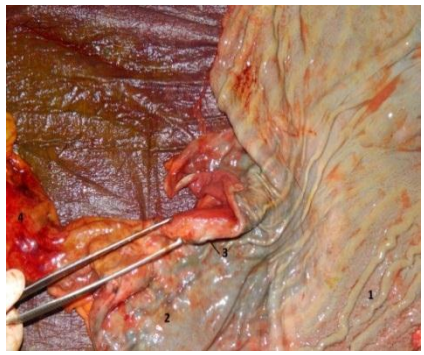

Fig. 4. Section through the pyloric region: 1- corpus ventriculi; 2- pars pyloric; 3- ostium pyloricus; 4- duodenum, pars cranialis

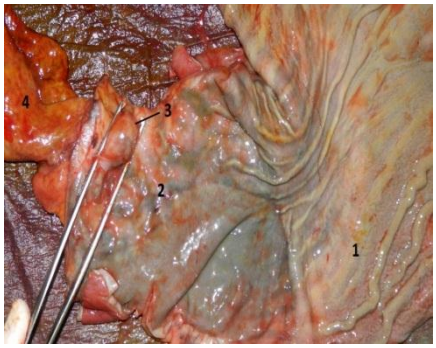

Fig. 5. The torus pyloricus: 1- corpus ventriculi; 2- pars pylorica; 3- torus pyloricus; 4- duodenum, pars cranialis

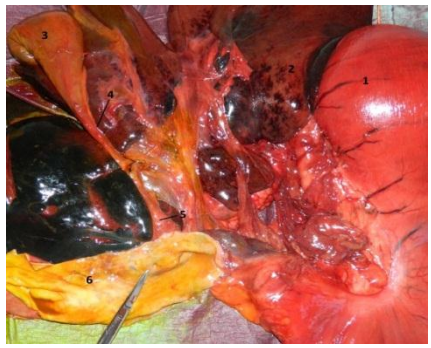

Fig. 6. The opening of the bile and pancreatic ducts: 1- gaster; 2- hepar; 3- vesica fellea; 4- ductus cysticus; 5- ductus choledochus; 6- duodenum, pars descendens

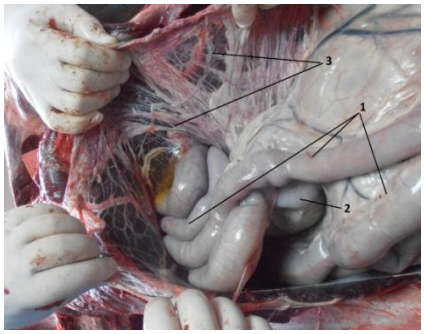

Fig. 7. Greater omentum : 1- jejunum; 2- doundenum, pars descendens; 3- omentum majus

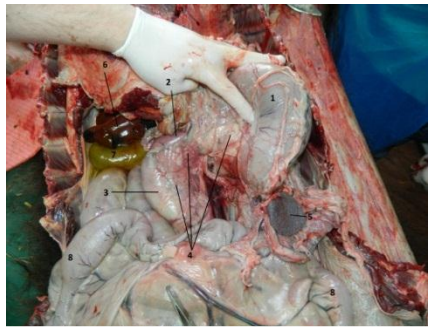

Fig. 8. Abdominal cavity, dorsal view: 1- gaster; 2- duodenum, pars cranialis; 3- duodenum, pars descendens; 4- pancreas; 5- lien; 6- hepar; 7- vesica fellea; 8- jejunum

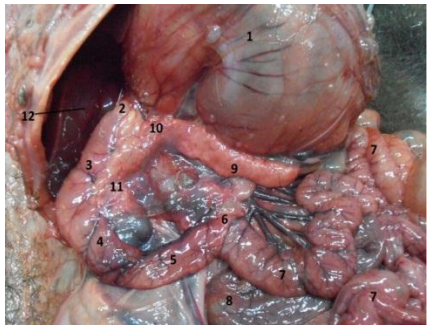

Fig. 9. The small intestine at a bear cub, 4 months old: 1- gaster; 2,3,4,5- duodenum (2- pars cranialis; 3- pars descendent; 4- pars transversa; 5- pars ascendens); 6- flexura duodenojejunalis; 7-jejunum; 8- colon; 9,10,11- pancreas (9- lobus sinister ; 10- corpus; 11- lobus dexter); 12 – hepar

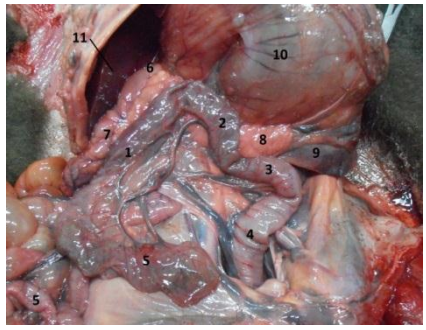

Fig. 10. The large intestine at a cub, 4 monthhs old: 1- colon ascendens; 2- colon transversum; 3- colon descendens; 4- rectum; 5- jejunum; 6- duodenum,pars cranialis; 7- duodenum, pars descendens; 8- pancreas; 9- lien; 10- gaster; 11- hepar

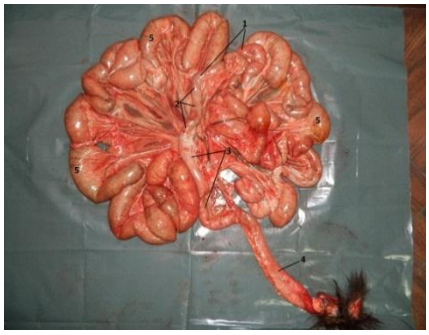

Fig. 11. The large intestine of an adult bear: 1- colon ascendens; 2 - colon transversum; 3- colon descendens; 4- rectum; 5- jejunum

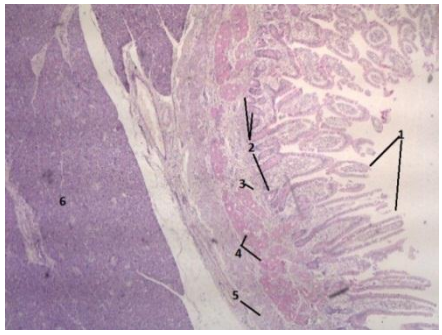

Fig. 12. The duoden near the pancreas :1- vilus; 2- Crypt of Lieberkühn; 3- muscularis mucosae; 4- Brünner' glands; 5- tela muscularis; 6- pancreas. PAS stain; x 40

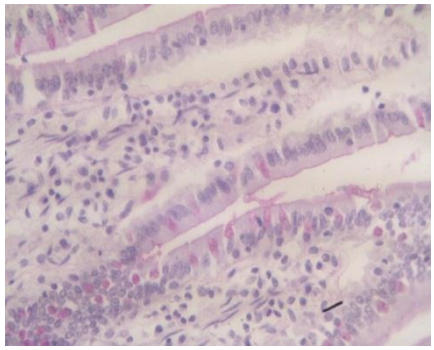

Fig. 13. Vilus from duodenum. PAS stain; x 400

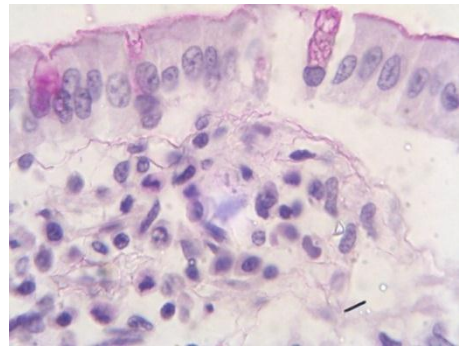

Fig. 14. Columnar epithelium of the vilus from duodenum. PAS stain; x 1000

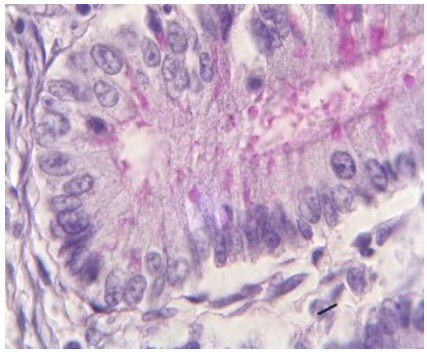

Fig. 15. Crypt of Lieberkühn from duodenum. PAS stain; x 1000

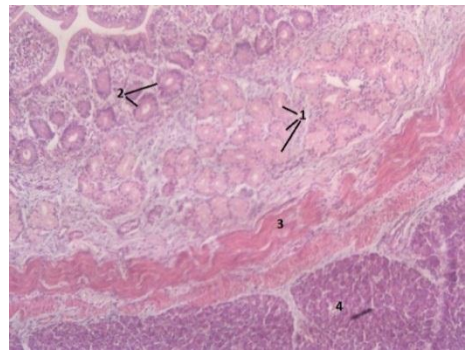

Fig. 16. Brünner' glands: 1- Crypt of Lieberkühn; 2- Brünner' glands; 3- tela muscularis; 4 – pancreas. HEA stain; x 100

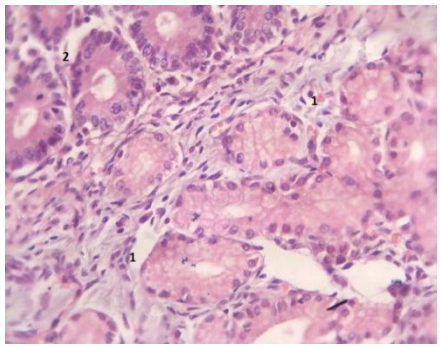

Fig. 17. Brünner' glands: 1- Brünner' glands; 2- Crypt of Lieberkühn. HEA stain ; x 400

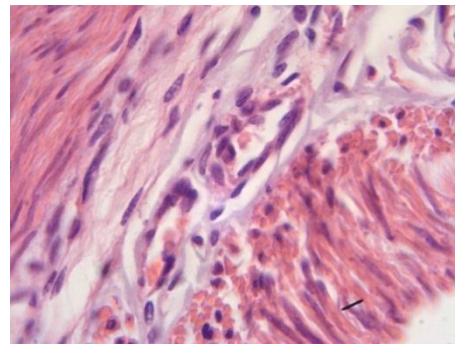

Fig. 18. Muscularis externa and interna from duodenum. HEA stain; x 1000

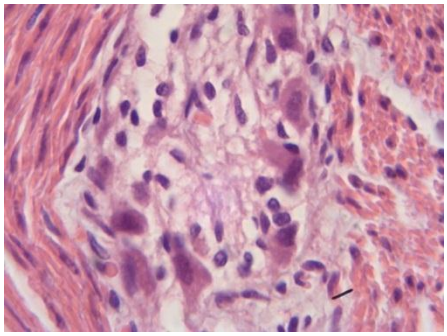

Fig. 19. Auerbach' s plexus. HEA stain; x 1000

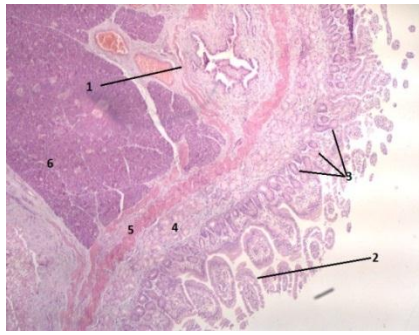

Fig. 20. Wirsung.'s duct: 1- Wirsung.'s duct; 2- vilus; 3- Crypt of Lieberkühn; 4- Brünner' glands; 5- tela muscularis; 6- pancreas. HEA stain; x 1000

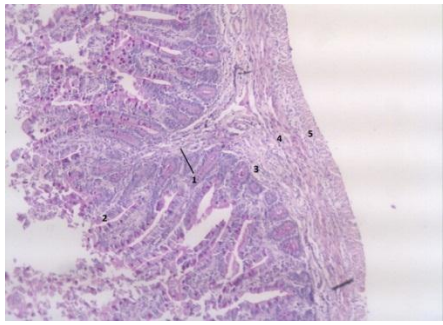

Fig. 21. Plicae circulares or Kerckring's folds: 1 - plicae circulares ; 2- vilus; 3- muscularis 4- tela submu ; 5- tela muscularis, PAS stain; x 100

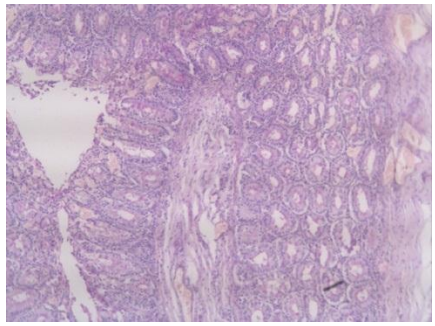

Fig. 22. Crypt of Lieberkühn from colon. PAS stain; x 100

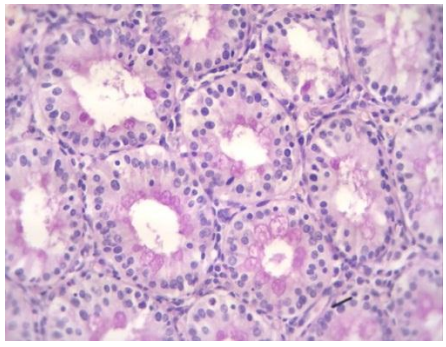

Fig. 23. Crypt of Lieberkühn from colon. PAS stain; x 400

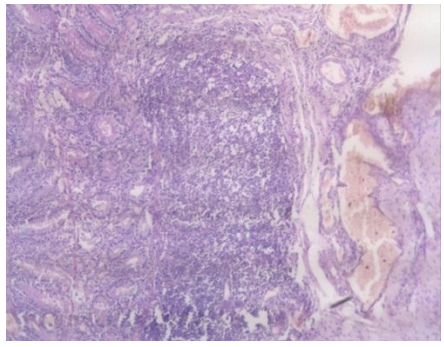

Fig. 24. Lymphatic nodule from colon. PAS stain; x 100

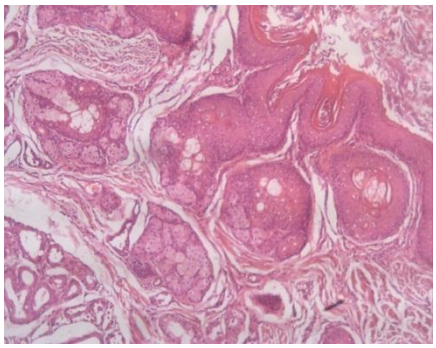

Fig. 25. Glands of the anal sac. PAS stain ; x 100

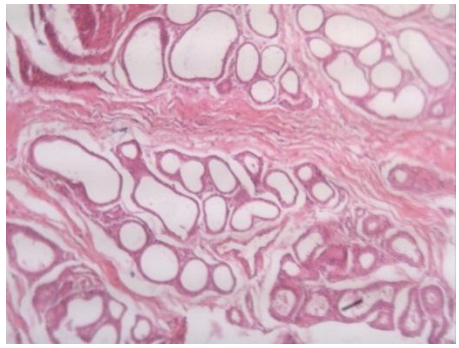

Fig. 26. Glands of the anal sac. PAS stain ; x 100

Conclusions

1. The stomach looks like a bag curved, "C"- shaped.
2. In terms of macroscopical, the type of the stomach mucosa, can be distinguish in two areas, fundic and pyloric.
3. Pyloric muscle is well developed and form a strong locking system, evident in wall thickness in this area. At the end of the pyloric canal (*Canalis pyloricus*), the pyloric opening (*Ostium pyloricum*) provide a torus pyloric (*Torus pyloricus*).
4. The duodenum has the same topography like the dog , a "U" shaped aspect.
5. *Jejunum* has a length of 8-10 m, is suspended by the great mesentery and separated from the ventral abdominal wall by interposing of the great omentum, only in the cranial portion of the abdominal cavity.
6. *Cecum* is absent.
7. Colon as a simple topographic,mainly like in the domestic carnivores.

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THE ORGANIC SELENIUM (SEL-PLEX) AND BIO-MOS PREBIOTIC ACTION ON PREGNANT SOWS ON PREVENTION OF NEONATAL DIARRHEA IN PIGLETS

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Abstract
The main goal of this study is to improve animals' health by using supplements based on probiotics and organic minerals. Here are presented data about the role of organic selenium and probiotic obtained on the basis of yeast culture (Sacharomyces cerevisiae) used in feeding of pregnant sows and their offspring. The benefits of using organic selenium (Sel - Plex), in the proportion of 0.5 kg / ton and of prebiotic Bio - Mos in proportions of 1 kg / ton feed, which has positive effects on laboratory and reproductive indices in pregnant sows in the last period of gestation and piglets during growth period are also presented. The positive action of the products is shown at the level of haematopoiesis by increasing erythrocytes and hemoglobin (P<0.05,) significant increase (P <0.01) of lymphocytes; reduction of AST activity (P <0.001) and diminution of urea concentration in plasma.
Key words: suckling piglets, Sel-Plex, Bio-Mos, neonatal diarrhea, profilaxy

Introduction

Ensuring animals' health, environment protection and food security are the dominant goals of the veterinary service. Investigations conducted in recent decades have shown that nowadays obtaining animal products contaminated with nitrates, nitrites, various pesticides, chemotherapuetic agents (antibiotics, anthelmintics, ectocide) presents one of the most important challenges facing humanity.

Today it is necessary to find solutions for improvement of animal health by increasing natural resistance to diseases thus avoiding the use of antibiotics. Our previous researches conducted in industrial conditions of swine breeding have clearly shown that organic selenium (Sel-Plex) due to the increased bioavailability and its biological activity, has important advantages in comparison with inorganic forms (such as sodium selenite) [1,2,3]. It has been found that supplementation of pregnant sows basic ration before parturition and during lactation period with organic Selenium improves animal performance and also have a positive influence on their health. Mortality losses in the experimental group from birth until weaning were lower (7.8% of actual) comparing to the control group (18%) [2].

In scientific literature we often find statements that the only way to combat antibiotic biopersistence is to develop new products, more potent and broader spectrum. But should remember that, trying to find the ideal product "that would combat super microorganisms" we cannot get away from "reality". Instead of „overcome" or reduce antibiotic resistance it is necessary only to decrease the number of recommendations of antibiotics [10]

Currently, according the new European context great efforts are made to substitute antibiotics with natural growth promoters, such as acids, prebiotics, probiotics, enzymes, etc. Thus, the goal is to correct and maintain an optimal intestinal environment for digestion and assimilation of nutrients.

Materials and methods

The research was conducted at the swine complex, SRLVerjecom on 7 pregnant sows and their offspring. The animals were divided into two similar groups: experimental group (1)-4 sows. Basic ration composition was given (by mixture of feed preparation unit) - Sel-Plex product containing organic proportion of 0, 5 кg / ton (50 g to 100 кg feed), and Bio-Mos product in proportions of 1 кg / tonne (100 g to 100 кg feed). Feed bags were assigned to manage manually, ranging from 20 - 21 day before and to day 42 after parturition.

Group II, witness consisted of 3 sows which were fed the same feed, except Sel-Plex and Bio-Mos.

Both groups of animals were maintained in the same compartment arranged with the similar technology, fully observing the requirements of microclimate conditions, feeding, watering and open space.

Basic ration per-total of 100 кg included in%: corn- 17%, barley-24% wheat-20%, wheat bran-10%, sunflower meal 10%, soybean meal-12%, meat-bone meal-5%, premix-1% sodium chloride-0, 3%, chalk feed-0, 7%.

Sows in both groups during first 7 days received daily fodder in two or three portions at about 2 to 2.5 кg per animal. it varied from-8th-42 up to day 5.4 кg.

Sel-Plex and Bio-Mos action on sows was monitored by assessing clinical status and indices such as bio, hematological and biochemical.

Body weight was measured by the gravimetric method on 1st, 10, 20 and 40 days of life.

Results and discussions

Supplementation of the basic ration of sows in the experimental group with organic selenium (Sel-Plex), 0.5кg/t of feed, and Bio-Mos, 1 kg /t of feed, during the 19 to 21days before and 42 days after parturition was resulted in the shortening of parturition period to 29.7 minutes and to the absence of any complications in the puerperal period. At sows in the control group, witch basic ration wasn't supplemented with probiotics during ante and post partum, parturition was significantly more laborious and in puerperium one of sow showed clinical symptoms of endometritis, and the other two had post partum genitalia involution period with 4 and 6 days longer respectively than in the experimental group of animals.

In the first days after parturition sows in both groups were clinically healthy. They showed care to offspring, consumed food and water, although the appetite was diminished. On average each sow has eaten in the first day after parturition 2.25 kg of food offered in dry form. On subsequent days the animals were healthy. Body temperature was in physiological limits and the appetite was increasing from day to day and from 6 - 7 day to 42 after parturition each sow from both groups consumed from 4.5 to 5.4 кg of food daily.

Basic ration for pigs from both groups in periods pre and post partum was identical, according to data from the literature (Gh.Pârvu et al, 2003). Digestible protein content in lactating sows ration made up 16.5%. Other ingredients - energy, minerals, vitamins were in accordance with current standards. The exception was only two prebiotics,Sel-Plex and Bio-Mos, which were administered only by animals from experimental group. Monitoring results of prebiotic action on general status and piglet growth and development are shown in Table 1.

Table 1. Action of organic selenium (Sel-Plex) and Bio-Mos products on morbidity, mortality and indices bio

Indices, units	***Group***		***Experimental group in % from control group***
	Control	**Experimental**	
	n M±n	**n M±n**	
Body weight of one piglet after birth, kg	33 1,148±0,90	42 1,265±0,06	110,19%
Number of piglets at the age of 40 days	29	42	
% storage	29 87,9%	42 100%	113,77%
Body weight at the age of 10 days 20 days 40 days	 33 3,950±0,48 32 5,93±0,84 29 9,34±1,68	 42 3,47±0,36 42 5,99±0,47 42 10,04±0,97	 87,85% 101,01% 107,49%
Total increase, kg	8,192	8,775	107,12
Medium daily increase, kg	0,205	0,219	106,83
Fodder costs / head / time / kg	280	254	90,31
Specific consumption of feed, kg / kg of weight gain	4,67	4,23	90,65

Data obtained in the research shows that the use of organic selenium (Sel-Plex) and prebiotic Bio-Mos has a favorable impact on clinical indicators, bioproduction of piglets, total weight gain and average daily weight gain. In the first day of life piglets from both groups weighed on average1.265 + 0.006 kg in experimental group and 1.148 + 0.90 kg in control group. At the age of 10 days body weight in piglets constituted 3.95 + 0.48 kg and 3.46 + 0.43 kg in the control and experimental groups respectively. Piglets in both groups were growing evenly. However, piglets from the control group during this period had a surplus of 49 g per head higher. At the age of 20 days the difference between groups was insignificant which also consists of only 9 g surplus piglets in the experimental group.

The tendency to better development preserved during the period from 20 to 40 days which ended with a surplus equal to 0.700 kg body weight ($P < 0.05$) in piglets in the experimental group. The positive effect of supplementing the diet with organic selenium and Bio-Mos on growth and development of piglets is confirmed by the percentage of diseases in studied groups of animals during calving and until parturition.

It was found that the maintenance of newborn piglets in the newly built rooms which are better prepared for parturition (disinfected, good ventilation system, etc.) and using a well-formed formulas of feeding in our opinion resulted in a lack of cases of diseases and gastrointestinal dysfunctions in newborns. Therefore the mortality rate was about 12% of pigs in control group.

At the age of 42 days there were retained 87.9% of piglets from the control group and 100% from the group 1 - experimental. Concomitant increase in total and average daily gain of piglets in experimental group was 7.12% higher than in the control group. Specific fodder consumption decreased from 4.67 kg to 1 kg of weight gain in the control group to 4.23 kg in the experimental group, or 9.35%.

Table 2 presents the results of investigation of the blood system in piglets from both groups.

Table 2. Hematological indices in piglets

INDICES	GROUP	INVESTIGATIONS			
		I 7nd day		II 32nd day	
		n	M±n	n	M±n
Hemoglobin g/L	I – experimental	5	107,6±0,55	5	143,26±0,55 **
	II – control	5	106,23±0,56	5	122,9±0,56
RBC (x 10^{12}/L)	I – experimental	5	4,55±0,56	5	7,08±0,55 *
	II - control	5	4,42±0,49	5	5,41±0,56
WBC (x 10^{9} / L)	I – experimental	5	7,76±0,49	5	6,98±0,56 **
	II - control	5	20,72±0,56	5	10,59±0,56

Legend: P<0,05; P<0,001***

According to the first research the haematological indices in piglets from both groups were similar, the difference was statistically unreliable (P> 0.05). According to the second research the number of erythrocytes and the amount of hemoglobin were increased essential in piglets in experimental group (P <0.05) and (P <0.001).

WBC in piglets in the experimental group had a stable amount which comprises 7.76 + 0.49 x 10^9 / L initially and 6.98 + 0.55 x 10^9 / L in the following assessment. Piglets in the control group had a decrease in leukocytes from 20.72 + 0.56 during the first research to 10.59 + 0.58 x 10^9 / L during the second research.

Table 3 presents data on some biochemical constants in sows.

Urea level in blood plasma in sows of both groups ranged from 8.16 to 9.12 mM / L. However in sows in the control group there were an 11.6% increase of the level of urea in the first and by 5.6% on the 2nd appreciation in comparison with the experimental group, where Sel-Plex and Bio –Mos were added in the ration.

AST is an enzyme found in all cells and organs, especially the myocardium (L. Lisi, 2007), it had a lower activity in experimental sows in group I (M =10.71+0.81 and 11,99+0.86 U / L) in the first and 2nd assessments, respectively.

In sows from group II there was an activity increase of AST by 41.2% to 50.45% from 1st and 2nd assessments (P <0.01). ALT activity (U / l) had a tendency to increase (statistically accurate - P<0.01) in animals of control group 1 and it fell on the 2nd of research, what's that comprising 16.41+1.01 u / l, 0.96 experimental and 15.0+0.96 u/l control group.

Table 3. Biochemical constants in sows

INDICES	GROUP	INVESTIGATIONS			
		I 7th day		II 32nd day	
		n	M±n	n	M±n
Glucose (mM/L)	I – experimental	5	4,910±0,55	5	4,35±0,52
	II - control	5	5,146±0,56	5	4,95±0,55
Total protein (g/L)	I – experimental	5	48,91±1,74	5	51,63±1,79
	II – control	5	48,54±1,73	5	48,18±1,73
AST (U/l)	I – experimental	5	10,71±0,81	5	11,99±0,86
	II – control	5	15,13±0,97	* 5	18,04±1,06
ALT (U/l)	I – experimental	5	9,66±0,77**	5	16,41±1,01
	II – control	5	16,18±1,00	5	15,01±0,96
Urea (mM/L)	I – experimental	5	8,16±0,71	5	8,64±0,73
	II - control	5	9,11±0,75	5	9,12±0,75

Legend: P<0,01; P<0,001***

The values of the energy and protein indicate an optimal level in both groups. Glucose and total blood protein had similar values quoted in the literature (Ghergariu S. et.Al., 2000).

Table 4 presents the results of hematological indices in sows. Blood samples were taken 20 days up to the 9th and 25th day after parturition.

Table 4. Hematological indices in sows

INDICES	GROUP	References S. Ghergariu et. al., 2000	RESEARCH					
			I 20th day Ante partum		II 9th day Postpartum		III 25th day Postpartum	
			n	M±n	n	M±n	n	M±n
Erythrocytes (x 10^{12}/L)	I – experimental	5,0-	5	6,34±0,136**	4	7,31±0,036*	4	7,411±0,039***
	II – control	8,6 (6,5)	5	5,713±0,08	4	6,90±0,060	4	7,077±0,027
Hemoglobin (g/L)	I – experimental	100-	5	127,28±12,76	4	155,81±8,59 *	4	144,28±4,20***
	II - control	160 (130)	5	141,89±14,68	4	132,3±8,237	4	124,34±1,14
Hematocrit (%)	I – experimental	32-	5	36,8±2,17	4	30,5 ±1,0	4	31,5±0,95
	II - control	50 (42,0)	5	31,4±2,19	4	36,25±2,5	4	38,75±1,25
Leukocytes (x 10^{9} / L)	I – experimental	11-	5	7,48±0,25 *	4	7,51±0,213	4	7,55±0,084 **
	II - control	22 (16,0)	5	6,62±0,17	4	8,06±0,22	4	8,237±0,08
Lymphocytes (%)	I – experimental	39-	5	30,8±2,61 **	4	38,75±1,54 **	4	41,0±0,707 * **
	II - control	62 (53,0)	5	44,0±2,59	4	26,0±1,779	4	26,75±0,75

Legend: P<0,05; P<0,01**; P<0,001****

At the first research group index difference between hemoglobin and hematocrit was statistically unreliable (P> 0.05), so the number of erythrocytes was 11% higher in the experimental group. Subsequently the number of erythrocytes increased in animals from both groups. However, at the end of experiment this index continued to be significantly higher (P <0.01) in sows in the experimental group which was supplemented with Sel-Plex and Bio-Mos.

It should be mentioned that the second and the third researches the number of red blood cells was increased essentially in sows from the experimental group (P <0.05) and (P <0.001). The same trend of increase was recorded in the amount of hemoglobin, which at the 2nd research was 17.7% higher and at the 3rd research was 16.03% higher. Hematocrit value was higher in the 2nd and 3rd researches in discretion to control group, but in both cases derived indices were not statistically reliable (P> 0.05).

WBC in sows from the experimental group were in a stable level which made 7.48+0.25 x 109 / L initial 7.51+0.21 x 109 / L at the 2nd, and 7.55+0.084 x 109 / L in the following assessment. In the sows of the control group there was a statistically reliable increase between groups (P <0.01) in the number of leukocytes. But essentially the number of lymphocytes increased in the 2nd (P <0.01) and the 3rd (P <0.001) researches in animals who received food with Sel-Plex and Bio-Mos.

The results clearly demonstrate a beneficial effect of organic selenium (Sel-Plex) in combination with probiotic Bio-Mos on clinical indicators, bioproductivity, hematological and biochemical indicators in sows and their offspring.

V. Cociu and others (2005) report that in the Republic of Moldova two essential trace elements - iodine and selenium are not included in the composition of premixes. Deficiency of these minerals usually manifest latent affecting animal productivity and quality of products achieved. However, these indices are not always taken into account by farmers.

Deficit of a single element such as selenium, which is necessary in trace amounts (0.5 to 0.7 ppm) causes a variety of morbid conditions in different animal species [5]. Rations containing large amounts of unsaturated fats and in the same time poor in protein, especially in sulfur amino acids, are predisposing factors that may cause disease [9].

P.F. Suray, 2007, pointed out that the content of selenium in the feed depends on the region where it was grown, the soil and a number of other factors. N. Abraham (1992) reports that the content of selenium in feed depends a lot on the amount of selenium in soil, and generally it depends on relation soil-plant-animal.

Recently, several studies have been devoted to testing the product Sel-Plex in maintaining antioxidant-prooxidant balance in the digestive tract and blood, and prevent the decline of productive and reproductive performance in sows and offspring [1,3,4]

The results obtained allow us to conclude that the inclusion in the basic ration Bio-Mos and Sel-Plex product have beneficial action on the health condition of lactating sows and their offspring, manifested by morbidity and mortality reduced.

Conclusions

1. Sel-Plex administered with food at a rate of 0.5 kg / tonne feed has a positive effect on reproductive and laboratory indices in sows during last term of gestation and growth of their offspring. The addition of Bio-Mos in the proportion of 1 kg / tonne

feed combined with Sel-Plex helped to increase reproductive indices (total number of piglets born alive, body weight at birth, infant piglet viability).

2. Sel-Plex exerts a complex action on hematopoietic status and enzyme reactions manifested by increasing significantly the number of erythrocytes and amount of hemoglobin ($P < 0.05$) significant increase ($P < 0.01$) the number of lymphocytes ; reducing AST activity ($P < 0.01$) and urea concentration in blood plasma.
3. The results demonstrate the necessity to replace the ratio of inorganic selenium to produce organic selenium the way Sel-Plex is.

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STUDY REGARDING THE EVOLUTION WITH AGE OF ULTRASOUND PROSTATE VOLUME IN ROTTWEILER MALE DOGS

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Abstract
The aim of this study is to analyze the evolution with age of the prostatic dimensions, serum testosterone level in Rottweiler dogs. For the veterinarians, these results could help to recognize more easily a pathological state of the Rottweiler dog's prostate, only by an ultrasound examination. After examining 38 Rottweiler dogs, for dogs of 2 to 4 years old, we obtained a mean prostatic volume of 19.92 cmc, for dogs between 5 and 7 years old the mean prostatic volume was 44.99 cmc and for dogs older than 7 years, the mean prostatic volume was 52.27 cmc. In conclusion, regarding the results, it can be affirmed that in Rottweiler dogs, prostate dimensions are increasing with age.

Keywords: age, dogs, prostate, reproduction, Rottweiler

Objectives

Prostate disease is a common problem in old dogs [6]. In humans, there are age-specific physiological ranges for prostatic volume. [1, 3, 9]

As long as in humans there are standards regarding the prostate dimensions on groups of age, and are also very useful for practitioner doctors in diagnose prostate pathology, we are trying to create the same kind of standards for Rottweiler dogs, on groups of age.

The pathology of the prostate is composed by the following diseases: benign hyperplasia, prostatitis, cysts, squamous methaplasia, atrophy and neoplasm. All these diseases are translated in prostatic volume changes. These changes mean increasing of prostatic volume or decreasing of prostatic volume. If there would be an accepted standard interval for physiological values of prostatic volume, any value that overcome that interval, could be a sign of prostatic disease. [4, 5, 7, 8]

The aim of this study is to contribute to the realization of age-related physiological standards regarding the prostatic volume for Rottweiler dogs and to obtain results that could be useful for the practitioner veterinarians in detecting pathological changes in the prostate of dogs.

Materials and methods

The ultrasonographic prostate volume was measured in 38 Rottweiler dogs. Dogs were selected, by anamnesis, to have no history of urinary trac or reproductive disease and not neutered. They were divided into three groups of ages: G1 between 2 and 4 years old; G2 between 5 and 7 years old, and group G3, consisting of 10 Rottweiler, over 7 years old, dogs. By dividing dogs in these three category of age, 14 of them were included in the group G1, 14 were included in the group G2, and 10 of them in the third group, G3.

The prostate volume was determined by a formula, using a 2D ultrasound. The mathematical formula was suggested in the literature by Kamolpatana et al., [2]. It was considered to be the most precise way for calculation of dog's prostate volume.

First, the length and height of the prostate were measured in longitudinal section. The second measurement was made in transversal plane, determining the weight and height. The height used in the formula was the average of the two measured values, longitudinally and transversally.

The applied formula is the following:

$$V= [1/2.6 (L * W * H)] + 1.8$$

Where:

L – Length of the prostate (longitudinally);

W – Weight of the prostate (transversally);

H – Average between the longitudinally measured height (D1) and the transversally measured one (D2) (see Fig. 1.).

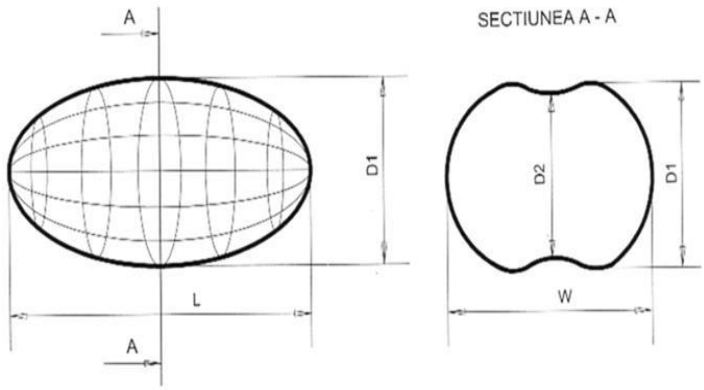

Fig. 1. Schematic representation of the prostatic dimensions measured by transabdominal ultrasonography

After calculating the prostatic volume for each examined dog, a mean value, with standard deviation, was calculated for each group of ages to observe the evolution with age of this factor. Statistical differences between the averages were processed using the ANOVA test.

Results and discussions

Results regarding the ultrasound measurements of the prostate gland in the 38 Rottweiler male dogs are exemplified in the figure 2 and 3 (fig.2 and fig.3) and are presented in the tables 1 and 2 (table 1 and table 2).

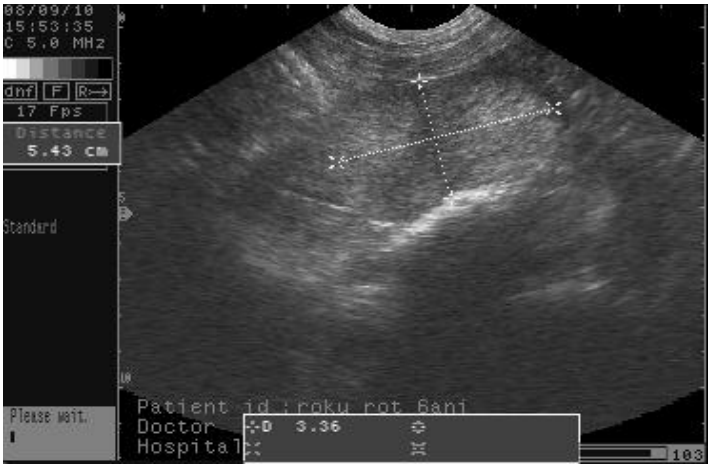

Fig. 2. Transverse section trough the prostate of a Rottweiler dog – ultrasound image

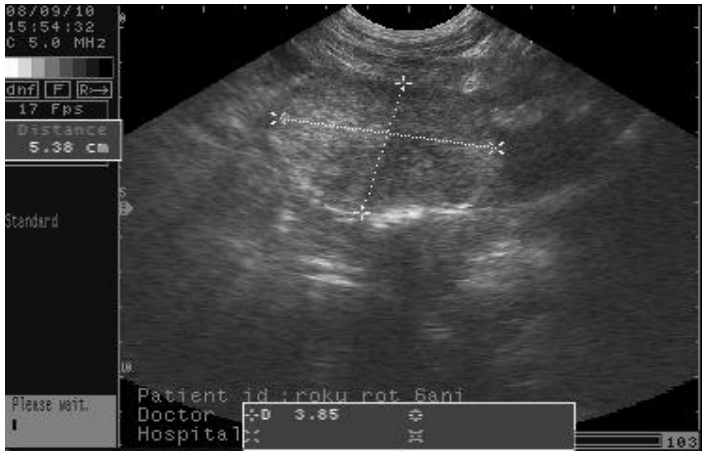

Fig. 3. Saggital section trough the prostate of a Rottweiler dog – ultrasound image

The obtained results regarding 2D-dimensions for the prostate in the examined Rottweiler dogs are presented as follows (Table 1.):

The length of the prostate:

- In dogs aged between 2 and 4 years - the average is 3.85 cm;
- In dogs aged between 5 and 7 years - the average is 5.69 cm;
- In dogs older than 7 years - the average is 5.74 cm;

The width of the prostate:

- In dogs aged between 2 and 4 years - the average is 3.53 cm;
- In dogs aged between 5 and 7 years - the average is 5.09 cm;
- In dogs older than 7 years - the average is 4.94 cm;

The height of the prostate:
- In dogs aged between 2 and 4 years - the average is 3.3 cm;
- In dogs aged between 5 and 7 years - the average is 3.87 cm;
- In dogs older than 7 years - the average is 4.32 cm.

Table 1. Prostate 2D dimensions in Rottweiler dogs obtained by ultrasound measurements

Group of age	Prostate length (cm)	Prostate weight (cm)	Prostate height (cm)
G1 (2-4 years)	3.85	3.53	3.3
G2 (5-7 years)	5.69	5.09	3.87
G3 (over 7 years)	5.74	4.94	4.32

Regarding the prostate volume, a 3D-dimension, for Rottweiler dogs with age between 2 and 4, we obtained a mean prostate volume of 19.92 cmc with a standard deviation of 7.32. For the older dogs, between 5 and 7 years old, we obtained a mean prostate volume of 44.99 cmc ±5.90; and for dogs included in group G3, aged over 7 years, the mean prostate volume was 52.27 cmc, with a standard deviation of 20.62 (Table 2.).

Table 2. Results of statistical interpretation regarding prostatic volume in Rottweiler dogs

Rottweiler male dogs			
Group of age	**G1 (2-4 years old)**	**G2 (5-7 years old)**	**G3 (over 7 years old)**
Mean prostate volume (cmc)	**19.92**	**44.99**	**52.27**
Standard Deviation	**7.32**	**5.90**	**20.62**
G1vG2 t(26)=9.98 p< 0.0001 Q=0.0000 G1vG3 t(22)=5.45 p< 0.0001 Q=0.0000 G2vG3 t(22)=1.26 p< 0.2205 Q=0.0000			

Analyzing the previous table we can affirm that the lowest mean prostate volume was found in group G1, growing strongly ensured statistically to group G2 ($p<0.01$). The highest value of mean prostate volume was noted in group G3 of dogs, although the difference between G2 and G3 is not ensured statistically ($p>0.05$).

Comparing the mean prostate volume of group G1 with that of group G3, the obtained difference is strongly ensured statistically ($p<0.01$), the value of G3 being much higher than that of the group G1.

The differences between values obtained for the three groups of age are represented graphically in the figure 4.

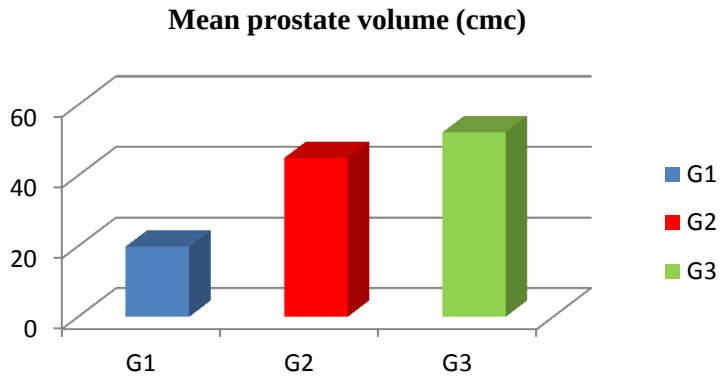

Fig. 4. Graphical representation of the mean prostatic volumes on groups of ages, in Rottweiler dogs

Conclusions

1. Prostate volume is significantly increasing in dogs between 5 and 7 years old, in comparison with those between 2 and 4 years old. Over age of 7 years, the growth of the prostate gland in Rottweiler dogs is insignificant in comparison with dogs of 5 to 7 years old.
2. Regarding the previous results and partial conclusions, it can be affirmed that the prostate volume of Rottweiler dogs is increasing along with age.

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HEMATOLOGICAL AND BIOCHEMICAL BLOOD ASPECTS FOR DOGS WITH CHRONIC CONGESTIVE CARDIAC INSUFFICIENCY (CCCI)

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Abstract
The RBC (red blood cells) exam performed for 10 dogs diagnosed with CCCI has revealed the existence of a deficiency anemia, that was also hypochromic and microcytic (No.RBC=5,7±2,3 mil/µl, Hgb=11,5±2,9 g/dl, Ht=35,4±3,1 %, MCV=58,6±3,3 μ^3, MCH=18,7±3,2 pg, MCHC=31,1±2,7 g/dl). The WBC (white blood cells) exam revealed leukocytosis with neutrophilia (No.WBC=17,3±2,4 thousands/µl, N=82±1,8 %). The biochemical blood profile confirmed that CCCI may evolve as a primary disease, without affecting in the compensated stage the basic physiology of the body. The parameters for this biochemical profile for the dogs with CCCI were included in the limits of the average reference values (TP=6,0±0,3 g/dl, AST=16,7±3,3 UI/L, ALT=25,2±3,1 UI/L, BUN=25,1±1,5 mg/dl, CRTN=1,3±0,5 mg/dl, Ca=9,9±1,2 mg/dl, P=5,4±0,5 mg/dl).
Keywords: cardiac insufficiency, dog, hematology, biochemistry

Method and materials

The researches were conducted on 10 dogs of different ages and breeds. By clinical exam, electrocardiography and by ultrasound exam these dogs were diagnosed with signs of CCCI.

The laboratory investigations consisted of performing the RBC exam with the ABC Vet automatic blood analyzer and the biochemical blood profile with the Comay Accent 200 automatic spectrophotometer.

Results and discussion

The results of the hematological exam are presented in the tables 1 and 2.

Table 1. Blood exam for the dogs with CCCI

Blood parameter	No. RBC	Hgb	Ht	No. WBC	WBC exam				
					N	E	B	M	L
Units	mil/ µl	g/dl	%	thousands/µl	%	%	%	%	%
Average reference values	5,4-7,8	13,0-19,0	37,0-54,0	6,0-17,0	30-75	62-83	0-3	3-10	12-30
Dogs with CCCI (n=10)	**5,7± 2,3**	**11,5± 2,9**	**35,4± 3,1**	**17,3± 2,4**	**82± 1,8**	**63± 1,1**	**1,5± 0,5**	**4,0± 0,7**	**14,0± 1,1**

From the two tables we can observe that the parameters had average values that were lower than the reference ones or close to the minimum value of the reference ones: No. RBC = 5,7±2,3 mil/µl, Hgb=11,5±2,9 g/dl, Ht=35,4±3,1 %. In the same time the calculated blood parameters had average values lower than the average reference ones: mean corpuscular volume (MCV)=58,6±3, 3 μ^3, mean corpuscular hemoglobin (MCH)=18,7±3,2 pg, mean corpuscular hemoglobin concentration (MCHC)=31,1±2,7 g/dl. The data characterizes the existence of an anemic sindrome: deficiency anemia, that is also hypochromic and microcytic.

Table 2. Blood parameters calculated for the dogs with CCCI

Hematological parameter	**MCV**	**MCH**	**MCHC**
Measurement units	μ^3	picograms (pg)	g/dl
Average reference values	64,0-74,0	22,0-27,0	34,0-36,0
Dogs with CCCI (n=10)	**58,6±3,3**	**18,7±3,2**	**31,1±2,7**

The WBC exam revealed the number of leucocytes and neutrophils with average values bigger or close to the superior limit of the reference ones: No. WBC =17,3±2,4 thousands/µl, N=82±1,8 %. The other parameters had resembling average values as the average reference ones: : E=63±1,1 %, B=1,5±0,5 %, M=4,0±0,7 %, L=14,0±1,1 %. These values characterize the diagnosis of leukocytosis with neutrophilia.

The results of the biochemical blood exam are presented in table 3.

Table 3. Biochemical blood profile for the dogs with CCCI

Biochemical parameter	**TP**	**AST**	**ALT**	**BUN**	**CRTN**	**Ca**	**P**
Units	g/dl	UI/L	UI/L	mg/dl	mg/dl	mg/dl	mg/dl
Average reference values	5,5-7,5	8,9-49,0	8,2-57,0	8,8-26	0,5-1,6	8,7-11,8	2,9-6,2
Dogs with CCCI (n=10)	**6,0± 0,3**	**16,7± 3,3**	**25,2± 3,1**	**25,1± 1,5**	**1,3± 0,5**	**9,9± 1,2**	**5,4± 0,5**

From this table we can observe that the values of the parameters from the dogs with CCCI find themselves between the limits of the average reference values: total proteins (TP)=6,0±0,3 g/dl, aspartate tranferase (AST)=16,7±3,3 UI/L, alanine transferase (ALT)=25,2±3,1 UI/L, blood urea nitrogen (BUN)=25,1±1,5 mg/dl, creatinine (CRTN)=1,3±0,5 mg/dl, seric calcium (Ca)=9,9±1,2 mg/dl, seric phosphorus (P)=5,4±0,5

mg/dl. These values confirm that CCCI can evolve as a primary disease without affecting, in the compensated stage, the basic physiology of the body. Later, in the decompensated stages, appear clinical or laboratory signs from other organs.

Conclusions

1. For the dogs diagnosed with CCCI the RBC exam had average values that were lower than the reference ones or close to the minimum value of the reference ones: No. RBC = 5,7±2,3 mil/µl, Hgb=11,5±2,9 g/dl, Ht=35,4±3,1 %, MCV=58,6±3,3 μ^3, MCH=18,7±3,2 pg, MCHC=31,1±2,7 g/dl. The data characterizes the existence of an anemic sindrome: deficiency anemia, that is also hypochromic and microcytic.
2. The WBC exam revealed the number of leucocytes and neutrophils with average values bigger or close to the superior limit of the reference ones: No. WBC =17,3±2,4 thousands/µl, N=82±1,8 %. These values characterize the diagnosis of leukocytosis with neutrophilia.
3. The values of the biochemical blood profile parameters for the dogs with CCCI were found between the limits of the average reference values: TP=6,0±0,3 g/dl, AST=16,7±3,3 UI/L, ALT=25,2±3,1 UI/L, BUN=25,1±1,5 mg/dl, CRTN=1,3±0,5 mg/dl, Ca=9,9±1,2 mg/dl, P=5,4±0,5 mg/dl.
4. These values confirm that CCCI can evolve as a primary disease without affecting, in the compensated stage, the basic physiology of the body. Later, in the decompensated stages, appear clinical or laboratory signs from other organs.

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THE INCIDENCE OF CHRONIC HEART FAILURE SYNDROME IN DOGS

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Abstract
Between 2006 and 2008, we calculated the incidence of chronic heart failure (CHF) in dogs, compared to the total number of cases examined, total morbidity, the type of CHF, age, gender and breed of the patient. The incidence of CHF in dogs was 7.03% compared with the total number of cases examined. Meanwhile, 69.49% (82 cases) had left CHF, 8.47% (10 cases) had right CHF and 22.03% (26 cases) had global CHF. The percentage of left sided CHF was the highest, representing approximately 2/3 of the total number of cases diagnosed with CHF, while de incidence of right sided CHF was less than 10% of the total number of CHF cases. In regard to age, the highest incidence of the syndrome of CHF was found in the age group 8-12 years, 72.03% respectively. As for gender, we found that the incidence of CHF syndrome was 75.42% (89 cases) in males and 24.57% (29 cases) in females. The CHF syndrome was diagnosed in all breeds of dogs directly proportional to the percentage of the breed in the number of cases examined.
Keywords: chronic heart failure, dog, incidence

Materials and methods

Following clinical, electrocardiographic (EKG) and ultrasonographic (EcoCord) examination, we categorized chronic heart failure (CHF) as left CHF, right CHF and global CHF.

Left CHF had polymorphic symptoms, including aortic stenosis endocardial murmur and mitral valve insufficiency, the lengthening of R wave (aortic stenosis) and QRS complexes with equal intervals (ventricular tachycardia), the presence of F waves (atrial fibrillation) in the severe dilation of the left atrium, the increase in duration of P wave, the thickening and hyperechogen aspect of the mitral valve both in its septal area and the parietal area, left heart dilation through the increase of the left atrium diameter compared to the aortic diameter (As/Ao > 1).

In right CHF, we noticed an increase in the systolic and diastolic diameter of the right ventricle with an asynchronous move of the interventricular septum, the existence of multiple cavity effusions, and the thickening and hyperechogenic aspect of the tricuspid valve.

Subsequently, the incidence of CHF in dogs during 2006-2008 was established based on data entered in the Register of Consultations and Treatments of the Internal Medicine Clinic. This was calculated compared to various factors of variation like the total number of cases examined, overall morbidity, type of CHF, patient age, gender and breed.

Results and discussions

The incidence of CHF compared to the total number of cases examined is shown in table 1 and figure 1.

Table 1. The incidence of CHF in dogs, compared to the total number of cases examined

Total number of dogs examined between 01.01.2006 – 31.12.2008	Total morbidity				Clinically healthy dogs	
	Dogs with CHF		Dogs with other conditions			
	Nr.	%	Nr.	%	Nr.	%
1.678	118	7,03	1267	75,50	293	17,46
	1385 (82,53%)					

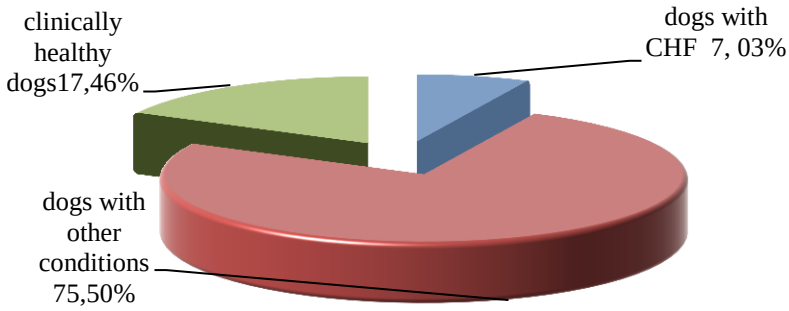

Fig. 1. The diagram of the incidence of CHF in dogs, compared to the total number of cases examined

Between 2006-2008 the incidence of CHF in dogs was 7,03%, 118 cases respectively, compared to the totalnumber of cases examined . This includes all types of CHF: left CHF, right CHF and global CHF. Meanwhile,75,50%, 1267 cases respectively, were dogs diagnosed with other medical conditions, and the rest of 17,46%, 293 cases respectively, were clinically healthy dogs.

The incidence of CHF syndrome compared to total morbidity is shown in table 2 and figure nr. 2.

Table 2. The incidence of CHF in dogs, compared to total morbidity

Total morbidity	Dogs with CHF		Dosg with other conditions	
1385	Nr.	%	Nr.	%
	118	8,52	1267	91,48

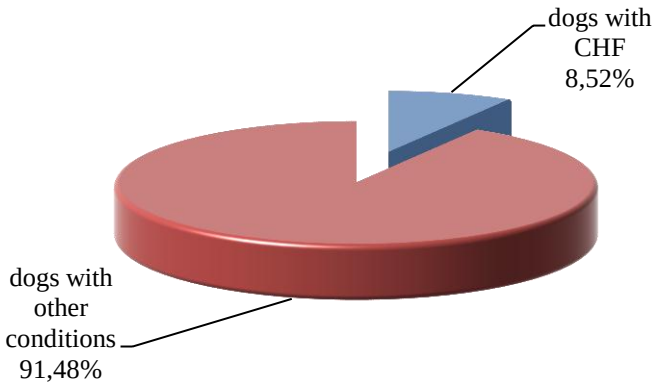

Fig. 2. The diagram of the incidence of CHF in dogs, compared to total morbidity

Of the 1385 dogs diagnosed with various medical conditions, 8,52%, 118 cases respectively, presented clinical or paraclinical signs of CHF syndrome, and the rest of 91,48%, 1267 cases respectively, were diagnosed with other medical conditions.

The annual incidence of the clinical types of CHF- left CHF, right CHF and global CHF- diagnosed in dogs between 2006-2008 is shown in table 3 and figure nr. 3.

As shown in table 3 an figure nr. 3, of th total number of cases diagnosed with CHF between 2006-2008, 69,49% (82 cases) had left CHF, 8,47% (10 cases) had right CHF and 22,03% (26 cases) had global CHF.

Table 3. The annual incidence of the clinical types of CHF

Year	Total number of dogs examined		Dogs with CHF		Dogs with CHF					
					Left CHF		Right CHF		Global CHF	
	Nr.	%	**Nr.**	%	Nr.	**%**	Nr.	**%**	Nr.	**%**
2006	**526**	100	**32**	6,08	21	**65,62**	3	**9,37**	8	**26,00**
2007	**559**	100	**39**	6,97	27	**69,23**	3	**7,69**	9	**23,07**
2008	**593**	100	**47**	7,92	34	**72,34**	4	**8,51**	9	**19,14**
Total	**1678**	100	**118**	7,03	82	**69,49**	10	**8,47**	26	**22,03**

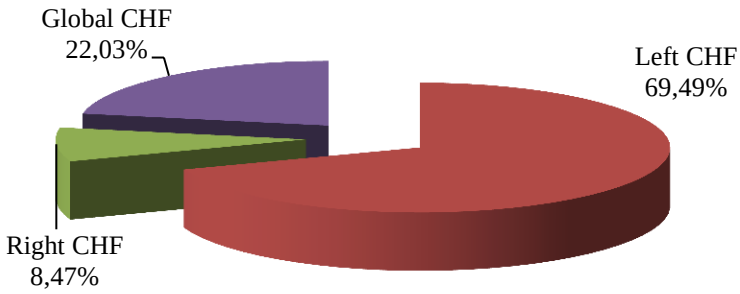

Fig. 3. The proportion of clinical types of CHF

The annual incidence of CHF is shown in table 3 and figure nr. 4 and 5.

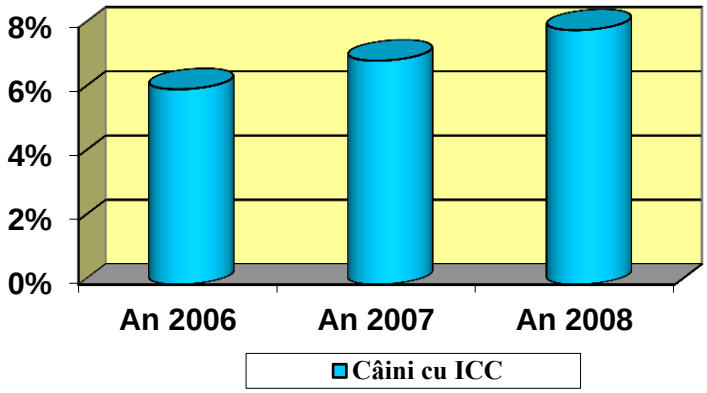

Fig. 4. The annual incidence of CHF syndrome

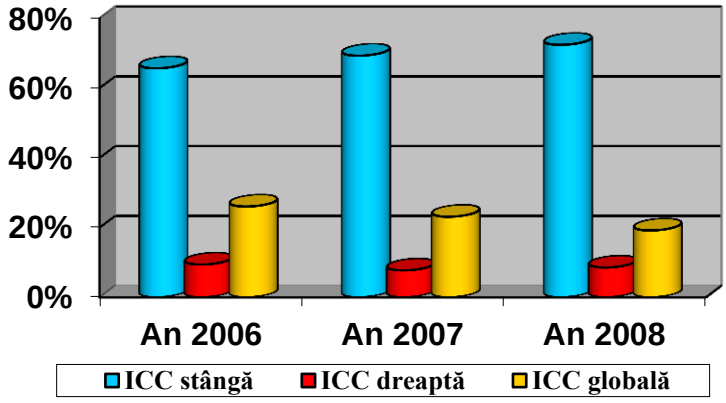

Fig. 5. The diagram of the annual incidence of CHF syndrome

Figure nr. 4 shows a progressive annual increase in the incidence of CHF, with 6,08% (32 cases) in 2006, 6,97% (39 cases) in 2007 and 7,92% (47 cases) in 2008 respectively.

Regarding the dynamic of the annual incidence of clinical forms of CHF (figure nr 5) we noticed a progressive incidence only of left CHF: 65,62% (21 cases) in 2006, 69,23% (27 cases) in 2007 and 72,34% (34 cases) in 2008 respectively. The incidence of right CHF was the highest in 2006, 9,37% respectively, and lowest in 2007, 7,69% respectively, compared to the total number of cases diagnosed with cHF syndrome. In 2008, the incidence of right cHF was 8,51%. The incidence of global CHF was decreasing from 26,00% in 2006, to 23,07% in 2007 and 19,14% in 2008.

However, every year, the percentage of left CHF syndrome was the highest, representing approximately 2/3 of the total number of cases diagnosed with CHF. If we add to the incidence of left CHF syndrome the incidence of global CHF, in which the left heart is also affected, we can deduce that the incidence of right CHF syndrome is less than 10 % of the total number of CHF cases.

The incidence of CHF according to age is presented in table 4 and figure 6.

Table 4. The incidence of CHF in dogs, according to age

Total number of dogs with CHF		Dogs with CHF- younger than 8 years		Dogs with CHF- 8-12 years		Dogs with CHF older than 12 years	
Nr.	%	Nr.	%	Nr.	%	Nr.	%
118	**100**	**12**	**10,17**	**85**	**72,03**	**21**	**17,80**

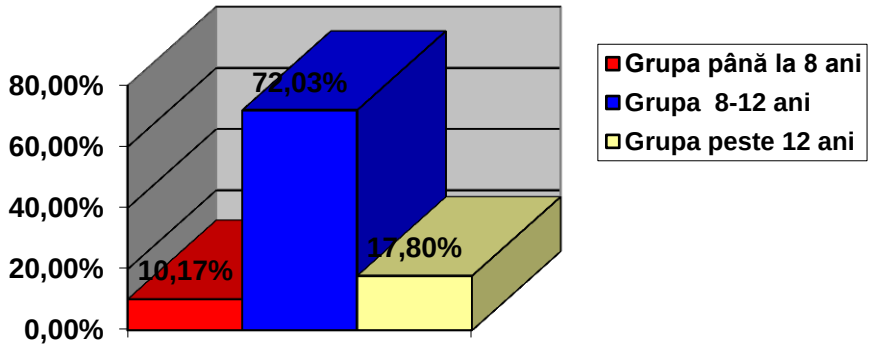

Fig. 6. The diagram of the incidence of CHF according to age

Table 4 and figure nr 3 show that of the total nu-mber of dogs diagnosed with CHF syndrome, the highest incidence was in the age group of 8 to 12 years, 72,03% respectively. In dogs older than 12 years, the incidence was 17,80%, and in those younger than 8 years, it

was 10,17%. Acquired heart conditions can develop in dogs of all ages, but the age interval most affected was 8 to 12 years.

The incidence of CHF according to the animals' gender is shown in table 5 and figure nr. 7.

Table 5. The incidence of CHF in dogs according to gender

Total number of dogs with CHF		Males		Females	
Nr.	%	Nr.	%	Nr.	%
118	**100**	**89**	**75,42**	**29**	**24,57**

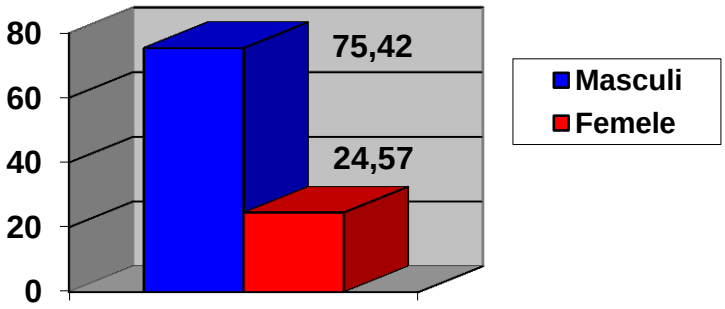

Fig. 7. The diagram of the incidence of CHF in dogs according to gender

Table 5 and figure 7 show that of the 118 cases diagnosed with CHF, 89 were males (75,42%) and 29 were females (24,57%). These statistics are similar to those presented in literature.

The incidence of CHF according to breed is presented in table 6 and figure nr. 8

Table 6. The incidence of CHF according to breed

Rasa	Număr cazuri	%
Ciobănesc german	**58**	**49,15**
Pechinez	**29**	**24,57**
Cocker	**7**	**5,93**
Caniche	**5**	**4,23**
Alte rase	**19**	**16,10**
Total	**118**	**100,00**

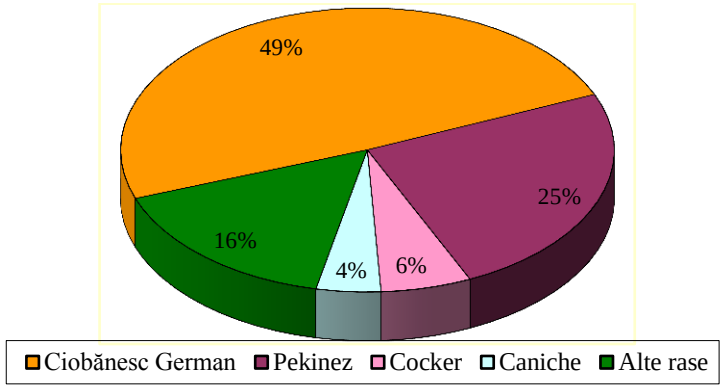

Fig. 8. The diagram of the incidence of CHF according to breed

According to breed, the incidence of CHF was highest – 49,15% (58 cases) in German Shepherd dogs and lowest – 4,23% (5 cases) in Poodles. CHF syndrome was diagnosed in all breeds presented for various medical reasons. These data show that the incidence of CHF syndrome does not depend on breed, being diagnosed directly proportional to the frequency of the breed in the total of cases presented for consultation.

Conclusions

1. Between 2006 and 2008, the incidence of CHF in dogs was of 7,03%, 118 cases respectively, of the total number of cases examined, which was 1678.
2. Of the total number of cases diagnosed with CHF between 2006 and 2008, 69,49% (82 cases) had left CHF, 8,47% (10 cases) had right CHF and 22,03% (26 cases) had global CHF.
3. Regarding the dynamic of the annual incidence of clinical forms of CHF (figure nr 5) we noticed a progressive incidence only of left CHF: 65,62% (21 cases) in 2006, 69,23% (27 cases) in 2007 and 72,34% (34 cases) in 2008 respectively.
4. Each year, the percentage of left CHF syndrome was the highest, representing approximately 2/3 of the total number of cases diagnosed with CHF, while the incidence of right CHF was less than 10 % of the total number of cases of CHF.
5. According to age, the highest incidence of CHF was seen in the group of 8 to 12 years, 72,03% respectively. In dogs older than 12 years, the incidence of CHF was 17,80%, and in those younger than 8 years, it was of 10,17%.
6. According to gender, we noticed that the incidence of CHF was of 75,42% (89 cases) in males and 24,57% (29 cases) in females.
7. According to breed, the incidence of CHF was highest – 49,15% (58 cases) in German Shepherd dogs and lowest – 4,23% (5 cases) in Poodles although CHF syndrome was diagnosed in all breeds of dogs.

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THE EVALUATION OF THE BODY CONDITION SCORE (BCS) ANTE AND POST PARTUM IN RELATION WITH THE INCIDENCE OF SOME REPRODUCTIVE DISEASES IN DAIRY COWS

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Abstract
The effects of body condition score (BCS) change and status ante- and postpartum (pp) on reproductive diseases in 250 Holstein cows from North eastern Romanian farm were evaluated. BCS was determined from 6 weeks antepartum until 6 weeks postpartum in a year interval. The incidence of various reproductive diseases, were assessed. We observed that both the losses higher than 0.5 BCS points and a low status of the BCS ante and post partum can be directly connected to the high incidence of the dystocia, due to the disorders in the uterine dynamics. Our observations reveal the fact that a low body condition score (BCS < 3) at parturition and during the early lactation period, as well as great body condition losses antepartum increase the risk for placental retentions or clinical endometritis to appear. The high incidence of cystic ovarian diseases could be noticed at females which suffered a massive loss of postpartum body condition points.
Key words: Holstein cow, body condition score, reproductive diseases

Body condition scoring is being used as a management tool to assess the energy reserves and thereby the nutritional status of dairy cows. In many investigations, body condition score (BCS) at calving, at varying time points during lactation or the change in BCS during early lactation were determined and related to fertility, animal health, metabolic parameters and milk yield. Ruegg et al. (1992b) did not find any association between numbers of days to first recorded oestrus or first breeding and BCS score at calving or amount of BCS loss, however, cows that calved with a BCS of ≥3.50 or lost >0.75 BCS points required more days to conceive. According to Domecq et al. (1997b), a BCS loss between calving and 4 weeks postpartum (pp) was related to a longer interval from artificial insemination (AI) to conception and a decreased likelihood of conception. Loeffler et al. (1999) considered a body score loss during early lactation as a significant predictor of pregnancy risk with a higher BCS loss being associated with a lower first service conception rate.

Rasmussen et al. (1999) found a link between BCS at calving and a higher risk of ketosis, whereas according to Ruegg and Milton (1995), BCS at calving was not significantly different between the cows suffering from various diseases such as cystic ovaries, metabolic diseases, lameness, reproductive diseases and mastitis and the cows that did not receive a disease diagnosis. As far as metabolic parameters are concerned, serum cholesterol values, but not serum urea nitrogen concentration, were inversely related to condition loss during early lactation (Ruegg et al. 1992a).

In addition, a marked BCS loss antepartum was related to lower serum cholesterol concentrations at month 1 of lactation, whereas serum triglyceride, glucose or urea nitrogen concentrations were not associated with a marked antepartal BCS loss (Kim and Suh 2003). With regard to milk yield, Ruegg et al. (1995) did not note any effect of BCS at calving and peak or 305 days milk yield. On the other hand, Domecq et al. (1997a) reported that an

increase in BCS during the dry period, was connected with higher milk yield during the first 120 days of lactation, whereas a high BCS at drying off had the opposite effect. There are only few studies dealing with the body condition during the dry period and its effects on the traits mentioned above (Gearhart et al. 1990; Markusfeld et al. 1997). Recently, Kim and Suh (2003) reported that severe body condition loss during the dry period resulted in a higher incidence of metritis and metabolic diseases postpartum, and increased numbers of days to first insemination.

Our researches evaluated the relationships that can appear between the modification of the body condition score ante and post partum and the incidence of the reproductive diseases that occurred at a farm in North-East Romania.

Materials and methods

The evaluation of the body condition score was determined at different time intervals, that is 6, 4 weeks antepartum, at parturition and 4, 6 weeks postpartum.

Body condition was assessed by the same technician and was based on the six-point scale described by Lowman et al.(1976), in which a score of 0 indicates severe emaciation and a score of 5 indicates obesity.

The females were grouped for the study according to the body condition score at the time intervals that were established, as well as according to the losses they suffered throughout the whole process.

The incidence of the various reproductive diseases was taken out of the gynecological files of the females, representing the total number of the known sickening at a certain time, or those traced during the research process, at the sickening time and afterwards.

The cows were placed into three groups, according to the modification of the body condition score degree at the mentioned time intervals, and the groups are: no body condition score loss, with losses of 0.5 body condition points and with losses bigger than 0.5 points (at least 1 point of body condition loss).

Results and discussions

Of the total number of females (n=250), almost 38% were primiparous and the remaining 62% were multiparous.

The average of the body condition score (BCS) at 6 weeks antepartum, at calving and 4 weeks postpartum was higher for the primiparous than for the multiparous, with 0.5 body condition points.

Comparing to the females with no body weight loss, more than a third presented a slight body condition loss, which was appreciated as lower than 0.5 points or a little over this value, from 6 weeks antepartum up to the parturition. The remaining females, however, registered a great loss in body condition points (> -0.5 points), yet only a few females presented losses over 2 body condition points.

The cows which presented a low average of the body condition (BCS) at 6 and 4 weeks antepartum did not register BCS losses during this interval, unlike the other cow category, which suffered losses over 0.5 points of the body condition, females that represented a body status superior to the first category and also higher at parturition (fig. 1).

In the group that had a slight body condition loss (BCS – 0.5), the average was constant during the two weeks antepartum, but then it registered a slight decrease onto the parturition.

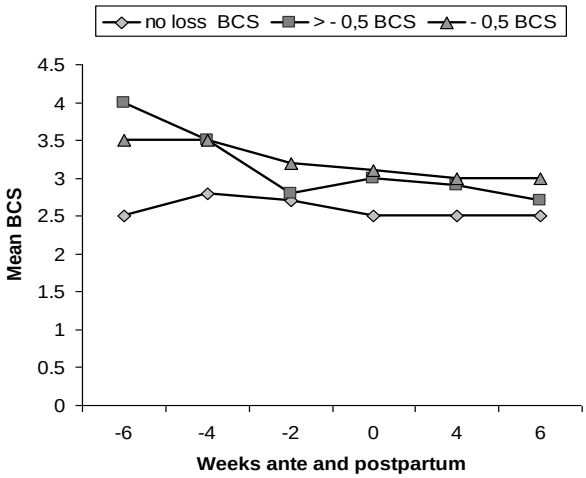

Fig.1. Mean of BCS in intervals 6 weeks antepartum – 6 weeks postpartum of cows without BCS loss and with 0.5 points and a greater loss in BCS

As for the postpartum period, the BCS average remained constant more or less for all the three groups. However, the cows with a slight loss in body condition points antepartum (-0.5), the BCS average postpartum presented a tendency of keeping high values, compared to the other two cow groups.

We evaluated the distribution of the different reproductive diseases (table 1), which we determined according to the body condition average for all three groups during the periods of time of the study, as well as the distribution of the reproductive diseases, according to the losses of points in the body condition that the cows suffered in the intervals between these periods (table 2).

Table 1. Distribution of different breeding diseases depending on the average BCS

Period	BCS	Dystocia %	Retained placenta %	Clinical endometritis %	Cystic Ovaries %
6 weeks a.p.	**< 3**	15	30	55	16
	≥ 3	14	13	35	11
0 (parturition)	**< 3**	10	20	70	7
	≥ 3	16	12	40	19
4 weeks p.p.	**< 3**	16	24	55	18
	≥ 3	12	10	22	13
6 weeks p.p.	**< 3**	20	20	56	14
	≥ 3	12	10	21	16

Table 2. Distribution of different breeding diseases depending on the loss of BCS

Period	BCS	Dystocia %	Retained placenta %	Clinical endometritis %	Cystic ovaries %
6 weeks a.p. – parturition	0	14	17	40	11
	- 0,5	10	10	43	15
	> - 0,5	17	21	48	13
0 (parturition) - 6 weeks p.p.	0	15	16	47	10
	- 0,5	12	13	39	14
	> - 0,5	7	6	25	20

The cows with a low status of the body condition presented a high incidence of placental retentions, endometritis, unlike the cows with a high status of the body condition (table 1).

The incidence of dystocia was higher for the females with a body condition lower than 3 points after parturition, similar to the high incidence of this disease notable for females with a better body condition status at parturition. Still, under the influenced of the body condition losses suffered by the maternal organism after calving, it could not be noticed a direct influence on the dystocia incidence, so the highest incidence was observed in females which lost more than 0.5 BCS points antepartum and in those cows which registered no body condition losses postpartum (table 2).

Considering that the body condition averages of the females with no losses were situated under 3 BCS points during the entire period of the study, we can come to the conclusion that the losses greater than 0.5 body condition points as well as a low BCS status ante and postpartum can be directly connected with the high incidence of dystocia, due to disorders in the uterine dynamics.

As for the ovarian cystic disease, there could not be established a correlation between the incidence of this disease and the body condition average at the time intervals that were studied, the highest value being observed in cows with excessive loss in body condition postpartum.

Our observations confirm the data in Markusfeld and coll.'s study (1997) who noticed a high incidence of placental retentions and endometritis in multiparous dairy cows with a low BCS status at parturition.

The high incidence of placental retentions was observed by the same author in cows who suffered body condition losses during the dry period.

Kim and Suh (2003) reported a high incidence of metritis in females with severe body condition loss during the dry period.

Pedron and coll. (1993) also observed the same high incidence of placental retentions in dairy cow groups with a low body condition status (BCS=3) at parturition, unlike other researches in which there could not be traced such statistically significant connections between these diseases and the body condition status at parturition (Gearhart and coll., 1990; Waltner and coll., 1993; Ruegg and Milton, 1995).

Rukkwamsuk and coll., Drackley (1999) find an increase in the disease incidence and in postpartum lipolysis in dairy cows which presented a sate of poor maintenance during the dry period.

Kim and Suh (2003) detected a large interval between the calving and the first artificial insemination in cows with severe body condition loss during the dry period.

Finally, our observations reveal the fact that a low body score (BCS < 3) at parturition and during the early lactation period, as well as great body condition losses antepartum can increase the risk of placental retentions and clinical endometritis. The high incidence of cystic ovarian diseases was detected in females which suffered massive losses of postpartum body condition points. We mention that the incidence of these diseases was studied on cows grouped according the body condition averages at certain time intervals, as well as according to the losses suffered and not separately on cows grouped according to the incidence of one single disease. Observing the high incidence of these reproductive diseases also in females without any body condition loss may be due to the fact that these females had already presented a poor body condition (<3) during the study period.

Conclusions

1. The body condition score (BCS) can be used as a management tool for dairy cows, with the aim of evaluating the energy resources and thus, the nutritional state of the females.
2. More than a third of the females presented a mild loss in the body condition, which we appreciated as lower than 0.5 points or slightly over this value from 6 weeks antepartum up to the parturition.
3. The cows which presented a low average of the body condition at 6 and 4 weeks antepartum did not suffer BCS losses during this interval, unlike the other cow category, which suffered losses greater than 0.5 body condition points, thus presenting a body score superior to the first category and also higher at parturition.
4. As for the postpartum period, the BCS average remained more or less constant for all the three groups.
5. For cows with a slight loss in body condition points antepartum (<-0.5), the BCS average postpartum presented a maintenance tendency at high values, compared to the other two cow groups.
6. This way, the females with a low body condition score presented a high incidence of placental retentions, endometritis, unlike the cows with a high body condition score.
7. The high incidence of endometritis and placental retentions could be detected also in females with severe loss in body condition points (BCS > -0.5).
8. The high incidence of dystocia due to disorders in the uterine dynamics could be noticed in females with a poor body condition antepartum and postpartum, but also in females with great losses (BCS > -0.5) in body condition points.
9. As for the cystic ovarian disease, there was not observed a direct correlation between the incidence of this disease and the average of the body condition at the time intervals that were studied, the highest value being observed in cows with excessive body condition loss postpartum.

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PERINEAL HERNIA REPAIR BY MUSCULAR TRANSPOSITION IN DOGS

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Abstract
Perineal hernia, caused by weakening of the muscular pelvic diaphragm, is a disease which induces discomfort to the animal, due to the abdominal or pelvic organs herniated at that level. Because of the complications, such as the absence of urination and defecation, the emergency surgical treatment consists in repositioning the herniated organs (urinary bladder, intestine, epiploon, prostate) in their cavity and repairing the defect in the perineum. Because, sometimes, repairing the pelvic diaphragm is difficult, due to atrophy of the tissue or its large rupture, the purpose of this paper is to present the results obtained after applying a new method of muscular transposition. The surgical procedure consisted in performing the standard herniorrhaphy, by suturing the perianal and perirectal soft tissues. In order to give more resistance to this suture, flaps from the superficial gluteal and the semitendinous were sutured, together with the external anal sphincter muscle. Thus, the muscle scar will be more resistant and will apply pressure on the herniorrhaphy, ensuring a good healing, with no recurrence.
Key words: dog, muscular transposition, perineal hernia, treatment

Introduction

Perineal hernia is more commonly seen in dogs living a sedentary life, in appartments (3). It results from failure of the muscular pelvic diaphragm to support the rectal wall (4), associated with constipation or diarrhea (1, 2, 5), extended ischio-pubic reposal (3), or muscular atrophy (3), which can be congenital or acquired.

No matter the cause, perineal hernia is a painful condition because of the internal organs moved at that level (urinary bladder, parts of the small intestine, prostate, epiploon), which apply pressure on the rectum (1, 2, 3). Prolonged compressions lead to rectal atrophy, which changes its position and diameter (3, 5).

Surgical correction is the only option for treatment, aiming to restore the organs back in their cavity and to repair the muscular pelvic diaphragm, by suturing the muscles which form it (the external anal sphincter, levator ani, coccygeus) (3, 5), by transpositioning a flap from the internal obturator muscle (1, 2, 6) or by applying a prosthetic mesh (1, 7).

The purpose of this paper is to describe the results obtained after attempting to reinforce the pelvic muscular diaphragm.

Material and method

21 dogs, from different breeds and with ages between 7 and 10 years old, submitted for consultations to the Surgical Clinic, Faculty of Veterinary Medicine Iaşi, Centrovet Clinic, Bucharest or Duo-Vet Clinic, Iaşi between 2009-2012, were included in this study. All animals presented clinical signs associated with perineal hernia: perianal swelling, tenesmus, proctitis.

Surgical intervention was dictated by local and general clinical signs. Emergency treatment was needed when the retroflexion of the urinary bladder generated postrenal uremia or a state of shock, due to strangulation of parts of the small intestine.

For these purposes, the next protocol was followed: preoperative evaluation of the overall status of the patient, regarding clinical health, the swelling (fig. 1), the herniated organ and the possibility of its manual repositioning, by moderate compressions; surgical intervention for reconstruction of the muscular pelvic diaphragm, in order to prevent recurrences; postoperative monitoring of the patient.

Because the most important part of this protocol consisted in repairing the muscular defect, after the herniated organs were anatomically repositioned, as to ensure the much needed future resistance, the research aimed to verify the possibility of providing better resistance by transpositioning muscle flaps from the superficial gluteal and the semitendinous.

Choosing these two muscles was based on their vicinity to the anal region and the fact that delimiting two flaps won't interfere with the animal's locomotion.

Induction was made with Domitor, and general anesthesia with Isoflurane. The surgical site was prepared by clipping the hair and placing sterile surgical drapes. The skin incision was made on the perianal swelling, parallel to and 3-4 cm away from the anus, from the base of the tail to the ischial tuberosity (fig. 2). The superior extremity of the incision was continued in an oblique fashion towards the iliac crest, in order to gain access to the superficial gluteal muscle.

The hernia was, then, opened and the organs were identified and replaced back into the abdominal cavity by moderate digital compression.

Care was taken as to preserve the pudendal artery, vein and nerve. Hemostasis was ensured by ligature. The urinary bladder was repositioned after cystocentesis. After identifying the soft tissues (the external anal sphincter, levator ani, coccygeus muscles, perineal fascia, the sacrotuberous ligament or the parietal peritoneum) which suffered a rupture or an atrophy, the standard herniorrhaphy (fig. 3) was performed in a continuous pattern using a 2/0 Vicryl suture.

For further tightness, a second suture is made using 1/0 Vicryl, in a simple interrupted pattern.

The following step is to identify, in the superior angle of the incision, the superficial gluteal muscle, from which a 2-3 cm flap is obtained (fig. 4), and the same procedure is applied for the semitendinous muscle (fig. 5). These two muscle flaps (fig. 6) are then sutured (fig. 7), together with the external anal sphincter, using 1/0 Vicryl. The skin is sutured with Silk (fig. 8).

Postoperative care implies a protective wound dressing and the Elizabethan collar. The physical exercise is restrained to a minimum.

All animals included in this study presented a unilateral hernia, the age of the affection varying between 2 and 7 weeks.

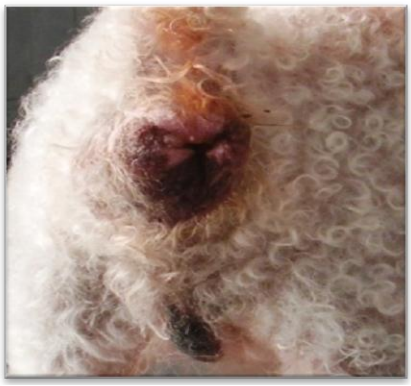

Fig.1 – Clinical aspect of the perineal hernia

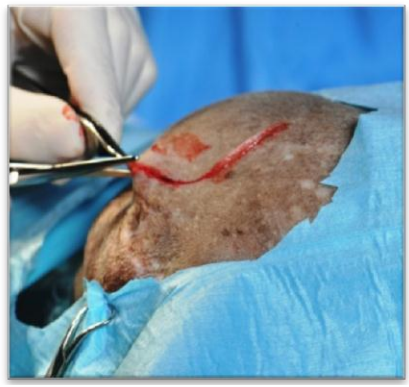

Fig.2 – The skin incision

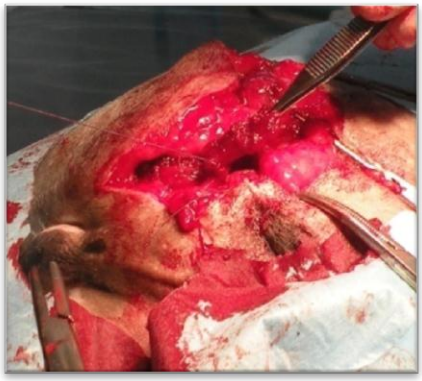

Fig.3 – Standard herniorrhaphy

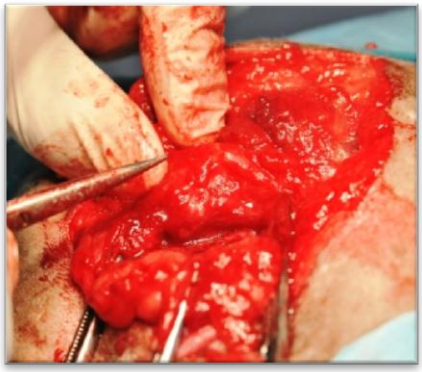

Fig.4 – Superficial gluteal muscle flap

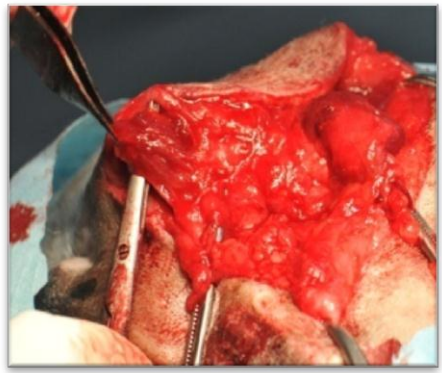

Fig.5 – Semitendinous muscle flap

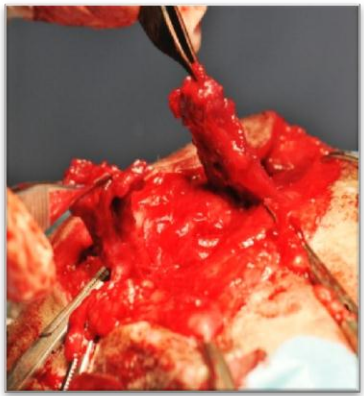

Fig.6 – Showing the two muscular flaps

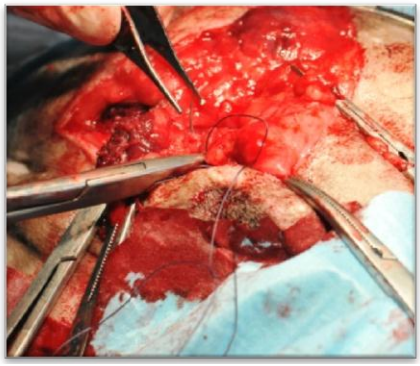

Fig.7 – Suturing the muscular flaps

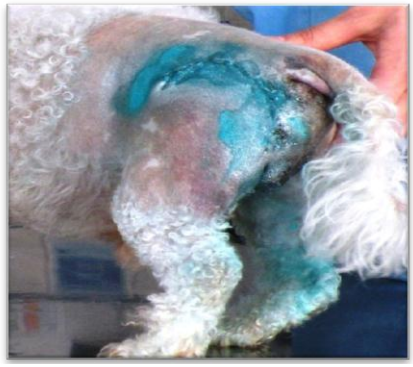

Fig.8 – Postoperative view

Results and discussion

Although perineal hernia is a painful condition, the owner fails to recognize the symptoms sooner, so the patient is presented late for consultation. Rectal tenesmus and animal anxiety are ignored, until the animal stops defecating or urinating, showing signs of uremia, as Hedlund, 1997, observes. This lack of interest from the owner allows the pelvic diaphragm to rupture and to cause difficulties for the later performed surgery, consisting in the impossibility of correct tissue apposition. This is why, in this paper, we compared the role of transpositioning muscle flaps for better postoperative suture resistance.

Advancing a flap from the superficial gluteal muscle is possible because of its topography, its thick bundles inserting distally on the sacrotuberous ligament and the ischial tuberosity. The semitendinous muscle originates caudally and ventrolaterally from the ischial tuberosity, between the heads of the biceps and semimembranous muscle. Both concur to the rump (tail) region (4), making possible their transposition. A 2-3 cm muscle flap was performed in all animals submitted for this study, and there was no negative influence on the locomotion. The slight incommodities in locomotion noticed in the first days following

surgery were due to the operative trauma. When standing, the animal rests on both hind legs, and when walking, a shorter stride length is observed for the operated leg.

Monitoring the patient after surgery submitted evidence, presented in table no.1, that supports the role of the muscular transposition comparative to the standard herniorrhaphy (suturing the external anal sphincter, the coccygeus, the internal obturator muscles along with the parietal peritoneum, perineal fascia and sacrotuberous ligament), as recommended by Dupre, 1993, and Bright, 1994.

The data from table 1 show that the success of the surgery depends on the age of the condition and the herniated organ. Recurrences were seen in the cases where the standard herniorrhaphy was performed, due to little resistance of the perianal soft tissues. Relapse is more important in earlier cases, because of the tissue atrophy. Even though a suture is attempted, it doesn't have the necessary strength. Anal paresis may concur to the lack of success, due to volume or position abnormalities.

This is why suturing the superficial gluteal and semitendinous muscle flaps over the standard herniorrhaphy gives the much needed resistance and more elasticity to the perianal region. As shown in table no. 1, from the 12 cases which underwent this combined method of hernia repair, only 2 suffered relapses, meaning 16,6%.

Table 1. Evolution of postoperative pelvic diaphragm recovery in perineal hernia in dogs

Type of surgery	Number of cases	Hernia age in weeks	Recurrence	Observations
Standard herniorraphy	2	2-3	1	Reintervention in cystocele
	4	3-5	2	Reintervention in cystocele and rectal deviation
	3	5-7	2	Reintervention in rectal modifications
Total	*9*	-	*5 = 55,5%*	
Standard herniorrhaphy and muscular transposition	3	2-3	-	
	6	3-5	-	
	3	5-7	2	Reintervention in prostate hernia and rectal deviation
Total	*12*	-	*2 = 16,6%*	
General total	**21**			

Surgical intervention was performed when rectal deviation was not corrected by colopexy or when prostatic benign hyperplasia was diagnosed. Rectal paresis favored further rectum deviation. The age of the hernia created problems for herniorrhaphy, but muscle transposition gave further resistance at this level.

The owner played a key role in the postoperative evolution of the patient, ensuring a balanced diet, in order to avoid constipation, and limited physical exercise, which lead to tissue toning.

Conclusions

1. Perineal hernia must be early diagnosed, because the age of the condition creates difficulties in reestablishing the continuity of the damaged tissues.
2. Difficulties in restoring the pelvic diaphragm are due to tissue rupture and atrophy, the standard herniorrhaphy method being followed by recurrence in 55,5% of cases.
3. Muscular transposition, by suturing two flaps obtained from the superficial gluteal and the semitendinous muscles, gives extra resistance to the herniorrhaphy, relapses being noticed in only 16.6% of the cases, due to incorrect management of deviated rectum or benign prostate hyperplasia.

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THE ANNUAL BOAR SPERMOGRAM IN A FARM FROM NORTH-WESTERN ITALY

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Abstract
To assess reproductive fertility of boar, semen quality control is essential. A male fertility depends on sperm quality. To know in detail the reproduction activity of boars from two farms and Offshore NV Italy, was prosecuted and made to ejaculate semen analysis to 8 boars during a year. Mean semen volume was 347.14 ml collected, obtained in winter, and 341.49 in summer. The highest values of the ejaculate volume was seen in winter. We note that depending on age and breed boars were obtained different values of concentration of ejaculate, the limits between 71.6 x109 (a Landrace 3 years) and 182.7 x109 (a MAXTER 1.5 years), average over the period under study being 109.4 x109 sperm. Note that in winter the number of cells throughout seminal ejaculation is much more than the results obtained in summer. This is explained in the literature, knowing that spermatogenesis is directly affected by the increasing ambient temperature. We say that seasonality has a severe impact on breeding boars processes.
Keywords: spermogram, seson, boar

Boars, evaluating the quality of semen is made by preparing complete and accurate boar sperm parameters of each part. Because of the importance it gives to, quality assessment semen striping to end compulsory microbiological semen analysis, identifying NTG / ml sperm, bacteria and fungi isolated pathogen susceptibility content and execution.

To assess reproductive fertility of boar, semen quality control is essential. Fecundity of a male depends on the quantity and quality of sperm.

Materials and methods

To know in detail the reproduction activity of boars from farms and mining, was prosecuted and made to ejaculate semen analysis to 8 boars during a year.

The data is monthly and seasonal calendar. Throughout the study of maintenance and feeding conditions were known and classified as the parameters.

For this study were followed several parameters: race, age, season, semen volume, its concentration per ml, concentration per ejaculate, and the number of doses of inoculated obtained.

Table 1 presents the number of absolute values of breeding parameters studied, and spermogramelor. Mean semen volume was 347.14 ml collected, obtained in winter, and 341.49 in summer. The highest values of the ejaculate volume was seen in winter, even if the volume point higher (477.6 ml) was encountered at a boar 2.5 years, the race Maxter during summer. Most other boars had the highest volume in winter, with appropriate boar Maxter of 1.5 years with a volume of 455.8 ml, followed by 4.5 years boar Maxter volume of 402 ml.

In contrast the minimum volume of ejaculate were obtained during summer. Thus the Durock breed boar 2 years was harvested this season, one of the smallest volume, 268 ml, and like the Landrace boar for three years, the volume was 206.4 ml.

Age boars ranged from 1 to 6 years. Note that depending on age and breed boars were obtained different values of concentration in seminal cells of the ejaculate, the limits between 71.6 $x10^9$ (a Landrace 3 years) and 182.7 $x10^9$ (a Maxter 1,5 years), average over the period under study being 109.4 $x10^9$ sperm.

Note that in winter the number of cells throughout seminal ejaculation is much more than the results obtained in summer. This is explained in the literature, knowing that spermatogenesis is directly affected by the increasing ambient temperature. We say that seasonality has a severe impact on breeding boars processes.

Table 1. Boar spermogram studying

No.	BOAR / SPERMOGRAM						
	bred / line	**age year**	**Seson**	**Vol.**	**Conc./ ml $_x10^9$**	**Conc./ ejaculate $_x10^9$**	**doses 3_x10^9**
1.	Pietrain	1	cold	270	0.420	113,4	38
			hot	340	0.215	73,1	25
			average yar	305	0,317	96,7	32
2.	Maxter	2,5	cold	385.6	0.310	119,5	39
			hot	477.6	0.188	89,8	30
			average yar	431,6	0.249	107,5	35
3.	Pietrain	6	cold	354.3	0.379	134,3	45
			hot	382.3	0.327	125	42
			average yar	368.3	0.353	130	44
4.	Maxter	4.5	cold	402	0.360	151,2	50
			hot	345	0.244	84,2	28
			average yar	373.5	0.302	112,8	39
5.	Landrace	3	cold	206,4	0,516	106,5	36
			hot	265	0,270	71,6	25
			average yar	235,7	0,393	92,6	31
6.	Duroc	2	cold	333	0,424	141,2	47
			hot	268	0,340	91,1	30
			average yar	300.5	0,382	114,6	39
7.	Marele alb	3,5	cold	370	0,441	163,2	54
			hot	284	0,364	101,9	34
			average yar	327	0,403	131,8	44
8.	Maxter	1,5	cold	455,8	0,403	182,7	61
			hot	370	0,304	112,5	37
			average yar	413	0,354	146,2	49
9.	average	-	cold	347.14	0,354	122,9	46
			hot	341.49	0,281	95,95	31
			yar	344.35	0,317	109,4	39

Average volume with the highest value was 431.6 ml, and was registered with a breed boar Maxter the age of 2.5 years, followed by boar Maxter of 1.5 years, with 413 ml. Similar values were obtained from Pieterain race of six years, where the average volume was 368.5 ml. The lowest average values were observed in a Landrace boar race three years old, the average volume of 235.7 ml.

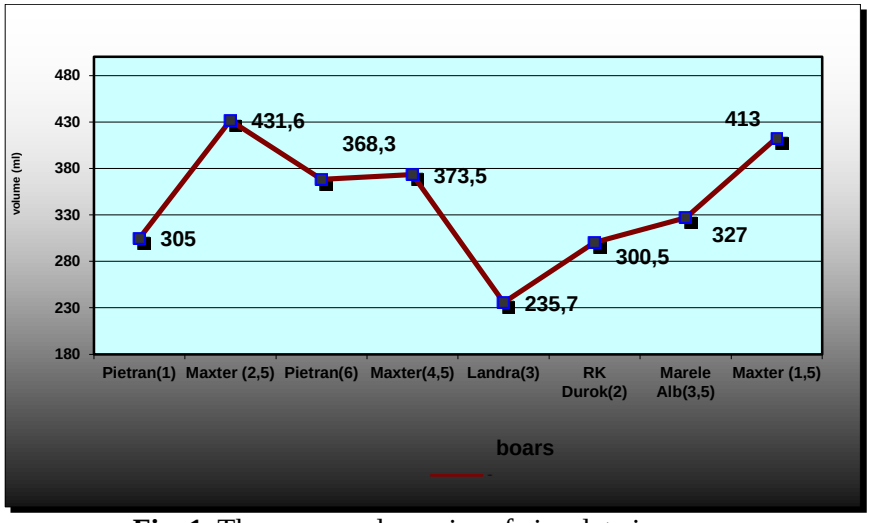

Fig. 1. The average dynamics of ejaculate in a year

On the average number of doses (with $3x10^9$) obtained from an ejaculate in one year, maximum of 49 doses was obtained from a boar Maxter of 1.8 years and 32 doses for a minimum of 1 year Pietrain . And mean dose was 39, fluctuations in the data values are influenced spermatogenesis and perfect adaptability and breeds (Fig. number 2).

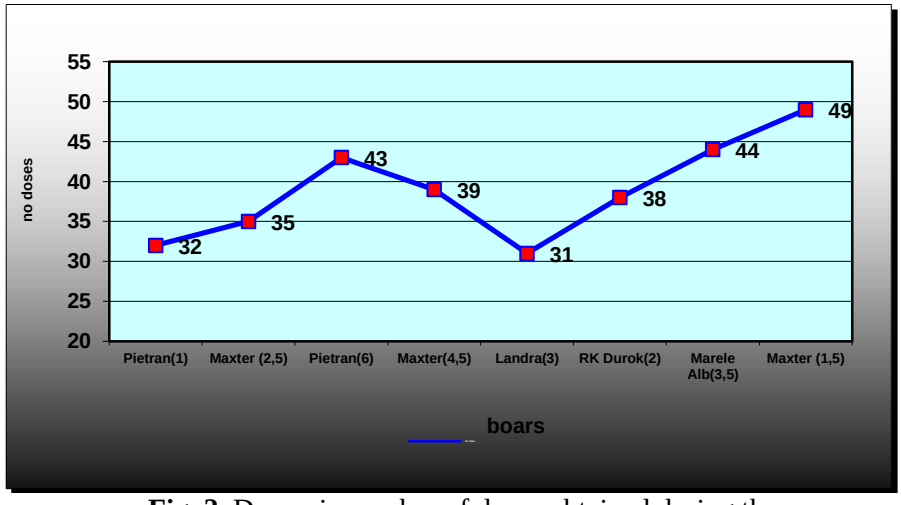

Fig. 2. Dynamic number of doses obtained during the year, depending on the breed

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Conclusions

1. 1 Depending on age and breed boars have obtained different values of concentration in seminal cells of the ejaculate, the limits between 71.6 $x10^9$ (a Landrace 3 years) and 182.7 $x10^9$ (a Maxter 1.5 years), average over the period under study being 109.4 $x10^9$ sperm.
2. 2 In the winter the number of cells throughout seminal ejaculation is much more than the results obtained in the summer. This is explained in the literature, knowing that spermatogenesis is directly affected by the increasing ambient temperature. We say that seasonality has a severe impact on breeding boars processes.
3. 3 The average of the highest value was 431.6 ml, and was registered with a breed boar Maxter the age of 2.5 years, followed by boar Maxter of 1.5 years, with 413 ml. Similar values were obtained from Pieterain race of six years, where the average volume was 368.5 ml.

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HEMATOLOGICAL AND BIOCHEMICAL INVESTIGATION OF RUMINAL ALKALOSIS IN CATTLE

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Abstract
Because the ruminal alkalosis is an acute or subacute, often chronic, primary food indigestion, characterized and induced by alkaline forestomach content and general disturbances due to metabolic alkalosis caused (according to history, a high protein feeding rations and poor in carbohydrates, but also to feed silage defects, which contained a high percentage of free ammonia or altered feed, suddenly introduced), is responsive for inducing obvious imbalances between ruminal microbial species. Thus, the mentioned causes has determined a ruminal microbial imbalance with excessive proliferation of germs, which leads to ammonia, ammonia salts and amine base increase, along with volatile fatty acid synthesis decrease, explaining the exaggerated alkaline rumen content and systemic-metabolic alkalosis. To confirm and establish a more precise diagnosis and to optimize the therapeutic approaches, the clinical data performed in animals from the studied group, were corroborated with the laboratory tests results (hematological examination, biochemical and ruminal contents examination). pH of rumen contents, collected from diseased animals (7.9 ± 0.1), present mean values significantly higher ($p < 0.05$) than control group (6.9 ±0.3). At the same time, on standard bicarbonate growth, occurs a blood pH increase (7.7 ±0.1), but without statistical significance

Keywords: cattle, ruminal alkalosis

Materials And Methods

For this study were selected, randomized, 8 cattle (n = 8) with general digerstive disorders, based on history that indicate their feeding with hyper – proteic and low carbohydrates rations but also with inadequately ensiled feed, which contained a high percentage of free ammonia, with pH above 5.5 or altered feed, especially if these rations were introduced suddenly.

Animals were selected in these groups, depending on the ruminal contents pH, obtained by ruminocentesis (1,6). The rumen content was harvested 4-6 hours after feeding and pH determination was made, with a portable pH meter and the pH indicator paper (Fig.1). Thus, LAlc group – was consisted of 8 cattle, which directly determined the ruminal content pH, immediately after harvest, have values between 7.5 and 8.3 (4).

Results of studies have shown that between blood pH and ruminal content are closely connected, relationship observed by other authors (3,4).

The animals from group LAlc, which had a ruminal content pH significantly higher ($p<0.05$) than the control group, were found to have in the same time an blood pH increase ($p>0.05$), with no statistically significant. Therefore, routine monitoring of rumen contents alkalinity, can be an efficient parameter in the diagnosis of ruminal alkalosis in cattle (4).

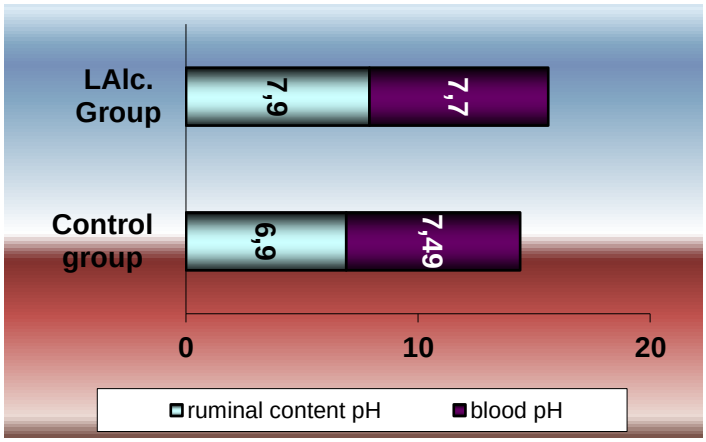

Fig. 1. The dynamic of mean values of blood, urine and ruminal content pH in cattle with ruminal alkalosis

Table 1. The mean values of hematological and biochemical parameters in cattle with a high pH of ruminal contents

Parameters	Control group	Experimental group LE-IBS	
Leucocytes *($x10^3/mm^3$)*	7.7±0.60	7.9±0.60*	min. 7,2
			max.8,6
Limphocytes *(%)*	53.5±3.8	53±3.8*	min. 49
			max.57
Neutrophils *(%)*	36±2.3	39±2.6*	min. 32
			max.46
Eosinophils *(%)*	4.7±0.35	4.8±0.2*	min. 3
			max.6
Basophils *(%)*	0.24±0.08	0.12±0.07*	min. 0
			max.1
Monocytes *(%)*	2.25±0.1	3.1±0.08*	min. 2
			max.4
Mg *(mg/dl)*	2.2±0.5	1.6±0.5**	min.1,4
			max. 1,8
Ca *(mg/dl)*	9.2±0.5	8.7±0.5*	min. 8,4
			max.9,0
HCO_3^- (mmol/l)	25±0.1	28.3±2.6**	min. 27,2
			max. 29,4

** p>0,05 – insignificant differences; ** p<0,05 - significant differences*

It is well known the role of magnesium in protein synthesis, being an activator of various fermentation processes (7).

Hypomagnesiemia registered in cattle from the investigated group (Table 1, Fig. 2,3), may be due to a low feed magnesium level or resorption disorders due to an excess of nitrogenous substances and can be clinically translated by nervous disorders.

In cattle from the investigated group, the hypomagnesemia is accompanied by a calcium serum decrease below normal values (with mean values between 8.7 ±0.5 mg/dl), but is a decrease without statistical significance.

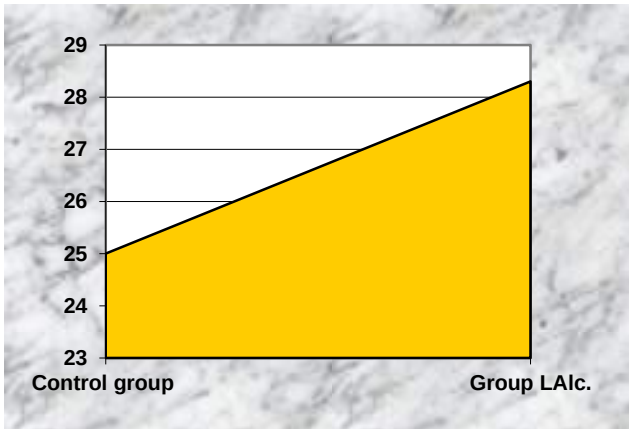

Fig. 2. The dynamic of mean values of bicarbonate serum levels in cattle from group LAlc

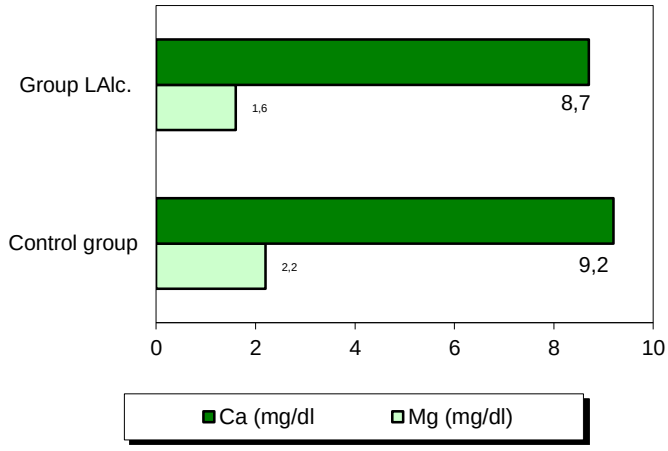

Fig. 3. The dynamic of mean values of magnesium and calcium serum levels in cattle from group Lalc

Conclusions

1. After hematological examinations in animals with ruminal alkalosis, we have registered an slight leukocytosis (7.9±0.60 x10^3/mmc blood) with neutrophilia (39%) without statistical significance ($p>0.05$), as due to superimposed infections (over these biochemical indigestion).
2. The performed biochemical evaluations revealed in animals with ruminal alkalosis an increasing of the serum bicarbonate (28.3±2.6 mmol/l) with statistically significant ($p <0.05$), and hypomagnesemia (1.6 ± 0.5 mg/dl), with mean values, significantly lower ($p <0.05$) than control group (2.2 ± 0.5 mg /dl).
3. Hypomagnesemia is accompanied by serum calcium decreasing (below normal values), in the investigated group, the registered values were between 8.7 and 0.5 mg/dl (without statistical significance).
4. The pH of the rumenal content, collected from diseased animals (7.9 ±0.1), present the mean values significantly higher ($p<0.05$) than in control group (6.9 ± 0.3) and in addition to standard bicarbonate increasing was recorded a blood pH increasing to 7.7 ±0.1 (without statistical significance).

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OBSERVATION REGARDING HEMATOLOGICAL AND BIOCHEMICAL INVESTIGATION IN RUMINAL ACIDOSIS IN CATTLE

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Abstract
Lack of early appearance of clinical signs with highly diagnostic specificity and their delayed appearance (even few weeks or months of onset of the disease), causes ruminal acidosis to be detected, usually late, especially in individual household system. In cattle with ruminal acidosis, after hematological and biochemical examination, the results revealed that additional to haemoconcentration (consecutive to excessive saliva secretion necessary to neutralize the ruminal pH decrease) have been noticed an increasing of the average values of red blood cells, hemoglobin and hematocrit (PVC), accompanied in severe cases (with superimposed infection) by a neutrophilia leukocytosis, and proteinuria, correlated with an apparent normoproteinemia and normoalbuminemia. Also the ruminal content pH values were in strictly correspondence blood and urine pH values.
Keywords*:* cattle, ruminal acidosis

Materials and methods

The selected animals were divided into 3 groups according to their pH ruminal contents, obtained by rumen puncture (1, 7). Ruminal content was taken 4-6 hours after forage intake and pH determination was performed using the portable pH meter and pH indicator paper (pH Box -Merck).

The cattle were divided into three groups as follows:

- LAc1 group – consisting of five cattle, the rumen contents had pH>5.8;
- LAc2 group - consisting of 9 cattle, the rumen contents had pH between 5.5 to 5.8;
- LAc3 group - consisting of 3 cattle, the rumen contents had pH<5.5 (range 5.0 to 5.5).

In animals from these three groups blood samples were collected from the jugular vein, and sent to the laboratory for hematologic and biochemical investigation. After blood collection (immediately), blood pH was determined, and for possible correlation was determined the urinary pH (2, 6).

Results and discussions

In animals from groups who had a low pH ruminal content, was performed a thorough clinical examination, noting a different symptoms depending on form of disease (4, 8).

The results showed that between the pH of blood, urine and ruminal content are closely connections (5).

Thus, in animals from group Lac 3, which had a pH of the ruminal contents, significantly ($p<0.05$) lower than the control group, it was observed that they presented, in the same time, lower blood ($p<0.05$) and urine ($p>0.05$) pH (Table 1).

Table 1. pH mean values of blood, urine and ruminal content in cattle with ruminal acidosis

Groups	pH mean values		
	Ruminal content	**Blood**	**Urine**
Control group	6.9±0.3	7.49	8.33
LAc 1	6.1±0.38	7.47	8.34
LAc 2	5.9±0.30**	7.44	8.27
LAc 3	5.7±0.1**	7.42**	8.13*

** p>0,05 – insignificant differences; ** p<0,05 - significant differences*

From this point of view can be appreciate the fact that the routine monitoring of urine pH may be an efficient parameter in the diagnosis of ruminal acidosis in cattle.

In all animals from the three experimental groups (which have low pH of the ruminal contents), the mean values of bicarbonate ion ($HCO3^-$), recorded statistically significant decreasing ($p<0.05$), compared with control group (Table 3).

In animals from control groups the decreasing of the pH level is accompanied by different degrees of haemoconcentration, decreasings of alkaline reserve, total protein levels, haemoglobin and hematocrit (Table 2).

Because the persistent biochemical rumen irritation, some animals developed rumenal inflammations (4), which worsened by the development of bacterial imbalances, and finally led to superimposed infection as reflected by leukocytosis (8.03 ± 0.6/mmc blood) with neutrophilia (39.3 ± 2.6%), with statistical significance, especially in animals of groups Lac 2 and Lac 3 (fig. 1).

Was statistically registered a significant decreasing ($p<0.05$) of the bicarbonate ion, in cattle from the experimental groups (which had a lower ruminal contents pH), due to increased cleavage of bicarbonates, which results in carbonic acid, which contributes the establishment of a metabolic acidosis.

Table 2. The mean values of hematological parameters in cattle with a low pH of ruminal contents

Parameters	Control group	Experimental groups		
		LAc 1 (pH=6,1±0,38)	**LAc 2 (pH=5,9±0,30)**	**LAc 3 (pH=5,7±0,1)**
Erythrocytes *(mil./mm^3)*	6.6±0.5	6.92±0,5*	7.24±0,6**	7.11±0,6**
		7.09±0,6**		
Hb *(g/dl)*	9.75±0.82	10.4±0,9*	10.7±0,9*	10.5±0,9*
		10.5±0.9*		
HCT *(%)*	36.1±3.1	37.7±4.2*	37.9±4.4*	38.6±4.6**
		38.06±4.6**		

MCV □□□□	54.5±4.6	54.4±4.9	52.2±4.2	54.2±4.7
		53.6±4.7*		
MCH *(pg)*	14.8±1.0	15.04±1.1	14.7±1.0	14.6±1.0
		14.7±1.0*		
MCHC *(g/dl)*	27.3±2,0	27.5±2.0	28.1±2.2	27.1±2.0
		27.5±2.0*		
Leucocytes *($x10^3/mm^3$)*	7.7±0.60	7.8±0.5	8.2±0.8**	8.1±0.6**
		8.03±0.6**		
Limphocytes *(%)*	56.5±3.8	54±3.9	53±3.8	52±3.7
		53±3.9		
Neutrophils *(%)*	36±2.3	37±2.6*	40±2.6**	41±2.6**
		39.3±2.6**		
Eosinophils *(%)*	4.7±0.35	5±0,4	4±0.3	3.6±0.3
		4.2±0.3*		
Basophils *(%)*	0.24±0.08	0.2±0.07	0.1±0.07	0.3±0.07
		0.2±0,07*		
Monocytes *(%)*	2.25±0.1	3.4±0.1	2.4±0.1	2.3±0.1
		2.5±0.1*		

** p>0,05 – insignificant differences*
*** p<0,05 - significant differences*

Table 3. The mean values of biochemical parameters in cattle with a low pH of ruminal contents

Parameters	**Control group**	**Experimental groups**		
		LAc 1 (pH=6.1±0.38)	**LAc 2 (pH=5.9±0.30)**	**LAc 3 (pH=5.7±0.1)**
Proteins *(g/dl)*	7.0±0.3	6.9±0.3	7.0±0.3	6.8±0.3
		6.8±0.3*		
Albumin *(g/dl)*	4.1±0.2	4.0±0.2	4.2±0.2	3.8±0.2
		4.0±0.2*		
Globulin *(g/dl)*	2.9±0.1	2.9±0.1	2.8±0.1	3.0±0.1
		2.9±4.6*		
HCO_3^- (mmol/l)	25±0.1	22.3±1.1	21.7±1.1	20.5±1.1
		21.5±1.1**		

** p>0,05 – insignificant difference; ** p<0,05 - significant differences*

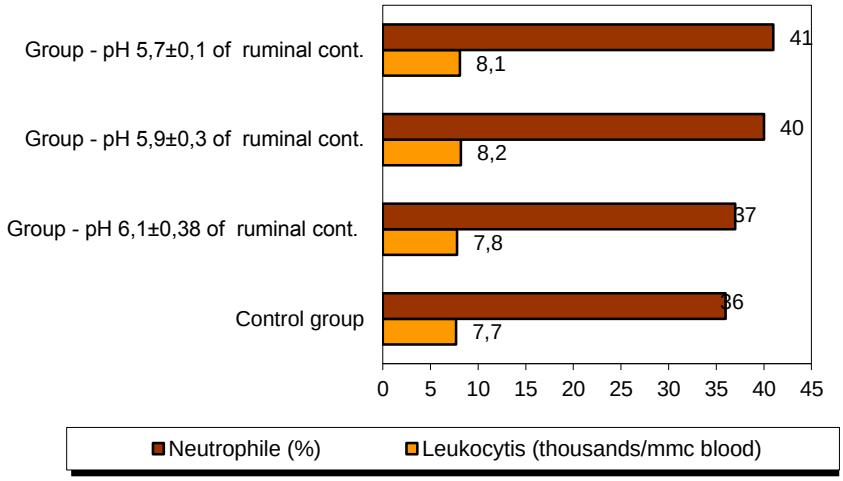

Fig. 1. The dynamics of average values of white cells and neutrophils in cattle from control and studied groups

Conclusions

1. After hematologic examination, in cattle with acid ruminal indigestion, shows that in such cases the lower pH ruminal content is associated with humoral changes, dominated by various degrees of haemoconcentration (reflected by increases in the average number of erythrocytes, hemoglobin and hematocrit), accompanied by proteinuria, correlated with an apparent normal level of albumin and total serum proteins.
2. Thus, due to persistent biochemical irritation of the rumen, some animals showed ruminal inflammation, exacerbated by the development of bacteria, which eventually led to superimposed infection, reflected by induction of neutrophilic leukocytosis of statistical significance.
3. Was registered an obvious bicarbonate ion decreasing, with statistically significant ($p<0.05$), in cattle with ruminal acidosis, due to increased cleavage of bicarbonates, which results in carbonic acid, responsible for establishing the metabolic acidosis.
4. The comparative analysis of the results revealed the existence of positive correlations between the pH of blood, urine and ruminal content, because cattle with significant decreasing of the ruminal pH ($p<0.05$), presented in the same time the most low blood pH ($p<0.05$) and urine pH ($p>0.05$), reasons which allow the recommendation of routine monitoring of the urine acidity, as a efficient screening in the diagnosis of ruminal acidosis in cattle.

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RESEARCH ON THE ANTIHELMINTHIC TREATMENT EFFICACY OF PRODUCTS BASED ON MACROCYCLIC LACTONES (IVERMECTIN) IN HORSES STRONGYLIDOSIS AND THE DYNAMIC OF EGGS COPRO-ELIMINATIONS, FOLOWING IMMEDIATELY ADMINISTRATION OF ANTIPARASITIC SUBSTANCE

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Abstract
Strongyl equine parasites are most prevalent group among populations of horses, both in our country and internationally. The measures to control these parasitoses are mainly based on regular deworming programs using different antiparasitic compounds. Numerous studies have revealed the installing of resistance phenomen to some of these antiparasitic compounds so in the case of Strongyls species and in the case of other digestive helmints of horses. In this study, we examined the efficacy of macrocyclic products (ivermectin) in strongylidosis using FECRT test (faecal egg count reduction test) while watching pre and post treatment copro-eliminations dynamic. We studied a total of six horses belonging to Iassy Equestrian Base, who were treated with an oral paste based on 1.87% ivermectin (Noromectin) at a dose of 0.2 mg / kgc. Using coproparasitological quantitative methods it was observed the increasing of egg load immediately after the treatment and gradually decreasing to extinction, around day 14. Based on these determinations, we obtained a 100% efficacy of treatment to all horses, and we observed the absence of Strongyls resistance phenomen to ivermectin in samples studied.

Key words : horses**,** strongylidosis, ivermectin, efficacy, resistence

Introduction

Of the horses parasitosis with digestive localization, Strongylidosis plays a crucial role both in their spread and in terms of their patogenicity. It is known that the Strongylidosis prevalence often reaches 100%, affecting all age groups. However, Strongyls carried out on hosts a variety of pathogenic actions, especially given to large number of parasite species and to many stages of development taking place in the same host in different body tissues and organs. Sometimes there is a high percentage of morbidity and even mortality, especially among youth treated, thus causing multiple damages of medical, economic and animal welfare concerns (Covașă, 2011). The presence of these parasites can alter digestive behavior, fertility, fitness, youth development, resistance to other pathogens and performance for which the animals are bred (Cernea, 2008).

Internationally, the study of equine Strongylidosis is a topical issue, particularly regarding the implementation of effective measures to control these morbid entities. Antihelminthic treatments are the basis for these purpose, so that it requires ongoing assesment of results abtained following administration of antihelminthic substances.

Development of species and parasites population resistant to one or more antihelminthic sustances is a very topical issue (Morariu, 2007).

The most current available and used antihelminthic substances used in parasitologic control belong to three main groups : benzimidazole, imidazole and avermectin (Uhlinger, 1992, Von Samson-Himmelstjerna, 2006).

Successful introduction of parasitological control programs, made to limit resistance development in populations of nematodes, depends in a certain degree on population availability and the methods used for detection and monitoring. For these purpose were designed numerous *in vivo* and *in vitro* tests for detection of resistance of major nematode populations groups to antihelminthic substances (Morariu, 2007).

In this paper, we propose an analysis based on ivermectin efficacy to combating Strongyls in horses, using qualitative and quantitative coproparasitological ovoscopic techniques, respectively FECRT test (faecal egg count reduction test).

Materials and methods

Coproparasitological research was conducted on a six horses belonging to Iassy Equestrian Base. Five of them were males and one female specimen, aged between 2 and 16,5 years. Dates on each animal are listed in Table 1. The research was conducted in April 2011.

Table 1. Dates on studies horses

Crt. no.	Breed	Age years	Sex	Growth mode
1	Frisian	4.5 ani	♂	Loose housing
2	Frisian	4 ani	♂	Loose housing
3	Sport roumanian horse	2 ani	♂	Loose housing
4	Sport roumanian horse	18 ani	♂	Loose housing
5	English thoroughbred	16.5 ani	♂	Loose housing
6	Metis	5 ani	♀	Loose housing

From each animal were collected samples (about 150 g each) of fresh faeces removed the day before administration antiparasitic substance (day 0), and in the near future, namely 24 hours, then 3, 5, 7 and 14 days.

The Willis and Teleman-Rivas qualitative ovoscopic methods and Baermann larvoscopic method are used for the identification of parasitic strongyls. McMaster quantitative method was used to determines the individual parasitic load of horses pre and post treatment, respectively EPG (eggs per gram of feces).

The studied animals were treated orally with ivermectin 1.87% (Noromectin) at a dose of 0,2 mg/kg, to verify the effectiveness of this substance on Strongyl nematodes. All horses were previously treated regularly twice a year with ivermectin based products.

Subsequently, based on EPG values, the test of reducing of eggs disposal FECRT (*faecal egg count reduction test*) was carried. Anthelmintic efficacy of the product was calculated using the formula:

E% = (Before treatment EPG (day 0) – day 14 EPG /EPG day 0) x100

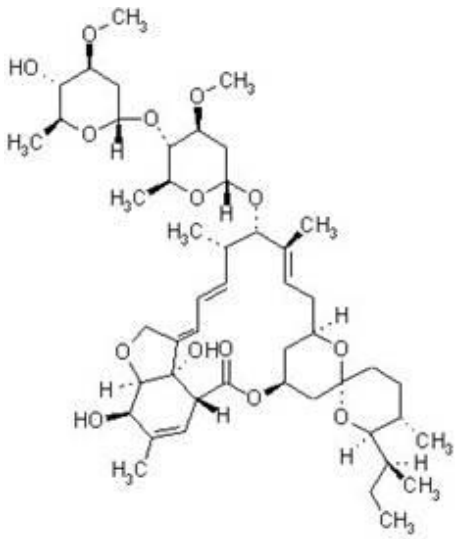

Fig.1. Chemical structure of ivermectin
(22,23-dihidroavermectin B_{1a} + 22,23-dihidroavermectin B_{1b})

Results and discussions

We identified parasitic strongyl elements, namely eggs of different sizes and L3 infestant larvae, based on coproparasitological analysis (fig. 2 and fig. 3).

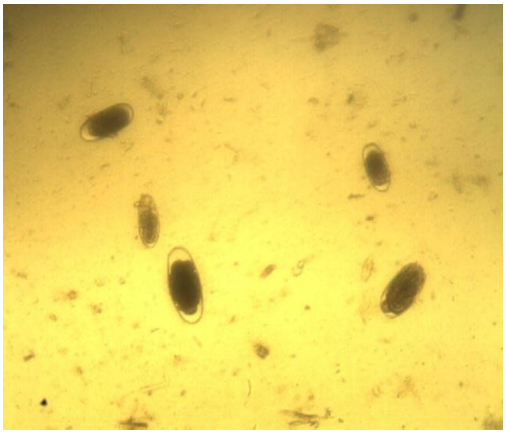

Fig. 2. Strongyl morulated eggs
x100

Fig. 3. L3 Strongyl infestant larva
x100

The average values of parasite loading the day before treatment (day 0) were between 100 and 800 EPG. 24 hours after the treatment were obtained higher values of EPG, between 300 and 1200, thereafter gradually decreasing to day 14, when their values was 0 (Table 2).

Table 2. Ante and post-treatment eggs coproeliminations dynamic

Crt. No.	EPG					
	DAY 0	**DAY 1**	**DAY 3**	**DAY 5**	**DAY 7**	**DAY 14**
1.	**800**	**1200**	500	250	100	0
2.	500	750	400	200	100	0
3.	450	650	400	150	0	0
4.	350	500	350	150	100	0
5.	350	600	300	100	0	0
6.	**100**	**300**	200	0	0	0
Average	**425**	**675**	**358**	**141**	**50**	**0**

It was observed that the effect of antiparasitic administration is an increase of eggs elimination immediately after the treatment, followed by the progressive decrease of the feces eggs number. There is a total reduction of EPG to a single individual, on the fifth day after treatment, until negative results on the 14 th day to all individuals .

After treatment with ivermectin FECRT results are shown in Table 3.

It can be seen that the parasite load is significantly reduced from the 7th day, when the lowest value was 87,5% FECRT until the 14th day when FECRT was 100% to all horses. These results confirm the good antihelmintic activity of ivermectin (effectively 100%). We can say also, that the strongyls helminths infrapopulations of studied horses, not shown signs of resistance to this substance.

Table 3. FECRT values obtained after ivermectin treatment

Crt. No.	Average EPG Day 0	Average EPG Day 7	FECRT %	Average EPG Day 14	FECRT %
1.	800	100	87,5	0	100
2.	500	100	87,5	0	100
3.	450	0	100	0	100
4.	350	100	87,5	0	100
5.	350	0	100	0	100
6.	100	0	100	0	100

However, nationally and globally, was found the antihelminthic substances resistance of horse strongyls and of other horse helminths. In our country, Cernea et al., noted in 2004 this phenomenon to horses from Bistrita Năsăud lands, where was reported ciatostomes resistance to albendazol treatment.

Resistance to benzimidazoles has been reported in North America, South America, South Africa, Australia, New Zealand, Turkey and several European countries (Çirak, 2004). If we analyze the spreading situation of anthelmintic substances resistance in Europe, we can notice a slight extension of the phenomenon from west to

east, affecting countries with tradition in raising horses, like England, Holland and Germany (Morariu, 2007). Thus, in some of these countries benzimidazoles resistance of ciatostomes reaches 70% or more in horse farms (Kuzmina, 2008). Recent studies have shown the installation of benzimidazole resistance in Ukraina and Turkey (Kuzmina, 2008; Çirak, 2004).

Due to increased effectiveness and broad spectrum of action, ivermectins tend to become the most widely used class of antiparasitic substances. In Europe, as in our country, there is little dates showing the emergence of Strongyls resistance to treatment with ivermectin. However, there are recent studies (Ricardo, 2010, Lyons, 2010) which showed only reduced efficacy of these compounds by reducing the period of eggs recurrence in faeces after treatment . This was particularly highlighted for ivermectin, maintaining good efficacy for moxidectin (Lyons, 2010). In exchange was reported in several studies, ivermectin resistance of *P. equorum*, both in Europe and in America (Kuzmina, 2008).

Conclusions

1. Ivermectin, like other antiparasitic compounds, has effect immediately after administration , on a feces load of parasitic elements increase, followed by a decline to extinction around the 14th day after treatment.
2. Although the study is relatively small, we can say that macrocyclic compounds exhibit good antihelminthic activity for equine strongylidosis, given 100% of FECRT test values obtained .
3. We believe that the absence of resistance to ivermectin in this case are due to regular annual deworming plan.
4. If internationally were reported numerous cases of chemoresistance, in our country the phenomenon is still under widespread, but ongoing.
5. We consider that low frequency of Strongylidae or other helmints resistance installation phenomenon to macrocyclic lactones and other antiparasitic substances has the right cause the recent use of these substances in antiparasitic treatment unlike most Western countries where they were put on the market longer.
6. There is a higher probability of antihelmintic resistance apparition in semiintensive growth system, due to applicattion of more or less of dewormings programs; in extensiv systems, resistance installation possibility is almost zero, due to total lack of such treatments.
7. As demonstrated in many previous studies, ivermectin remain, especially in our country, a higher class of antiparasitic compounds, both in terms of spectrum and treatment effectiveness.
8. Investigation results of antiparasitic treatment is a main direction in the future of equine parasitology.

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ETIOLOGICAL AND EPIDEMIOLOGICAL DETERMINATIONS OF INFESTATION WITH STRONGYLIDAE IN HORSES

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Abstract
Strongylidosis and ciatostomosis are the most common digestive parasitoses in horses, most animals being affected more or less. This study reveals etiological and epidemiological situation of these parasitoses for equines coming from some areas of Moldova, Iassy and Neamt respectively. It was made a comparative study on these issues between horses coming from extensive and semiintensive growth system. Using coproscopic methods it was determined a 100% strongylidosis prevalence, with large and small strongyls among horses studied. Invasion intensivity was generally medium, with values between 100 and 2200 OPG. It was observed a medium to low level in horses from farms and a medium to slightly raised in horses from the extensive system. There were no significant differences in the influence of race, age, sex. Aanalysis of faeces collected helmints revealed the following species: we met frequently Strongylus vulgaris from the category of large strongiyls and Cylicostephanus longibursatum the most common nematode from the category of ciatostomes. With a low incidence and relatively similar we met also species like Cylicostephanus calicatus, Cyathostomum pateratum, Cyathostomum catinatum and Coronocycus labiatus caracterised by female sex dominance.

Key words : horses**,** strongylidosis, extensivity, intensivity, etiology

Introduction

Equine strongyles (Nematoda, Strongylidae) are a large group of intestinal nematodes of relevant importance in equine clinical practice (Traversa, 2010). This group encompasses two subfamilies, Strongylinae ("large strongyles") and Cyathostominae ("small strongyles"), is known so far 19 genera and 64 species (Lichtenfels, 2008). The occurrence of small strongyles is associated with various types of colics (Murphy and Love, 1997; Mair et al., 2000) and with decreased rates of performance, rough hair coat and debilitation (Uhlinger, 1991). More importantly, the simultaneous reactivation of larval stages encysted in the gut wall may lead to a clinical syndrome called "larval cyathostominosis", represented by a severe inflammatory enteropathy affecting the caecum and colon. This disease is a colitis characterized by weight loss, severe diarrhoea, loss of proteins, subcutaneous edema, with a 50% fatality rate even if a timely anthelmintic treatment is administered (Giles et al., 1985; Eysker et al., 1989, 1990; Love and McKeand, 1997).

Whereas these parasitoses affect all age groups, the diversity of economic, medical or welfare damages is more pronounced. Infestation with these digestive parasites can alter behavior, fertility, fitness, youth development, or may decrease resistance to other pathogens (Cernea, 2008).

Strongyls equine study both in our country and internationally, occupies an important place on the equine digestive parasites in order to improve control measures of such morbid entity. Various evolutionary aspects require to be permanently analyzed, including knowledge of some epidemiological indicators or the parasites etiology. Folowing coproparasitological tests we studied the extensivity and intensivity of strongyls infestation and the etiological diversity existing in horse population from the studied areas.

Materials and methods

Strongylidosis diagnosis was made with coproparasitological methods on faeces from horses coming from extensive and semi-intensive system too. Horses studied from the extensive system belonged to population from Iassy limtrofe areas, a total of 29 animals aged between 9 months and 26 years being examinated. Six horses from semi intensive system aged between 2 and 16.5 years came from Iassy Equestrian Base and the remaining 23 horses aged between 3 and 20 years, were from the Stallions Store Dumbrava, Neamt County. Animals belonging to these two equestrian centers regularly received antiparasitic treatment, twice a year, while the other did not receive such treatment.

From each animal were collected two samples of faeces per day (about 150 g each), freshly removed morning and evening, for three consecutive days. Faecal samples were examined qualitatively and quantitatively. Parasitic elements were identified using microscopic direct examination of simply or with Lügol solution slides, Willis and Teleman-Rivas ovoscopic methods and Baermann larvoscopic methods. McMaster and Stoll methods were used to quantify these parasitic elements (Cosoroabă, 2002). Identification of macroscopically visible helmints from faecal and intestinal contents, was performed by harvesting, fixation in 70% alcohol solution and then clarification in lactic acid followed by subsequent microscopic examination of morphological characters.

The research was conducted during 2010 and 2011, and tests or analyzes were carried out in parasitology laboratory of the Faculty of Veterinary Medicine Iassy.

Results and discussions

Following coproparasitological qualitative analysis, we observed and identified strongyl parasitic elements, that eggs of different sizes and L3 infestant larvae (fig. 1 and fig. 2).

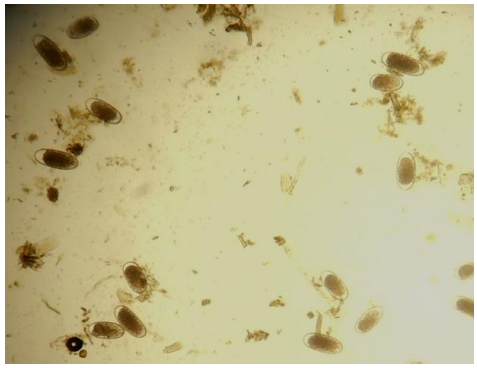

Fig. 1. Strongyl type morulated eggs, x100

Fig. 2. Strongyl infestant larva L3, x100

From all horses were seen eggs with strongyls or ciatostomes specific morphological characteristics, larvae being more difficult to distinguish. It found a widespread infestation with strongyls on horses coming from these areas, that an 100% extensivity. In terms of quantity, the mean level of parasitic load (OPG – eggs/gram of faeces) was between 100 and

2200 OPG (Table 1 and 2). There were no major variations between the results obtained in the 3 days under study, values being close or similar.

Table 1. Average OPG values obtained on horses coming from extensive system

Crt. no.	O.P.G.values			Breed	Age	Sex	Growth mode
	Day 1	Day 2	Day 3				
1	150	150	200	Metis	13 years	♂	Loose housing and grazing
2	400	420	480	Metis	6 years	♂	Loose housing and grazing
3	500	600	600	Metis	7.5 years	♂	Loose housing and grazing
4	600	660	700	Metis	7 years	♂	Loose housing and grazing
5	450	350	400	Metis	4 years	♂	Loose housing and grazing
6	600	630	650	Metis	8.5 years	♀	Loose housing and grazing
7	450	350	400	Metis	9 months	♀	Loose housing and grazing
8	300	400	330	Metis	3 years	♀	Loose housing and grazing
9	400	300	350	Metis	19 years	♀	Loose housing and grazing
10	100	200	200	Metis	8.5 years	♀	Loose housing and grazing
11	320	300	350	Metis	20 years	♂	Loose housing and grazing
12	300	300	400	Metis	15 years	♂	Loose housing and grazing
13	750	650	700	Metis	13.5 years	♂	Loose housing and grazing
14	430	500	400	Metis	10 months	♂	Loose housing and grazing
15	500	500	450	Metis	3 years	♂	Loose housing and grazing
16	530	500	550	Metis	5 years	♂	Loose housing and grazing
17	450	450	500	Metis	4.5 years	♂	Loose housing and grazing
18	600	650	650	Metis	7 years	♂	Loose housing and grazing
19	500	400	400	Metis	10 years	♀	Loose housing and grazing
20	800	780	750	Metis	13 years	♀	Loose housing and grazing
21	540	600	550	Metis	17 years	♂	Loose housing and grazing
22	450	370	400	Metis	9 months	♂	Loose housing and grazing

23	400	300	400	Metis	2.5 years	♀	Loose housing and grazing
24	550	570	600	Metis	8 years	♀	Loose housing and grazing
25	650	600	550	Metis	1 year	♂	Loose housing and grazing
26	900	900	880	Metis	25 years	♀	Loose housing and grazing
27	950	900	900	Metis	26 years	♂	Loose housing and grazing
28	1900	2200	2000	Metis	8 years	♂	Loose housing and grazing
29	650	750	700	Metis	6 years	♂	Loose housing and grazing

The values between 150 and 2200 OPG with an average around 800 OPG were obtained on horses from family households. These values shows generally a moderate or slighty increased infestation. No differences were observed in representative regarding age and gender factors, the values being close.

Table 2. Average OPG values obtained from horses bred in semi -intensive system

Crt.no	**O.P.G.values**			**Breed**	**Age years**	**Sex**	**Growth mode**
	Day 1	**Day 2**	**Day 3**				
1	750	800	700	Frisian	4.5	♂	Loose housing
2	450	450	400	Frisian	4	♂	Loose housing
3	450	400	550	Sport roumanian horse		♂	Loose housing
4	350	300	340	Sport roumanian horse	18	♂	Loose housing
5	320	340	330	English thoroughbre	16.5	♂	Loose housing
6	100	150	130	Crossbreed	5	♀	Loose housing
7	300	330	300	Semi hard	10	♂	Loose housing
8	500	420	590	Semi hard	10	♂	Loose housing
9	150	150	200	Semi hard	6	♂	Loose housing
10	100	150	150	Semi hard	8	♂	Loose housing
11	450	550	550	Semi hard	3	♂	Loose housing
12	300	300	300	Semi hard	3	♂	Loose housing
13	200	200	250	Sport roumanian horse	8	♂	Loose housing
14	430	400	380	Sport roumanian horse	5	♂	Loose housing
15	100	100	200	Sport roumanian horse	9	♂	Loose housing
16	350	300	300	Sport roumanian horse	8	♂	Loose housing
17	400	330	450	Sport roumanian horse	13	♂	Loose housing
18	250	200	200	Sport roumanian horse	15	♂	Loose housing

19	400	400	450	Lipiţan	20	♂	Loose housing
20	350	400	450	Lipiţan	10	♂	Loose housing
21	300	350	300	Lipiţan	10	♂	Loose housing
22	550	650	600	Lipiţan	7	♂	Loose housing
23	230	180	200	Lipiţan	4	♂	Loose housing
24	400	450	400	Lipiţan	6	♂	Loose housing
25	400	400	400	Lipiţan	9	♂	Loose housing
26	300	350	250	Lipiţan	10	♂	Loose housing
27	300	300	300	Lipiţan	10	♂	Loose housing
28	250	300	350	Lipiţan	18	♂	Loose housing
29	200	200	200	Bucovina horse	3	♂	Loose housing
30	300	300	300	Bucovina horse	3	♂	Loose housing

Strongylid infestation of horses bred in semi-intensive system had OPG values between 100 and 800 , with an average around 500 OPG. One speaks in this case by a medium to low infestation. Again, the influence of intrinsic or extrinsic factors such as sex, age, race, or the growth mode of animals was insignificant, all types of horses being affected similarly by these parasitoses.

In terms of strongylid identified population, we found an increased incidence of *Strongylus vulgaris* (fig. 3, 4, 5) as representative for large strongyls. *Cylicostephanus longibursatus (Trichonema longibursatum)* (fig. 6, 7) was the most common species of cyatostomes. With a lower rate and a similar prevalence, was found *Cylicostephanus calicatus* species (fig. 8, 9), *Cyathostomum pateratum (*fig. 10, 11, 12), *Cyathostomum catinatum* (fig. 13) and *Coronocycus labiatus* (fig. 14, 15). Female sex predominance was found in a ratio of 3/1 for *Strongylus vulgaris* and 4/1 for cyatostomes.

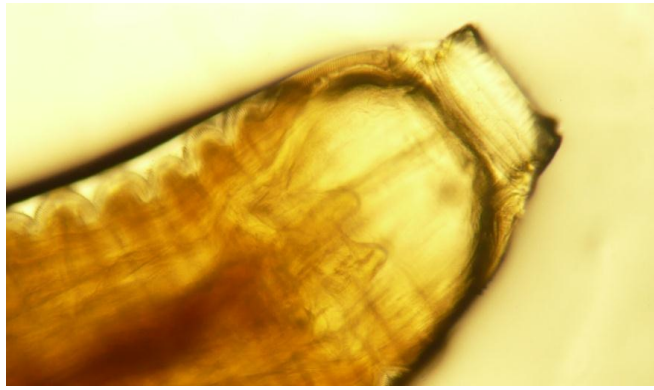

Fig. 3. *Strongylus vulgaris,* anterior extremity - buccal capsule, x100

Fig. 4. *Strongylus vulgaris,* anterior extremity-buccal capsule, x100

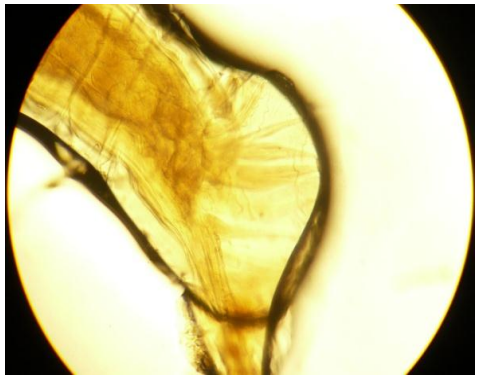

Fig. 5. *Strongylus vulgaris,* male, posterior extremity - caudal bursa x100

Fig. 6. *Cylicostephanus longibursatus (Trichonema longibursatum);* cephalic extremiy; x400

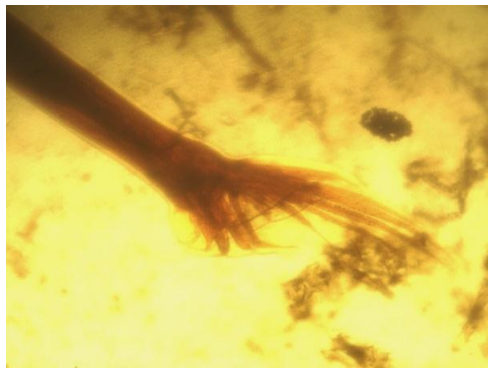

Fig. 7. *Cylicostephanus longibursatus (Trichonema longibursatum)* ♂*;* posterior extremity - caudal bursa; Col. sol. Lügol x 100

Fig. 8. *Cylicostephanus calicatus;* cephalic extremity; x400

Fig. 9. *Cylicostephanus calicatus;* posterior extremity; ♀; x100

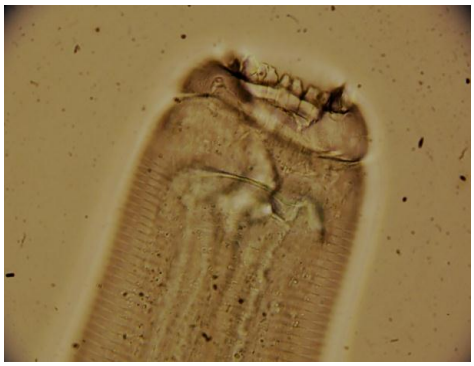

Fig. 10. *Cyathostomum pateratum;* cephalic extremity; x400

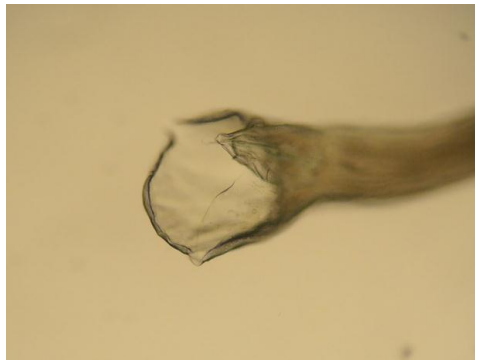

Fig. 11. *Cyathostomum pateratum;* posterior extremity – caudal bursa, ventral aspect; ♂; x100

Fig. 12. *Cyathostomum pateratum;* posterior *extremity - caudal bursa, lateral aspect; ♂; x100*

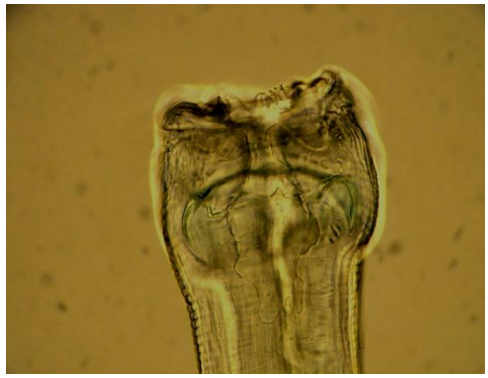

Fig. 13. *Cyathostomum catinatum;* cephalic extremity; x400

Fig. 14. *Coronocyclus labiatus;* cephalic extremity; x400

Fig. 15. *Coronocyclus labiatus;* posterior extremity; ♀; x100

Conclusions

1. Strongylid infestation know a large extensivity among horses, the studied population being 100 % positive on horses bred in extensive and semi intensive systems too.
2. Most animals are affected by strongyloidosis and ciatostomosis too, both parasitoses evolving with incidence and prevalence ratios close.
3. Intensivity of infestation is generally medium, with relatively small differences in terms of horses origin. We have a medium to low infestation on horses bred in semi intensive system and a medium to slightly increased infestation on horses bred in extensive system.
4. Although the farm horses benefit from regular deworming program, this maintain a low level of infestation, without total deworming . Medium to high level of infestation in horses from farm households is due to antiparasitic treatment and maintenance on pasture from spring to autumn.
5. Strongylid population examined is represented by *Strongylus vulgaris* and ciatostome species identified like belonging to the genera *Cylicostephanus, Cyathostomum* and *Coronocyclus*, with predominance of *Cylicostephanus longibursatum*. Female sex predominance was found in a ratio of 2/3 for *Strongylus vulgaris* and 3/4 for ciatostomes.

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RESEARCH ON THE LESIONS OF SOME DIGESTIVE PARASITOSIS IN HORSES

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Abstract
Digestive organs of horses are often invaded by many parasitic species with diverse local and general pathogenetic action and polymorphic lesions. Research on these issues has been achieved in present study following the necropsy of 20 horses from the Iassy and Bacău county. They were diagnosed following digestive parasitose: gasterophilosis, strongylosis, ciatostomosis, parascaridosis, oxiurosis and cestodosis. Macroscopic lesions was dominated by the presence of various shapes and sizes of gastrointestinal mucosa ulcers from the stomach to the rectum. Other lesions consisted of mucosalthickening and hypertrophy, congestions, more or less abundant catarrh, lesions which show a strong irritant local action.It was also observed along the large intestine the presence of parasitic granulomas with dimensions of several milimeter in diameter. In terms of histopathological, the local changes were represented by the mucosal discontinuities made by ulcers or the parasites implantation in mucus, hyperkeratosis, epithelial peeling and dystrophies mucinous calciforme cells of intestinal epithelium. It was observed the presence of granulomas containing parasitic larvae of strongyls surrounded by leukocyte reaction, eosinophilic and eosinocitar infiltration diffuse or focal, limfohistiocitar hyperplasia, blood vessels ectasi, in mucosa corion and in submucosa.

Key words : horses, digestive parasitoses, macroscopic lesions, histopathology

Introduction

The horses digestive tract develops a wide range of parasitic agents, which make that diseases often involved in digestive pathology of this species to be represented by digestive parasitosis. If parasitic protozoa is a relatively rare group who needs special epizootiological conditions, trematodes, cestodes and nematodes especially, frequently colonizes the digestive tract of horses, reaching very high epidemiological indices, such as *Strongylidae* family whose prevalence is often 100% (Covașă, 2011). In almost all segments and digestive organs of horses can be located one or more parasites, which further diversifies morpholesionale aspects, besides the multiplicity of genera and species which have like development support these ecological niche.

Evolution most often associated of parasitic diseases in horses, causes multiple health digestive and economic damages which aimes to animal welfare. It is known that the etiological agents of horses colic syndrome are *Anaplocephala perfoliata* (Veronesi, 2009) as cestodes or, very often, different Strongyls species. From medical damages we noted a high percentage of morbidity and sometimes mortality, damages that can be equally considered economic too. Digestive parasites can alter behavior, fertility, fitness, youth development, resistance to other pathogens or behavior performance for which they are breed animals (Cernea, 2008).

Materials and methods

Morphological investigations were carried out in the Faculty of Veterinary Medicine and at the slaughterhouse from Nicolae Bălcescu village, Bacău County. They were examined a total number of 20 horses over the years 2010, 2011 and 2012, from Iassy and Bacău counties territory. Horses age ranged from 9 months to 16 years, all were raised in intensive system (Table 1).

It was realised necropsy of segments and digestive organs, looking at macroscopic lesions,then samplingthe representative samples of tissue for histopathological investigations. Histopathological examination was performed on permanent histological preparations obtained by method of paraffin inclusion and stained with HEA general guidance method.

Table 1. Dates on horses taken for study

Crt. no.	Breed	Age	Sex	Growth mode
1.	Common	2 years	♂	Loose housing and grazing
2.	Common	4 years	♂	Loose housing and grazing
3.	Common	9 months	♂	Loose housing and grazing
4.	Common	7 years	♀	Loose housing and grazing
5.	Common	10 months	♂	Loose housing and grazing
6.	Common	13 years	♀	Loose housing and grazing
7.	Common	14 years	♂	Loose housing and grazing
8.	Common	10 years	♀	Loose housing and grazing
9.	Common	16 years	♂	Loose housing and grazing
10.	Common	1 years	♀	Loose housing and grazing
11.	Common	15 years	♀	Loose housing and grazing
12.	Common	8 years	♀	Loose housing and grazing
13.	Common	6 years	♂	Loose housing and grazing
14.	Common	8 years	♀	Loose housing and grazing
15.	Common	7 years	♀	Loose housing and grazing
16.	Common	10 years	♂	Loose housing and grazing
17.	Common	14 years	♂	Loose housing and grazing
18.	Common	7 years	♂	Loose housing and grazing
19.	Common	6 years	♀	Loose housing and grazing
20.	Common	3 years	♀	Loose housing and grazing

Results and discussions

As a result of the study we diagnosed the following digestive parasitosis : gasterophilosis, parascaridosis, oxiurosis, strongylosis, trichonemosis and cestodosis. Were affected following segments of the digestive tract : stomach, duodenum, jejunum, ileum, cecum, colon and rectum. We have identified several parasite species to all animals, so that we can speak about a widespread of parasitic associations in this species, with lesional implications more or less pronounced on the digestive tract.

Macroscopic lesions were dominated by the presence of ulcers in digestive mucosa from the stomach to the rectum, ulcers caused by fixing formations of digestive parasites. These ulcers have different shapes and sizes depending on the parasitic agent.

We observed isolated but generally grouped ulcers caused by deep implantation of *Gasterophillus spp.* larvae in the stomach mucosa. Most numerous larvae, ulcers respectively

were found in the antrum, pyloric orifice and canal, in the folded edge and the gastric bag or bottom (Figure 1). Ulcers were rare, isolated in the body stomach. These ulcers have the aspect of "craters" (Paul,2001), the mucosa beeing turgid, hypertrophied and with a hard texture (Figure 2).

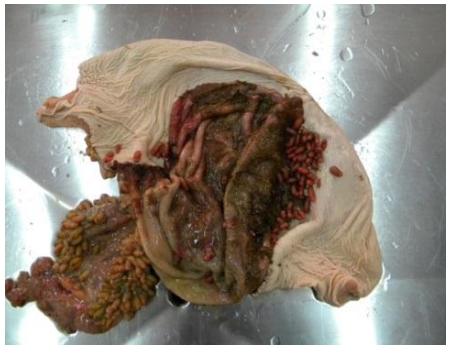

Fig. 1. Many *Gasterophilus spp* larvae present in the folded edge and gastroduodenal area

Fig. 2. Crater shape ulcers grouped in the folded edge

Histopathologically, the lesions of gastric mucosa are characterized by epithelial discontinuities representing the space for mucosa mounting of larvae, the microscopic corespondent for the craters shape ulcers (Figure 3), stratum corneum hyperkeratosis and peeling in this layer (Figure 4), eosinocitary infiltration in chorion mucosa through the fibers of connective tissue (Figure 5). Little parasites on stage of implantation in mucosa were observed (Figure 6).

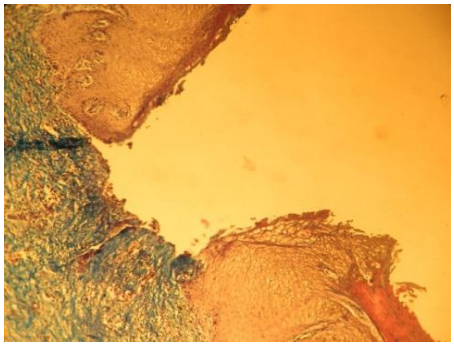

Fig. 3. Discontinuity of the epitelial layer in gastric mucosa; Stain HEA x100

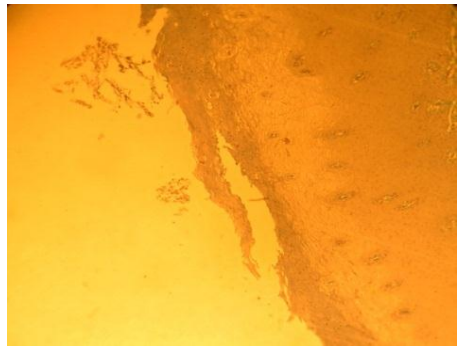

Fig. 4. Stratum corneum hypercheratosis and epithelial scallings; Stain HEA x100

In the intestine, we found ulcers generally small, reddish, formed as a result of ciatostomes and strongyls fixation on mucosal surface and ulcers much lower numerically, formed as a result of cestodes scolex fixation. Although *Anaplocephala spp.* infestation was more pronounced around the ileo-caecal orifice, we noticed the presence of these cestodes in the jejunum, ileum and cecum, but in small numbers. Lesions formed as a

result of *Strongylidae* action were spread throughout the large intestine, until to descending colon.

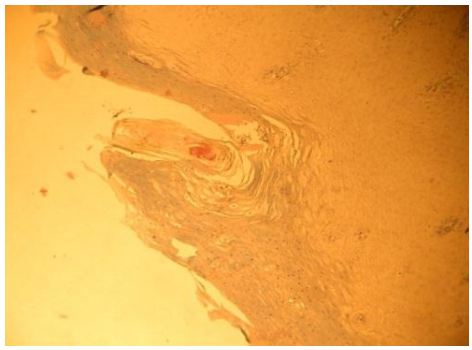

Fig. 5. *Gasterophillus* larva at the place of fixation in gastric mucous membrane; Stain HEAx100

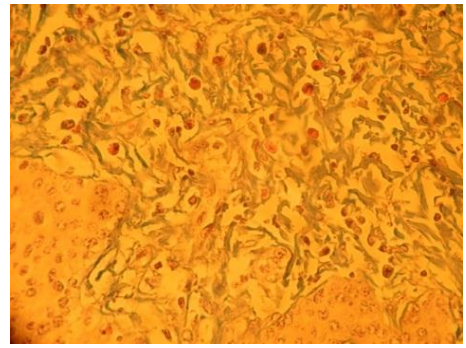

Fig. 6. Eosinocitary infiltration in gastric submucosa; Stain HEA x 100

However, the strongest infestation was found at the tip and body of cecum, where the lesions were more pronounced and the helminths were larger than those from other segments of the colon (Fig. 7, 8, 9, 10, 11, 12). All macroscopically, in the intestinal segments, we observed small gray or red granulomas on mucosal surface (Figure 9).

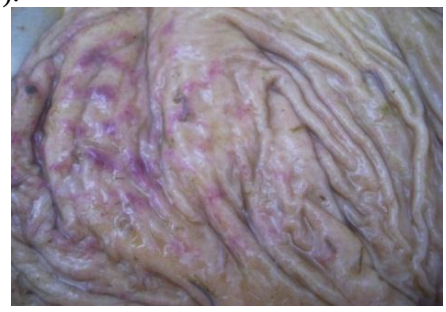

Fig. 7. Congestions, ulcers and catarrh of the nose caecal mucosa on strongylosis

Fig. 8. Adult helminths and strongylosis lesions on the body caecal

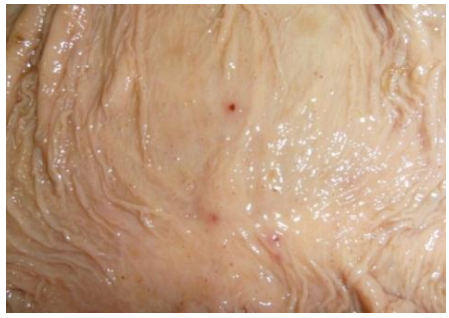

Fig. 9. Congestive ulcers and strongylic parastic granulomas in caecal mucosa

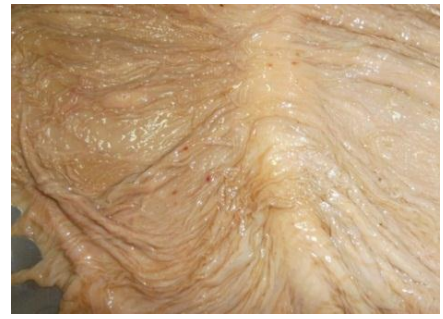

Fig. 10. Strongylic ulcers spread on the surface of caecal mucosa

Fig. 11. Strongylic ulcers point-like on the colon mucosa

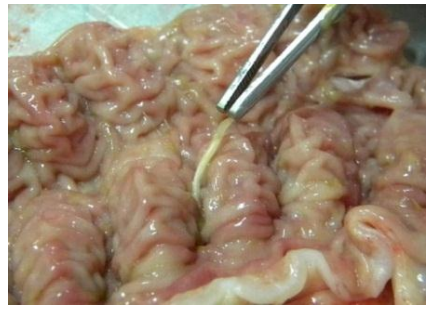

Fig. 12. Strongyl fixed on colon mucosa; congestion, thickening catarrh of mucosa

Histopathologically, the presence of ciatostoma larvae inside granulomas, surrounded by lymphoeosinophilic reaction, were found present (Fig.13, 14).

Eosinophilic infiltrations and limfohistiocitary diffuse or focal, was highlighted in most of the large intestine, on submucosa and chorion (Fig. 15, 16, 17, 18).

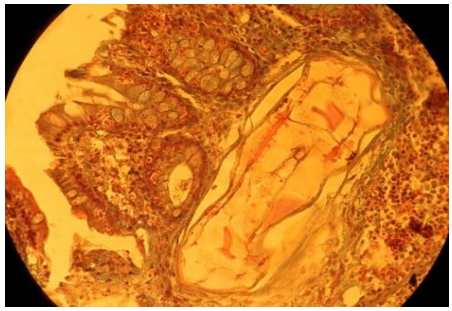

Fig. 13. Parasitic granuloma on thick intestine which contain a ciatostoma larvae; adjacent limpho-eosinophilic enterite. Stain HEA x 400

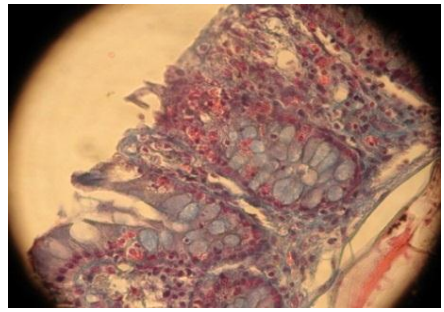

Fig. 14. Eosinocitary reaction in lamina propria surrounding parasitic granuloma; Stain HEAx400

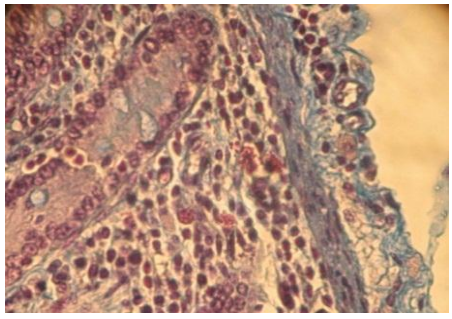

Fig. 15. Eosinophilic infiltration in cecum chorion Stain. HEA x400

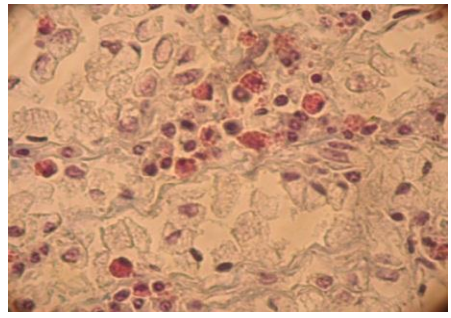

Fig. 16. Eosinophilic focus in colon submucosa; Stain HEAx400

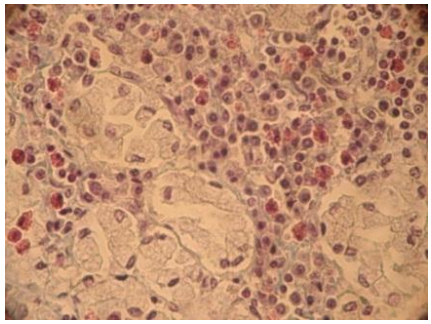

Fig. 17. Eosinophilic infiltration in colon submucosa characteristic to strongylydae parasitism Col.HEAx400

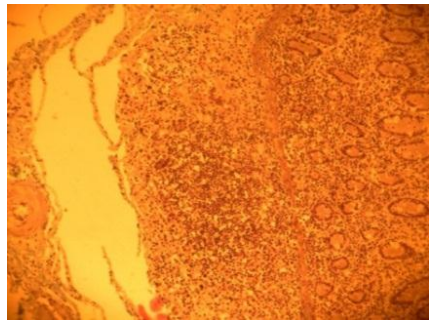

Fig. 18. Limphohistoicitary hiperplasia on lamina propria and colon submucosa Col.HEAx100

Predominant lesions in the small intestine were congestions, tumefactions and the presence of abundant catarrh, lesions attributable largely to parascaridosis, which was the main parasitose diagnosed at this level (Figure 19, 20), and less to cestodosis, diagnosed much lower percentage and generally with a few helmints (Figure 21, 22).

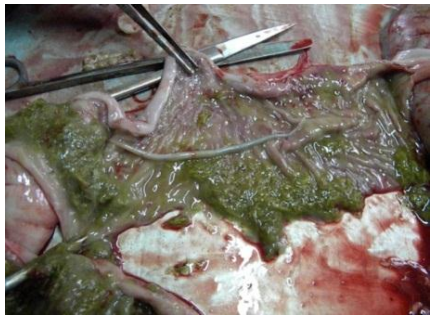

Fig. 19. *Ascaris* nematod in duodenal lumen; congestion and mucosa catarrh

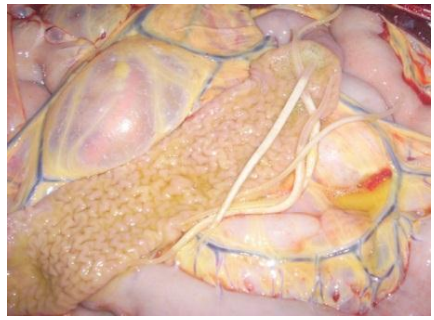

Fig. 20. *Ascaris* nematodes in jejunal lumen

Fig. 21. Parasitism with cestodes on ileo-cecal orifice; thickening and congestion of mucosa

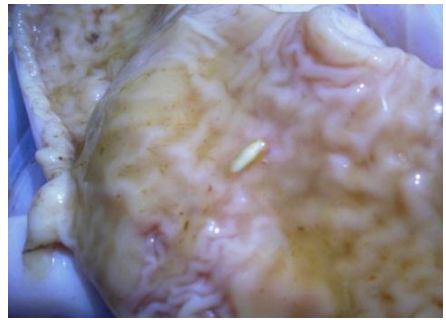

Fig. 22. Cestode on jejunal mucosa

Microscopically, these lesions were translated by observing many ectasiate vessels in small intestine submucosa and mucinous dystrophy of calciforme cells (Fig. 23, 24).

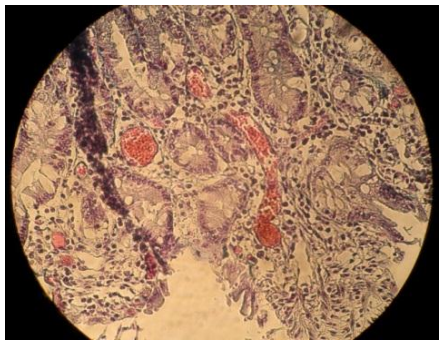

Fig. 23. Congestion in jejunal submucosa; Stain HEAx100

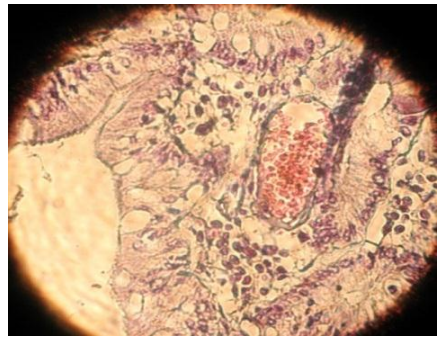

Fig.24. Vascular ectasy in submucosa and mucinous distrophie of calciform cells; Stain HEAx400

Oxiurosis was also diagnosed (fig. 25, 26) in a relatively high percentage, but most often in association with strongilosis and ciatostomosis, so common lesions present on colon are mainly attributed to the latter.

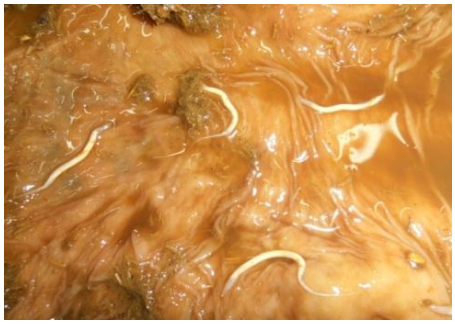

Fig. 25. Oxiurosis on descendant colon

Fig. 26. Numerous *Oxiuris* nematodes localised on colon

Conclusions

1. It can be seen a relatively intense parasitism of the digestive tract in horses from Iassy and Bacău county, particularly with agents belonging to miases, cestodes and especially nematodes.
2. In all cases there was a trend of several related parasitosis to the same individual, with lesional implications on multiple digestive segments.
3. Most severe traumatic actions of the parasites was observed in the stomach, mucosa beeing affected by numerous crateriform ulcers as resulting effect of gasterophillus implantations.
4. More discrete ulcerative lesions were observed in the small and large intestine, as result of Strongylidae or cestodes action. Irritant action of parasitic agent manifested by thickening and hypertrophy of the mucosa, congestion and intestinal catarrh. Specifically we found a granulomatous enteritis in the large intestine.
5. Histopathologically it met an eosinophilic or eosinocitary infiltration of chorion and digestive submucosa and limphohistoicitary hiperplasia outbreaks like nonspecific

and almost ubiquitous response and strongylic granulomas from cecum and colon submucosa like specific lesions were described.

6. We can say that multiple pathogenetic action gived by the associated parasitisme of digestive organs in horses results in a diverse morpholesional aspect with features conferred by the digestive sequence and parasitary phenomenon intensivity.

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ROLE OF MULTIPLANAR REFORMATTING IMAGING IN THE ASSESSMENT OF THORACOLUMBAR DISC HERNIATION IN DOGS

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Abstract
Computed tomography (CT) is an imaging modality that uses X-rays and powerful computers to construct cross-sectional images of a patient. CT software also has the important capability of converting the axial sections in sagittal, dorsal, and oblique planes. In dogs intervertebral disc herniation is one of the most common affection of the thoracolumbar spine and the main indication for spinal imaging. Thirty-eight CT studies with a diagnosis of intervertebral disc herniation of the thoracolumbar region in dogs were retrospectively evaluated. The multiplanar reformatting (MPR) imaging was proven to be a valuable tool mainly regarding the localization of lesion. Accurate localization of the site of intervertebral disc herniation is important for surgical planning.
Keywords: thoracolumbar, multiplanar reformatting imaging, dog

Objectives

Intervertebral disc herniation is common in dogs and CT is an accurate imaging procedure for dogs with suspected thoracolumbar disc herniation (1, 3, 5).

The aim of this study was to assess the role of the multiplanar reformatted (MPR) CT-imaging for characterizing thoracolumbar intervertebral disc herniation in 38 patients.

Materials and methods

The dogs with disc herniation were selected from the last three years CT studies performed at the Interdepartmental Center of Radiology, Naples. In order to assess the intervertebral disc herniation, 38 studies made on dogs of different breeds, weight and age were analyzed (Table 1).

The CT studies were performed using a helical (single layer) CT scanner (GE Prospeed). The dogs were placed under general anesthesia and positioned in dorsal recumbency. No intrathecal or intravenous contrast medium was used. In order to localize the lesion site, a series of single subtle slices were made, passing through the intervertebral spaces from T3-T4 to L4-L5.

Table 1. The patient's sex, age, weight and breed

Male	27	
Female	11	
Mean Age (±St.Dev.)	6.9 (±2.6)	
Mean Weight (±St.Dev.)	18 (±14.3)	
Breed	Mixed-breed	12
	Dachshund	6
	German Shepherd	5
	Bichon Maltese	3
	Cocker Spaniel	2
	Dalmatian	2
	Pekingese	2
	Italian Spitz	1
	Jack Russell	1
	Labrador Retriever	1
	Lagotto Romagnolo	1
	Rottweiler	1
	Yorkshire Terrier	1

Once the affected spinal segment was found, it was studied with multiple contiguous subtle slices. Slice thickness varied between dogs (1 or 3 mm), depending on body size and length of the studied segment. CT settings were 120 kV, 100 - 160 mA**,** with a scan time of 2 seconds per slice. CT window level (WL) and window width (WW) settings varied according to the examined tissues. CT image sets for each study were converted into Digital Imaging and Communications in Medicine (DICOM) format and transferred via Ethernet to a CT workstation (Merlino, Theorem@ Medical Software, Italy). Multi-planar reformatted CT images for each dog were created using the workstation's image analysis software.

Axial computed tomographic images were compared with sagittal and dorsal reformations to evaluate the diagnostic usefulness of MPR for the characterization of thoracolumbar disc herniation.

Results

A total of 80 disc herniations (27 Hansen type I disc extrusions and 53 Hansen type II disc protrusions) were found by CT study. The most commonly affected sites were the spaces near to the thoracolumbar junction (Fig. 1).

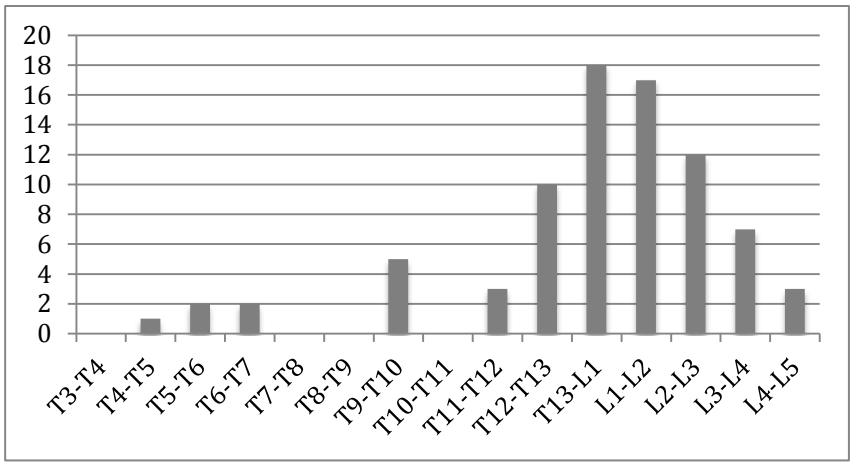

Fig. 1 Distribution of thoracolumbar disc herniations

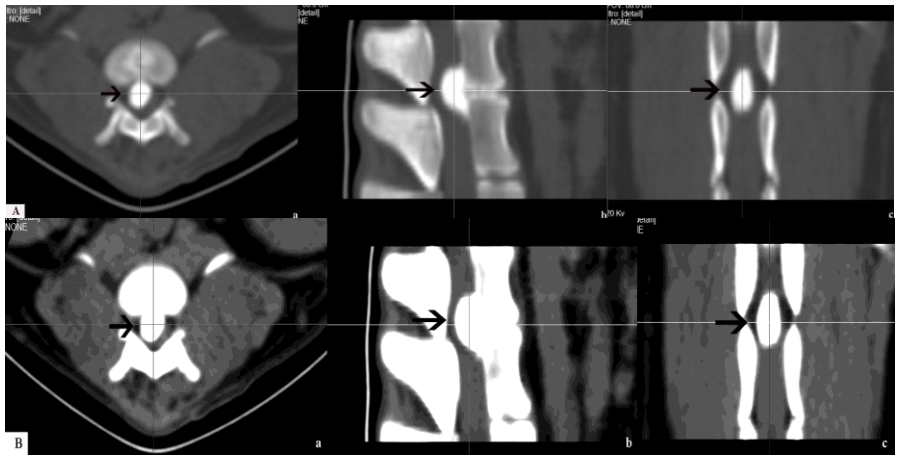

Fig. 2 Multiplanar reformatting images- bone (A) and soft tissue (B) windows
a- transverse scan; b- sagittal scan; c- dorsal scan. Extruded disc material is visible as a circular mineral opacity in the ventral vertebral canal at L1-L2 (black arrow)

The sagittal reconstruction showed the size (length and height), the displacement (cranial or caudal), the shape and the number of herniation (single or multiple) (Fig. 2b). Sagittal reconstruction images are particularly useful in the case of multiple disc herniations. The shape can be useful to differentiate the disc extrusion from disc protrusion.

Dorsal and/or curved MPR images are useful to demonstrate the lateralization of herniated disc material and to show the relationship between disc material and vertebral foramina (Fig. 2c).

Another advantage of MPR imaging is the possibility of being able to correct the plan of reformation in cases where the patient is not correctly positioned on the CT table.

Marked breath and/or peristaltic movements may produce motion artifacts that on sagittal MPR images can hide some spinal cord portions.

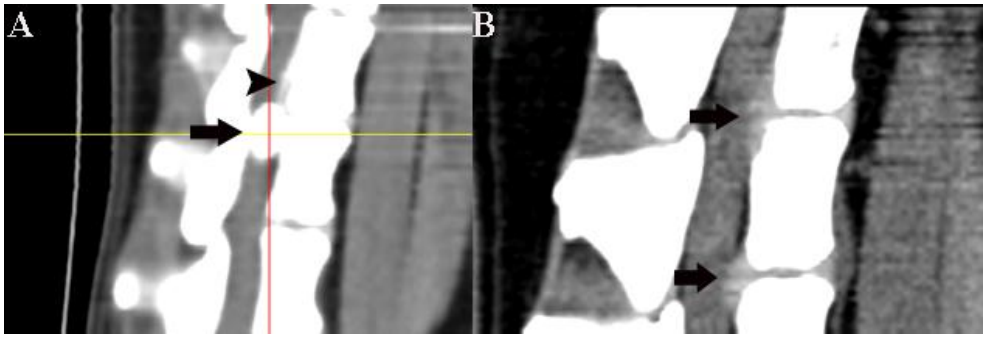

Fig. 3 MPR sagittal scan: differences between Hansen type I (A) and Hansen type II (B) disc herniation (black arrow), and presence of subdural hemorrhage (black arrowhead)

There are some disadvantages of MPR images: difficulty in distinguishing between hemorrhage and extruded disc material, chronic disc protrusion and extrusion, and subjective interpretation of the severity of spinal cord compression when multiple sites are damaged.

In our experience, for treatment planning, surgeons needs to evaluate all the studies in MPR images due to the easiest assessment of spatial displacement and degree of compression of the herniated material and its relationships with anatomic landmarks. This is in agreement with the results of King et al. (4). However, our protocol is significantly different from those proposed in other authors studies of predetermined spinal segments [i.e. from mid T-10 to mid-L3 (4) or from cranial T2 to L4 (2)]. In our opinion, the protocol that we used permits a reduced total radiation dosage, since it allows localizing with great precision the site of the lesion hence, restricting the scan area.

Conclusions

CT study confirmed to be a valuable imaging technique in the diagnosis and characterization of intervertebral disc herniation without using contrast media. Although axial scans could be considered sufficient for an expert radiologist, MPR imaging made easier, more intuitive and more accurate the characterization of the disc herniation.

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COMMON ERRORS IN THE MANAGEMENT OF DIABETES MELLITUS IN SMALL ANIMALS

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Abstract
Due to the increased number of cases with diabetes mellitus we consider it necessary to list the most frequent errors in the management of diabetes, caused by doctors and owners. Purpose of this paper is to recognize pets with diabetes, treatment optimization and to highlight the most common mistakes in diagnosis and treatment of diabetes so that diagnostic errors are to be avoided. Feline diabetes affects between 1 in 50 and 1 in 400 cats, depending on the population. In 80-95% of diabetic cats, their diabetes appears analogous to human type 2 diabetes.Environmental factors which predispose pets to diabetes include low physical activity and being kept entirely indoors (cats), high body condition score, dental disease, chronic or recurring health problems. Chronic high demand for insulin secretion as a result of high carbohydrate diet may predispose to diabetes.Early intervention with good glycemic control reverses or decreases B-cell glucose toxicity. Diabetic dogs are at increased risk for developing bacterial infections particularly of the urinary tract. Routine bacterial culture of urine is recommended.

Key words: diabetes mellitus, insulin, hypoglycemic drugs, insulin, diabetic.

Introduction

The signs of early diabetes are frequent urination, drinking lots of water, a large appetite, and unexplained loss of weight. The laboratory findings are high glucose levels in the blood and urine.

In more advanced cases there is lethargy, loss of appetite, vomiting, dehydration, weakness, and coma. Cataracts are common in diabetic dogs. Ultimately, diabetes is a disease that affects all organs. Diabetic dogs will have enlarged livers, be susceptible to infections, and often develop neurological problems if not treated.

Diabetic ketoacidosis is a condition associated with severe hyperglycemia in which ketones (acids) build up in the blood. Ketones are products of the metabolism of fat. In diabetic ketoacidosis, fats are metabolized for energy because sugar is unavailable. Diabetic ketoacidosis can be recognized by weakness, vomiting, rapid breathing, and the odor of acetone on the breath (fruity odor).

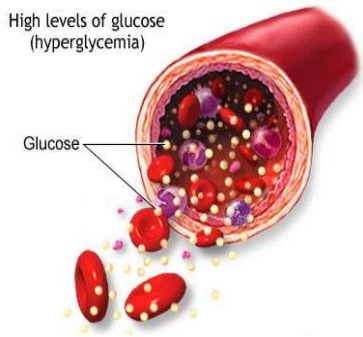
Aim of this work is to summarize which problems are encountered during treatment, helping owners to adjust with the new lifestyle of diabetic pet and to know what to do when good glycemic control is not achieved.

Materials and methods

The cases were studied and treated at the Medical Clinic of the FMV, Bucharest.

For these cases, the steps in diagnosis and treatment were as following:

- Case history
- Clinical exam
- Blood exam and biochemistry exam
- Abdominal ultrasound
- Cardiologic exam
- Ophthalmologic exam



Following cases examined since 2007-2011, in Clinic FMVB we found that there are some mistakes that are repeated frequently in the treatment of the diabetic patients. During four years of study we followed 82 cases.

For this, dogs and cats with diabetes mellitus were followed during the course of the disease, trying to stabilize blood glucose 110-150 mg / dl (sometimes 200 mg / dl), with dietary compliance, an appropriate lifestyle (physical activity), and the appropriate treatment and, not least by educating owners of diabetic animals.

Case studies, we identified it and watched it, is outlined in Table 1 and the two figures attached, systematized by sex and gonadal integrity.

Table 1. Case studies

Total cases with diabetes	**Total females with diabetes**	**Total males with diabetes**
Dogs	35	13
	73%	27%
Cats	11	11
	50%	50%

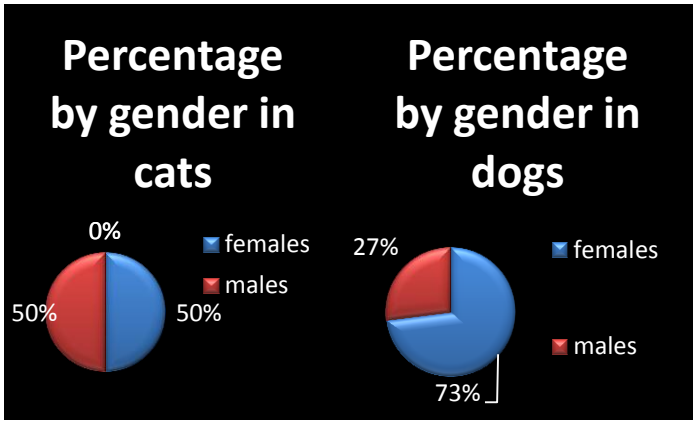
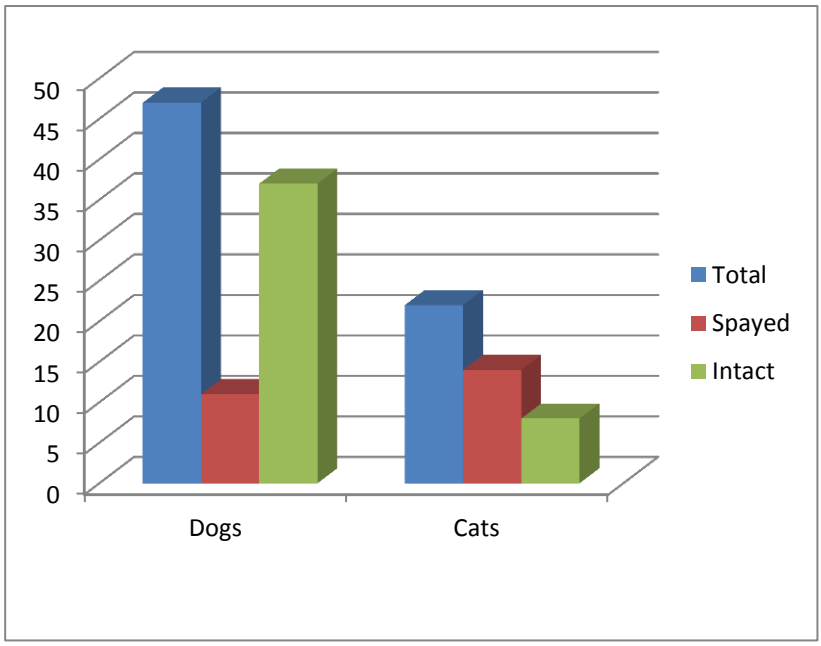


Fig.1. Systematized by sex



In dogs, the total number of cases, 11 were castrated and intact 37, while in cats, a total of 14 were castrated and 8 were intact.

The literature shows that, in dogs, diabetes is at least twice as common in females as in males, whereas in cats, male cats neutered is highest risk category for diabetes. Obesity was found in nine dogs and seven cats.

Some dog breeds such as the Samoyed, Schnauzer Miniature, Poodle Miniature, and Boxer are more prone to diabetes than other breeds, while in the cats the most sensitive breed is Burmese. (2, 3)

We found that pets can get diabetes at any age, but the pike is represented by those with an average age of eight years.

For determination of BSL (blood sugar level) the classical methods were used, usually starts with colorimetric determination using o-toluidine method, and enzymatic methods with hexokinase and glucose dehydrogenase. Venous blood measurements are total, total capillary or venous plasma. Besides these methods can be used glucometers. Glucose determinations will be made in the morning, fasting ("a jeun"), after a break food for 8-10 hours.

Polyuria polydipsia, the most important sign of diabetes, at dog appeared in a number of 43 cases, and in cats, to a total of 17 cases.

Ophthalmological and cardiological examination was performed at the department of ophthalmology and cardiology of FMVB Clinic.

Cataracts, another important sign of diabetes in dogs, were observed in 17 dogs. In cats we found only one case of cataract, the patient had type I diabetes.

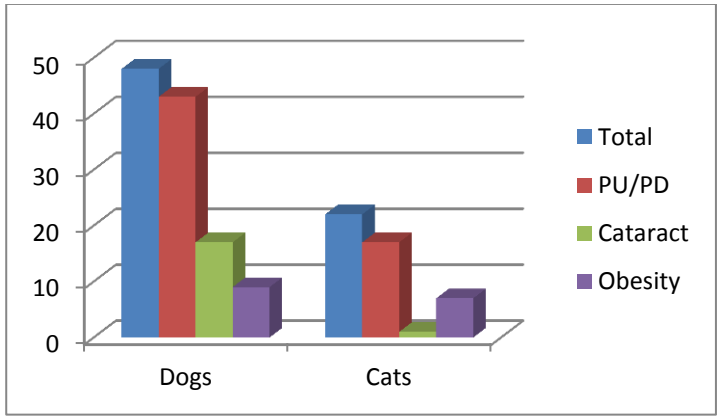


Fig. 3. Total number of cases in the study presenting polyuria-polydipsia, cataract and obesity

Results and discussions

This paper is a further study of my doctoral dissertation entitled "Contributii privind optimizarea tratamentului in diabetul zaharat la carnivore".

Those cases were sent to the clinic for examination. We performed clinical examination after a detailed history, examination of blood, urine, ophthalmology, cardiology, ultrasound and blood insulin dosing. These findings have emerged in the study and led to some errors in diagnosis or treatment. Some of the errors are due to owners regarding technical aspects of the treatment and other errors are made by the veterinarians.

These findings were correlated with literature and some have been related with human medicine (some errors are common).

We tried that through this work to elucidate certain aspects of clinical and therapeutic approach of DM in small animals. We believe that these aspects are necessary for patients with DM, veterinarians and animal owners.

Top 11 mistakes in the management of diabetic patients

Errors of the owners with diabetic pet

1. Late presentation to the doctor of patients with symptoms such as: polyfagia, polyuria, polidypsia, recent weight loss. (2)
2. One of the most important periods in the owner`s care of a diabetic pet is the time during which the veterinarian or the nurse teaches the technical aspects of the treatment. The owner must be able to mix the insulin correctly (gentle rolling, not shaking), load a syringe without air bubbles, administer an injection subcutaneously on the lateral wall of the chest or in the flank, know how to deal with such problems as injection pain or bleeding. (2)
3. Check if the insulin used by the owner is not outdated, has not been diluted, frozen or heated. Also check the syringe used by the owner: U-40 or U-100. (2)

4. The owner must know to not administer another dose of insulin if injection had been made into the fur rather than the subcutis. The insulin dosage or the type of insulin used for treatment is choosed only by the doctor . (2)
5. In case of vomiting after administration of oral hypoglicemic drugs, the owner should not administer another pill whithout the consent of the doctor.
6. Insulin should be stored in the door of the refrigerator to maintain a consistent environment for the insulin preparation.
7. The owners should know that the diabetic patient requires 7-10 days to equilibrate to changes in the insulin dosage or preparation. (2,3)
8. The owner has to follow diet(quality and quantity) recommended by the doctor. The owner has to know that exercise plays an important role in the maintenance of glycemic control and pet`s exercise should be done daily at the same hour.
9. The owner has to check blood glucose level when the doctor recommends. Check if the test-strip used by the owner is not outdated. (2)
10. Artifactual hypoglicemia becasue of innapropiate handling of portable glucose meters, insufficient application of blood,despite „beep" given by the device as an indication of the opposite. (2,3)
11. The owner of a diabetic patient has to recognize sympotms of hyper/hypoglicemia, recurrence of polyuria and polydipsia, and symptoms of diabetic ketoacidosis, and that this requires consultation with the doctor. If any changes occurs in the patient`s behavior the owner has to report to the doctor. (2,3)

Errors of the doctors in managing diabetic patients

1. Properly taken history of the patient (sex, age, breed, if neuterized or not, other underlying diseases, other diseases in history). A genetic predisposition, infection, insulin-antagonistic diseases anddrugs (anti-inflamatory, hormonal drugs such as estrogen, progesterone), obesity, immune-mediated insulitis, and pancreatitishave been identified as inciting factors. (1,2)
2. The doctor has to establish appropiate and timely treatment. (1,2)
3. Differential diagnosis of DM type I, type II or hyperglycemia.For differential diagnosis between diabetes mellitus type I or type II, blood levels of insulin must be measured.
4. The doctor has to follow specific protocols regarding dosage of oral hypoglicemic drugs and insulin. (1,2)
5. Supportive treatment should be administered .
6. To know the side effects of oral hypoglicemic drugs (correct dosage only if the diabetic patient is in a good health, drug is given after meal).
7. Routine hematology, plasma or serum biochemistry, urinalysis, and urine culture should be performed. Also performe cardiologic exam,abdominal ultrasound and ophthalmologic exam.
8. The doctor must know how to prevent short-term complications (hypoglicemia and ketoacidosis), how to deal with complications of diabetes mellitus and to intervene promptly to their occurrence. (1,2)
9. To know that obesity decreases insulin sensitivity. Obesity induced- insulin resistance is reversibile and even slight to moderate weight loss improves metabolic control.(2)

10. Recommend specific diet and excercise. Exercise must be adjusted according to patient.
11. Perform a glycemic curve before and after establishing a treatment protocol. (1,2)

Conclusions

1. Avoid treatment decision based on clinical signs.
2. Corectly establish differential diagnosis.
3. Prevention of obesity and maintenance of optimal body condition are to date the the best way to minimize the occurence of insulin resitance.
4. An established diagnosis and treatment of DM at the right time may save the life of the diabetic patient.
5. A relationship based on trust and co-operation between veterinarian and client invariably leads to the most satisfactory outcome, while key to success is individuallization of the advice to suit both diabetic patient and owner.
6. Particular attention to the diabetic patient overall health and nutrition is required, and strategies employed to treat the various complications of diabetic patients.

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APPROACH TO THE NEUROLOGICAL EXAM

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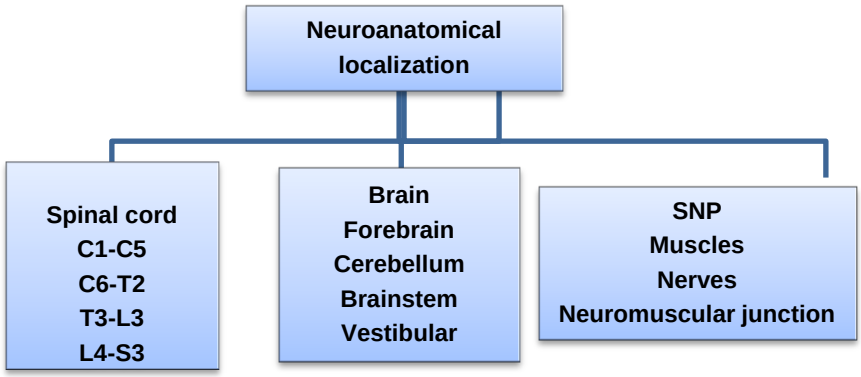
Abstract
Due to the increased number of cases with neurological disorders (cats and dogs) we considered it necessary to introduce neurological examination as part of the general examination. We opted to introduce the neurological sheet, taken from the books of veterinary neurology because we found that in the last three years it was really useful in approaching patients presenting neurological disorders.
Key words: neurological exam, cranial nerves, posture, gait ,panniculus.

Introduction

The neurological examination based on the neurological sheet, helped us to solve three major problems:

1. We established the localization of the lesion whether is in the SNC or SNP.
2. We can determine a precise localization. We can establish if the lesion is focal, multifocal or diffuse.
3. We can make a differential diagnosis based on VITAMIND:
   V – Vascular
   I – Infectious, inflammatory
   T – Traumatic
   A - Anomalous
   M – Metabolic, toxic
   I - Idiopathic
   N - Neoplastic
   D – Degenerative



Materials and methods

The cases were studied and treated at the Medical Clinic of the FMV, Bucharest; the clinical and neurological exams done in the Medical Clinics.

For these cases, the steps in diagnosis and treatment were as following:

- Case history
- Clinical exam
- Neurological exam
- Blood exam and biochemistry exam

We have taken under study 80 cases in the past three years, all with neurological disorders; we examined animals after a careful clinical history. We had 58 dogs and 22 cats presenting neurological disorders. Only 12 out of 58 dogs and 4 out of 22 cats had orthopedic disorders. When establishing neuroanatomical localization from a total of 46 dogs with neurological disorders, 42 had affection of SNC (24 with brain disorders and 18 had affection of the spinal cord), and 4 had affection of SNP. From a total of 18 cats with neurological disorders, 1 cat had affection of SNP and 17 had affection of SNC (6 with spinal cord disease and 11 with SNC affection).

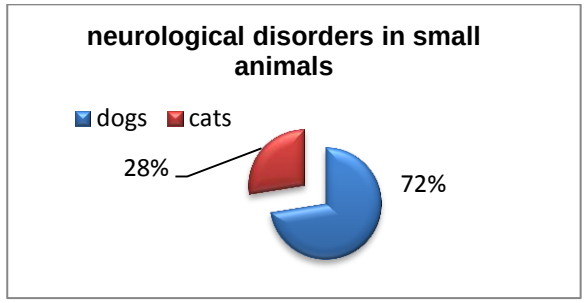


Fig. 1. Neurological disorders by species in small animals

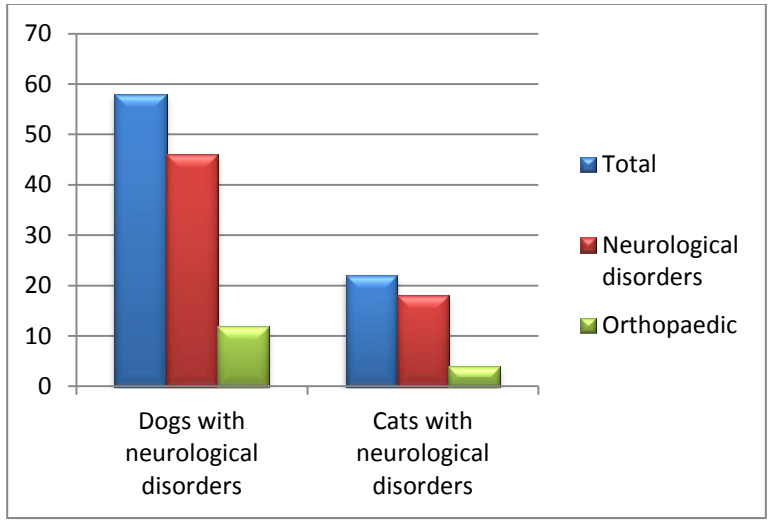


Fig. 2. Neurological vs. orthopedic disorders in dogs and cats

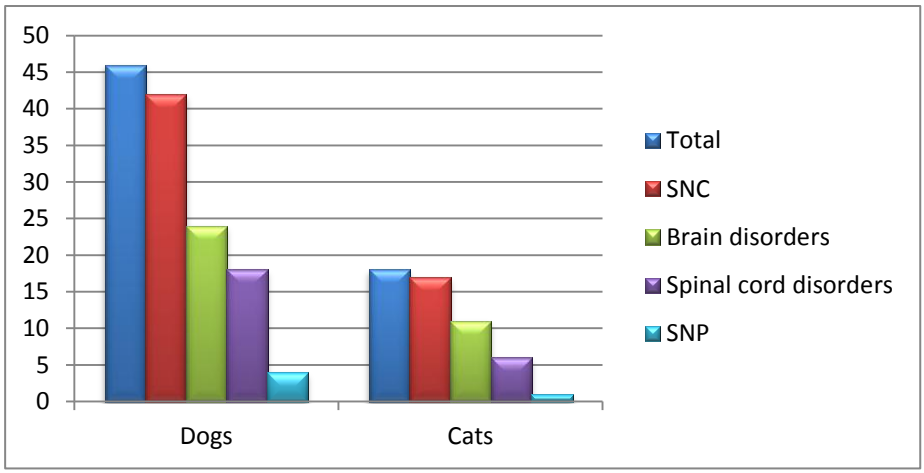


Fig. 3. Total cases of neurological disorders and lesion localization

Results and discussions

Aims of the neurological examination

1. Do the clinical signs observed refer to a neurological or orthopedic disorder? Or both?
2. What is the location of this lesion within the nervous system? SNC or SNP?
3. What are the main types of disease process that can explain the clinical signs?
4. How severe is the disease?

I. Objectives

- Evaluate mental status, behavior, gait, and posture for abnormalities
- Evaluate higher level integration through postural reactions and responses
- Evaluate cranial nerve function
- Use spinal reflexes to assess specific nerves and spinal cord segments
- Accurately assess sensory perception

II. Patient Assessment

- Signalment
- History
- General physical examination
- Neurological exam
- Lesion localization
- Differential diagnoses VITAMIND
- Diagnostic/treatment planning
- Prognosis

III. Neurological examination

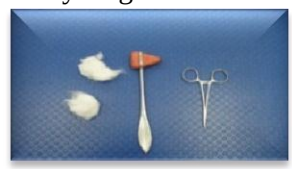
- Determine presence/absence of neurologic disease

Determine neuroanatomical localization

- Intracranial
  - Forebrain, brainstem, cerebellum

- Spinal cord
  - ♦ C1-C5, C6-T2, T3-L3, L4-S3
- Neuromuscular disease
  - ♦ Peripheral nerve, muscle, NMJ
- Multifocal disease
  - ♦ Deficits cannot be explained by single lesion

Tools for neurological examination



- ♦ Pleximeter
- ♦ Hemostats
- ♦ Trans-illuminator / LED light
  - Penlight often not bright enough
- ♦ Cotton swab

Neurological examination (components)

- ♦ Observation
  - Mentation, behavior, involuntary movements
- ♦ Gait, posture
- ♦ Postural reactions
- ♦ Reflexes
- ♦ Cranial nerve evaluation
- ♦ Palpation
- ♦ Sensory evaluation

Mental status classification

- ♦ Normal
- ♦ Depressed
- ♦ Obtunded
- ♦ Stupors
- ♦ Comatose

Posture

- ♦ Head position
  - With respect to gravity/ground (horizon)
  - With respect to axis of body
  - With respect to direction of movement
  - Tilt, turn, ventroflexion, dorsiflexion
- ♦ **Trunk and limb position**
  - Position of body with respect to gravity
  - Leaning, falling, rolling
  - Wide-based (or narrow-based) stance
  - Decreased/increased tone
  - Deviation of spine
    - If recumbent, also consider

- Decerebrate rigidity
- Decerebellate rigidity
- Schiff-Sherrington

Gait

- Lameness
- Paresis (weakness) or plegia (paralysis)
- Ataxia (incoordinated, irregular gait)
- Dysmetria
- Circling
- Pacing
- Exercise intolerance

Ataxia clasification

- Proprioceptive
- Vestibular ataxia
- Cerebellar ataxia

Postural reactions

- Conscious proprioceptive placing (CP)
- Hopping
- Tactile placing
- Visual placing
- Hemistanding/hemiwalking
- Extensor postural thrust
- Wheel barrowing

Spinal reflexes

- Myotatic reflexes
- **Thoracic limb** :Biceps,Triceps,Extensor carpi radialis
- **Pelvic limb :**Patellar,Cranialtibial,Gastrocnemius
- Flexor withdrawal reflexes
- Cutaneous trunci reflex
- Perineal reflex

Sensory evaluation

- Hyperesthesia
- Paresthesia
- Anesthesia
- Superficial pain perception
- Deep pain perception

Cranial nerve evaluation

- Know cranial nerves and their functions
- Know components of pathway involved
- Approach CN exam as a mini problem list
    - List components of every abnormal CN test

- Compare to find common component
- Localize to one lesion if possible
- If not possible, consider multifocal disease

Conclusions

1. Neurological examination must be performed in any patient with neurological signs.
2. Neurological examination will help us to establish neuroanatomic localization and diagnosis.
3. Clinical signs are related both to history and other tests: laboratory (blood, urine), ophthalmology, ultrasound, etc. Rx.
4. Differential diagnosis (VITAMIN D) is required in each case to avoid diagnostic errors and treatment.
5. Specialized examination (RMN and CT) are extremely important for accurate neurological diagnosis.

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PERIODONTAL DISEASE: CLINICAL FINDINGS ON A POPULATION OF DOGS IN BERLIN, GERMANY

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Abstract
The aim of this study was to determine and clasify the clinical stages of periodontal disease in a population of 100 dogs of different breeds and ages. The dogs were randomly selected from the patients of a small animal surgery hospital in Berlin, Germany, between April and August 2011. The results show that 93% of the examined dogs showed signs of periodontal disease.
Keywords: periodontal disease, dog

Periodontal disease is very common in small animal patients. [2] This causes pain and/or localized and systemic infections. However, this condition has little to no obvious clinical signs, thus diagnosys is not typically made until the later stages of the disease. [4]

Periodontal disease is a multi-stage process of tooth attachment loss, that ends with the loss of the affected tooth. [5] Periodontitis is defined as the active disease state of the periodontium. Periodontitis usually develops from gingivitis, but not all untreated gingivitis leads to periodontitis. [1, 3]

Materials and methods

The study was accomplished on 100 (n=100) dogs of different breeds, genders and ages, randomly selected from the patients of the Small Animal Surgery Clinic, of the Free University Berlin, Germany. Exclusion criteria: age less than 1 year.

The clinical examination was performed under general anesthesia with Midazolam®Braun, Polamivet®Intervet (*methadon*) and Narcol®CP-Pharma (*propofol*).

Plaque Index (PI), Gingival Index (GI), Sulcus Bleeding Index (SBI), Calculus Index (CI), Percentage of Attachment Loss Index (PAL), Furcation Exposure (FE) were used for the diagnosis and staging of the periodontal disease, as described by Wiggs et al. (1997)

Results and discusion

The percentage of each stage of the periodontal disease was determined after the analisys of the clinical data. The results are summarized in Table 1.

Table 1. Summary of the stages of periodontal disease found in the examined dogs

Periodontal Disease Stage	Localized (%)	Generalized (%)	Total (%)
STAGE 0 – Healthy gingiva	-	-	**7**
STAGE I – Early gingivitis	39	7	**46**
STAGE I - Moderate gingivitis	5	12	**17**
STAGE II – Early periodontitis	11	2	**13**
STAGE III – Moderate periodontitis	6	3	**9**
STAGE IV – Advanced periodontitis	4	4	**8**

- **7%** of the total examined dogs had a **healthy gingiva** and no sigh of periodontal disease;

- The first stage of periodontal disease is represented by gingivitis. **46%** of the dogs had a Gingival Index of 1 and no bleeding on probing, signs of an **early gingivitis**. **85%** of them had only a localized gingivitis on the canins and upper forth premolars, with little or no calculus and plaque deposits;

- **17%** of the subjects had a Gingival Index of 2 and bleeding on probing, signs of a **moderate gingivitis**. Unlike the early gingivitis, the moderate gingivitis tends to be generalized, with bigger calculus and plaque deposits;

- the **advanced gingivitis**, localized or generalized, reprezented by Gingival Index of 3 and strong bleeding on probing, was found in the most cases of periodontitis, and thus was not included as a separate entity in this statistic;

- the second stage of periodontal disease, the **early periodontitis**, was found in **13%** of the dogs. As with the early gingivitis, the early periodontitits is a localized lesion, in most cases affecting one or two neighbouring teeth;

- **moderate periodontitis**, the third stage of periodontal disease, was diagnosed in **9%** of the dogs;

- advanced periodontitis, the forth stage of periodontal disease, was diagnosed in **8%** of the cases. In half of the cases the advanced periodontitis was localized to a few neighbouring teeth, and in the other half of the patients it was a generalized condition.

Conclusions

1. From the total of 100 dogs that were examined, **93%** showed signs of periodontal disease, from early gingivitis to advanced periodontitis, severe attachment loss and edentations. **32%** of the total dogs had periodontitis stage II, III or IV.
2. The first stage of periodontal disease was diagnosed in **63%** of the dogs, the second stage in **13%**, the third stage in **9%**, and the forth stage in **8%** of the cases.
3. The first stage of periodontal disease is characterized by exsudative inflammation of the free gingiva.
4. In the second stage of periodontal disease, the macroscopic leasions are mostly alterative, with signs of destruction of the free gingiva and retraction of the jonctional epithelium.
5. The advanced stages of periodontal disease are characterized by root exposure, high tooth mobility and edentations.

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CORRELATIONS BETWEEN THE AGE AND BREED AND THE CLINICAL STAGES OF PERIODONTAL DISEASE IN DOGS

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Abstract
*The aim of this study was to determine the correlations between two favorising factors of the periodontal disease, the **breed** and **age** of the dog, and the clinical stage of the periodontal disease. The dogs were randomly selected from the patients of a small animal surgery hospital in Berlin, Germany, between April and August 2011.This study showed that more severe periodontal disease signs are found in smaller breeds and that the evolution of periodontal disease is proportional to the age of the dogs.*
Keywords: periodontal disease, dog, breed, age

Periodontal disease manifests itself in almost every dog, but some of them show more severe signs than others. [1,2] Some of the factors influencing the severity of the periodontal disease include the breed and the age of the dogs.[5]

Periodontal disease includes all the stages of inflammation, from early gingivitis to advanced periodontitis, that lead to the loss of the affected tooth. [3] Periodontitis represents the inflammation of the supporting tissue that surrounds a tooth. [4]

Materials and methods

The study was performed on 100 (n=100) dogs of different breeds, genders and ages, randomly selected from the patients of the Small Animal Surgery Clinic, of the Free University Berlin, Germany. Dogs under 1 year were not included in this study.

The clinical examination was performed under general anesthesia with Midazolam®Braun, Polamivet®Intervet (*methadon*) and Narcol®CP-Pharma (*propofol*).

Plaque Index (PI), Gingival Index (GI), Sulcus Bleeding Index (SBI), Calculus Index (CI), Percentage of Attachment Loss Index (PAL), Furcation Exposure (FE) were used for the diagnosis and staging of the periodontal disease, as described by Wiggs et al. (1997).

The owners filled a questionnaire with information about the dogs.

Results and discusion

The dogs were distributed to 4 groups, depending on the size, after the standards of the American Kennel Club [***].

The ***toy*** breeds represented **9%**, the ***small*** breeds **32%**, ***medium-size*** breeds **18%**, and **large** breeds **41%**.

In correlation with the **breed** of the dogs and the stage of the periodontal disease, the following results were obtained (*Table 1*):

- **77,7%** of the ***toy breeds*** were diagnosed with periodontitis;
- of the total of ***small*** breeds dogs, **40,6%** had periodontitis, and almost half of the dogs with gingivitis had generalized lesions;
- ***50%*** of the ***medium-size*** dogs had a healthy gingiva or only early gingivitis localized at the canins and upper forth premolars;

- the same pattern was found in the ***large*** breeds of dogs: **66%** of them had a healthy gingiva or only early gingivitis localized at the canins and upper forth premolars.

Table 1. Percentage of the stages of periodontal disease in correlation with the size of the dogs

Stage of periodontal disease / **Size**	**Toy (%)**	**Small (%)**	**Medium-size (%)**	**Large (%)**
Healthy gingiva	0	6.25	5,55	9,75
Localized early gingivitis	11,11	21,87	44,44	**56,1**
Generalized early gingivitis	0	3,12	11,11	9,75
Localized moderate gingivitis	11.11	6.25	5,55	2,44
Generalized moderate gingivitis	0	21,87	11,11	7,32
Localized early periodontitis	22.22	15,62	16,67	2,44
Generalized early periodontitis	11.11	0	0	2,44
Localized moderate periodontitis	11,11	9,37	0	4,88
Generalized moderate periodontitis	0	6.25	5,55	0
Localized advanced periodontitis	0	6.25	0	4,88
Generalized advanced periodontitis	**33,33**	3,12	0	0
TOTAL	**9**	**32**	**18**	**41**

Depending on the age factor, the dogs in this study were grouped in 4 categories:

- A: 1 – 3 years (**14%**);
- B: 4 – 6 years (**26%**);
- C: 7 – 9 years (**33%**);
- D: 10+ years (**27%**).

In correlation with the **age** of the dogs and the stage of the periodontal disease, the following results were obtained (*Table 2*):

- **64%** of the dogs up to 4 years had healthy gingiva or early gingivitis localized at the canins and upper forth premolars. Only 2 cases (**14%**) had localized early periodontitis;

- **34,5** of the dogs with the age between 4 and 6 years had periodontitis: **23%** early, **7,7%** moderate and **3,8%** advanced;

- only **3%** of the dogs with the age between 7 and 9 years had a healthy gingiva. **67%** of them had gingivitis, **9%** early periodontitis, **15%** moderate periodontitis and **6%** advanced periodontitis;

- no dogs over 10 years had healthy gingiva. Gingivitis was found at 67% of them; the advanced periodontitis was diagnosed in **18,5%** of the cases;

Table 2. Percentage of the stages of periodontal disease in correlation with the age of the dogs

Stage of periodontal disease / Age	1 – 3 A	4 – 6 B	7 – 9 C	≥ 10 D
Healthy gingiva	28,57	7,69	3,03	0
Localized early gingivitis	35,71	34,61	39,39	44,44
Generalized early gingivitis	7,14	7,69	6,06	7,4
Localized moderate gingivitis	7,14	3,84	3,03	7,4
Generalized moderate gingivitis	7,14	11,54	9,09	7,4
Localized early periodontitis	14,28	23,07	6,06	3,7
Generalized early periodontitis	0	0	3,03	3,7
Localized moderate periodontitis	0	3,84	9,09	7,4
Generalized moderate periodontitis	0	3,84	6,06	0
Localized advanced periodontitis	0	3,84	0	11,11
Generalized advanced periodontitis	0	0	6,06	7,4
TOTAL	**14**	**26**	**33**	**27**

Conclusions

1. More severe periodontal disease signs are found in smaller breeds.
2. **77,7%** of the ***toy*** breed dogs and **40,6%** of the ***small*** breed dogs were diagnosed with, while **55,5%** of the ***medum-size*** dogs and **66%** of the ***large*** breed dogs were diagnosed with early gingivitis.
3. The evolution of periodontal disease is proportional to the **age** of the dogs.
4. Periodontitis was diagnosed in **14%** af the dogs up to 4 years old, in **34%** of the dogs with the age between 4 and 6 years, in **30,3%** of the dogs with the age between 7 and 9 years, and in **33,3%** of the dogs with the age over 10 years.

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THE INFLUENCE OF THE TYPE OF FOOD AND ORAL HYGIENE TO THE PERIODONTAL DISEASE IN DOGS

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Abstract
The aim of this study was to determine the effects of the type of food the dog receives on the clinical stage of the periodontal disease. Another aim of this study was to determine the level of oral hygiene the dogs receive from the owners. The dogs were randomly selected from the patients of a small animal surgery hospital in Berlin, Germany, between April and August 2011.This study concluded that dogs that receive mostly or only soft food show more severe forms of periodontal disease. Also, more than half of the dog owners from this study give no attention to the pets` oral hygiene, although 20% of tese dogs had periodontitis.
Keywords: periodontal disease, dog, type of food, oral hygiene

Periodontal disease can manifest from just a mild gingivitis, up to an advanced periodontitis, and is a very common health problem of the dog [3]. The type of food received is also a factor in the appearance and evolution of the periodontal disease [2, 5].

The periodontal disease is a very common health problem of the dogs, although many of them, especially large breeds, only have a mild gingivitis trough their whole life [1, 4]. Periodontitis represents the inflammation of the periodontal ligaments that attach the tooth to the alveolar bone, which in time leads to bone resorption and edentation. [5]

Materials and methods

The study was performed on 100 (n=100) dogs of different breeds, genders and ages, randomly selected from the patients of the Small Animal Surgery Clinic, of the Free University Berlin, Germany. Dogs under 1 year were not included in this study. The dog owners filled a questionnaire with information about the dogs, the type of food administered and the oral hygiene habits.

The clinical examination was performed under general anesthesia with Midazolam® Braun, Polamivet® Intervet (*methadon*) and Narcol® CP-Pharma (*propofol*). Plaque Index (PI), Gingival Index (GI), Sulcus Bleeding Index (SBI), Calculus Index (CI), Percentage of Attachment Loss Index (PAL), Furcation Exposure (FE) were used for the diagnosis and staging of the periodontal disease, as described by Wiggs et al. (1997).

Results and discussion

According to the type of food received, the dogs were distributed to three groups (*Table 1*): dry food, mixed food (dry and wet) and soft food (both canned and home cooked).

Table 1. Percentage of the stage of periodontal disease in correlation with the type of food received by the dogs

Periodontal Disease Stage	Type of food		
	dry (%)	mixed (%)	wet (%)
STAGE 0 – Healthy gingiva	13,63	3,84	0
STAGE I – Early gingivitis	47,72	42,3	46,67
STAGE I - Moderate gingivitis	22,72	7,69	16,67
STAGE II – Early periodontitis	11,36	23,07	6,67
STAGE III – Moderate periodontitis	4,54	15,38	10
STAGE IV – Advanced periodontitis	0	7,69	20
TOTAL	**44**	**26**	**30**

- **70,5%** of the dogs that eat only dry food had signs of early or moderate gingivitis and **16%** had periodontitis;

- **50%** of the dogs that eat only dry food had signs of moderate gingivitis and **46%** of them had periodontitis (early periodontitis **23%**);

- of the dogs that eat only soft food, **63%** were diagnosed with gingivitis and **37%** were diagnosed with periodontitis. Advanced periodontitis was diagnosed in 20% of the cases.

The attention given by the owners to the pets` oral health was graded in three groups:

- A: none;

- B: the occasional administering of oral health products or professional veterinary dental prophylaxis;

- C: increased care for the pets` oral hygiene and frequent professional veterinary dental prophylaxis (Table 2).

Table 2. The attention given by the dog owners to the pets` oral hygiene

Periodontal Disease Stage	Dogs` oral hygiene (%)		
	A	B	C
STAGE 0 – Healthy gingiva	10,17	3,22	0
STAGE I – Early gingivitis	54,24	38,71	20
STAGE I - Moderate gingivitis	15,25	22,58	10
STAGE II – Early periodontitis	11,86	16,13	10
STAGE III – Moderate periodontitis	5,08	9,67	30
STAGE IV – Advanced periodontitis	3,39	9,67	30
TOTAL	**59**	**31**	**10**

- **59%** of the dog owners give no attention to the pets` oral hygiene, although **20%** of these dogs had periodontitis;

- **31%** of the dog owners occasionally give oral health products (mostly chewing products) or request professional veterinary dental prophylaxis. **35%** of these dogs had different stages of periodontitis;

- only **10%** of the dogs owners are consistently taking care of their pets` oral health, by regular professional veterinary dental prophylaxis, teeth brushing and administering dental health. This is probably because **70%** of these dogs had periodontitis, with **60%** of them having moderate and advanced forms.

Conclusions

1. The consistency of the food received represents an important factor in the appearance and evolution of the periodontal disease. Dogs that receive mostly or only soft food show more severe forms of periodontal disease.
2. This can be argued by the fact that some dogs receive soft because they have edentations after suffering from periodontitis.
3. 59% of the dog owners give no attention to the pets` oral hygiene, although **20%** of these dogs had periodontitis.
4. Introducing regular oral examination as a common practice in the veterinary clinics and counceling is paramount to the widespread of the awareness from the pets` owners. This will lead in time to fewer cases of more severe periodontitis and a general improvement of the dogs wellbeing.

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CRYOPRESERVATION OF BOAR SEMEN AND ASSESSMENT OF ITS EFFECTIVENESS BY LABORATORY METHODS

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Abstract
Our research was designed to determine the effectiveness of cryopreservation using two standard extenders as well as other original extenders, to estimate the changes in sperm during freezing in terms of mobility, viability and morphology, as well as to express the temporal evolution of thawed semen quality indexes according to the type of extender used for cryopreservation. A short term extender (BTS) as well as a long term extender (ANDROHEP) were used as such, as well as originally improved by our research team. Boar semen was collected, evaluated, diluted, frozen and thawed at various intervals, when its quality was assessed using standard procedures. Both for short term as well as for long term cryopreservation, the originally improved extenders yielded better results than their commercial counterparts. The original short term extenders based on BTS were able to maintain the mobility of semen for a period of two days (85%), while after thawing the straws on the second day of the protocol, we found satisfactory percentages of live sperm in both proposed experimental extenders (86%). For the long term cryopreservation, ANDROHEP yielded a mobility of 58% while the two original extenders provided a 65% and 68.50% mobility. Preservation of boar semen for more than two days allows its transportation on considerable distances for artificial insemination thus facilitating the genetic progress in all breeds.

Key words: semen, dilution, cryopreservation, thawing, evaluation, qualitative indices

Introduction

Boar semen cryopreservation itself is not an entirely new technique, but advances are always made in order to make it more efficient and to improve fertility rates. Benefits arising are multiple: ensuring the appropriate flow of semen for artificial insemination of sows, creating semen banks collected from valuable or endangered livestock breeds or lines, the assurance of gene stock for genetically tested males and capitalized after their productive life is over, overcoming restrictions on the transport of genetic material, preventing the transmission of illnesses, etc. [1-25].

Our research was designed to coagulate a sum of efforts and results in a well defined purpose: to determine the effectiveness of cryopreservation using two standard extenders as well as other original media, to estimate the changes in sperm during freezing in terms of mobility, viability and morphology, morfocytometry as well as to express the temporal evolution of thawed semen quality indexes according to the type of extender used for cryopreservation.

Material and method

Biological material

The research was conducted during 2007-2010 using 36 semen samples from 20 boars representing approximately 60% of the total harvest. We considered and accepted for processing only the samples whose mobility was evaluated to be over 75%. The origin of

samples in the order of harvesting was as follows: first harvest, using specific techniques and equipment dedicated to this purpose, provided a total of 13 samples, the second harvest provided 12 samples, and the third harvest a number of 11 samples.

Preparation of boar semen for cryopreservation

After assessing the concentration, mobility and morphological normality of sperm, the effectiveness of two known extenders was tested: BTS® extender, that allows cryopreservation of boar semen for two days and ANDROHEP®Plus extender that allows cryopreservation of semen boar for seven days.

Subsequently, each extender was originally modified in terms of the proportion of standard components, thus representing our experimental variants, two for each extender (Table 1 and 2). Preparation of the actual extenders followed the well established steps: weighing, (fig. 1) mixing constituents (fig. 2), homogenization (fig. 3), pH determination (fig. 4), calculation of absolute and relative density (fig. .5), the establishment of osmotic pressure (fig. 6) proper 1:2 dilution, etc.

Fig. 1
Weighing of chemical compounds

Fig. 2
Mixing the constituents

Fig. 3
Homogenization

Fig. 4
pH determination

Fig. 5
Determination of absolute and relative density

Fig. 6
Establishment of osmotic pressure

Table 1. Composition of BTS ® extender and of original experimental variants

		BTS	**BTS_1**	**BTS_2**
No.	***Component***	***Proportion* gr/100 ml**	***Proportion* gr/100 ml**	***Proportion* gr/100 ml**
1	Glucose	3,70	3,70	3,70
2	Sodium citrate	0,60	0,60	0,60
3	EDTA	0,125	0,125	0,125
4	Sodium bicarbonate	0,125	0,125	0,125
5	Potassium chloride	0,075	0,075	0,075
6	BSA	-	-	**0,050**
7	Neomycin sulfate	0,050	**0,055**	**0,055**
TOTAL		**4,675**	**4,680**	**4,730**

Table 2. Composition of ANDROHEP® Plus extender and of original experimental variants

		A	**A_1**	**A_2**
No.	***Component***	***Proportion* gr/100 ml**	***Proportion* gr/100 ml**	***Proportion* gr/100 ml**
1	Glucose	2,600	2,600	2,600
2	Sodium citrate	0,800	0,800	0,800
3	EDTA	0,240	0,240	0,240
4	Sodium bicarbonate	0,120	0,120	0,120
5	HEPES	0,950	**0,570**	**0,665**
6	BSA	0,250	**0,150**	**0,200**
7	Neomycin sulfate	0,100	**0,080**	**0,080**
TOTAL		**5,060**	**4,560**	**4,705**

Cryopreservation of boar semen

The cryopreservation of semen was performed after each harvest, applying the extender for each dilution in each experimental variant proposed. The experimental variants followed the protocols described by Pena et al. 2003, Bianchi et al. 2008 with the amendments proposed by us. The stages were: 1:2 dilution with semen extender, preheated to 32.5ºC and dispensed in Falcon tubes, the mixture was kept one hour at room temperature (22ºC), centrifugation (300-500 rpm / min) using a centrifuge with adjustable temperature 15ºC for 3 hours; the sediment was resuspended in 80 ml ß-lactose and 20 ml egg yolk (medium C) while the sample temperature was gradually reduced from 5ºC to 0.2º C / minute. The third dilution was made using 89.5 ml of medium C and 9 ml glycerol 3% (medium D) maintained at 4ºC followed by packaging, freezing in liquid nitrogen vapors and transferring of straws into liquid nitrogen containers.

Thawing of boar semen

Semen thawing was performed by plunging the straws in a water bath at 38ºC for 20 seconds. After thawing we assessed the mobility, viability, and morphological changes of sperm.

Results and discussions

Quality testing of short time extenders (BTS, BTS1, BTS2) was performed on a total of 13 ejaculate samples after the first harvest of boars. Initial values, day 1 and day 2 after thawing are presented in Table 3.

After 24 hours (day 1) we noticed equal values in terms of motility in all three tested media (86%). After 48 hours (day 2) the BTS extender had an average mobility of 77.5% while the experimental variants (BTS1 and BTS2) had an average mobility of 82.5% (Fig. 1).

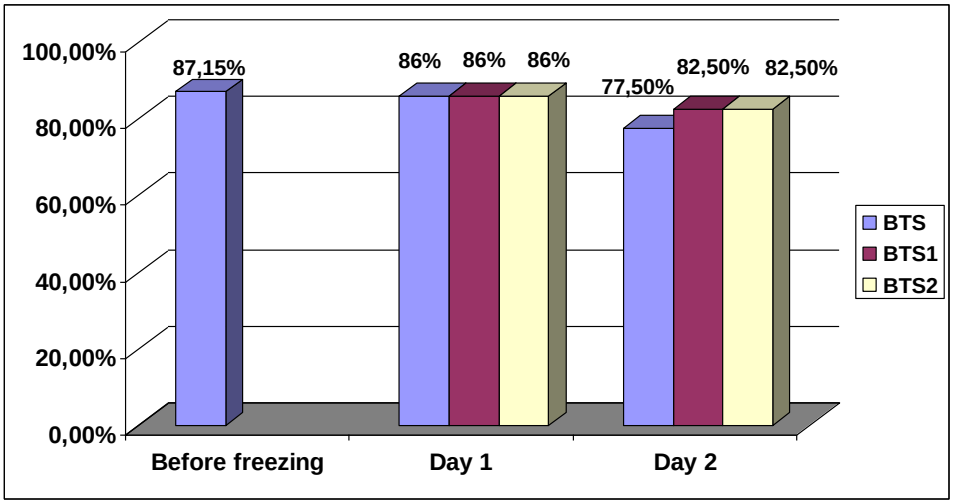

Fig. 1. Changes in mobility for BTS, BTS1 and BTS2 extenders

Regarding the viability all extenders, provided an average of over 85% viable sperm after the first day of conservation, (BTS - 88%, BTS1 - 89%, BTS2-90%). After 48 hours of freezing we observed close values of sperm viability in the original versions, 86% to 85.50% respectively. The commercial extender led to a decrease of approximately 20% of viable sperm (70%) (Fig. 2).

The percentage of dead sperm obtained after 24 hours, were within the physiological limits, ranging from 10-12%. In the second day, there was a considerable increase in the percentage of dead sperm for BTS extender, up to 30%. The average values obtained in our experimental variants were between 14 to 14.5% dead sperm.

Testing of the long-term ANDROHEP ® Plus (A) extender and of the proposed experimental variants (A1, A2) was performed on a total of 11 samples of semen. Initial values, day 5 and day 9 after thawing are shown in Table 4.

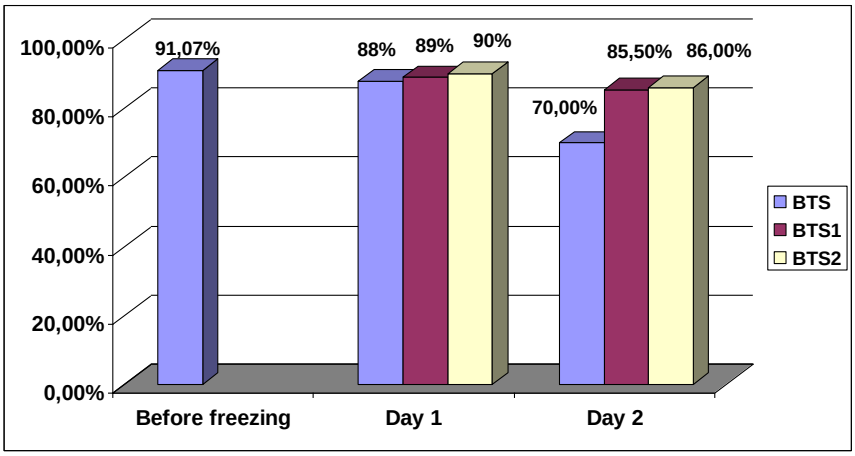

Fig. 2. Changes in sperm viability for BTS, BTS1 and BTS2 extenders

On day 5 of the protocol we found that the average mobility values were of 74%. with the commercial extender. For the experimental variants we obtained values that fall within physiological limits of mobility (72% motile sperms). On the ninth day, the centralized data showed a dramatic decrease when the commercial extender was used, reaching an average of 58% motile sperms. In A1 extender the mobility reached an average of 65% and in the A2 extender, the percentage was of 68.50% (Fig. 3).

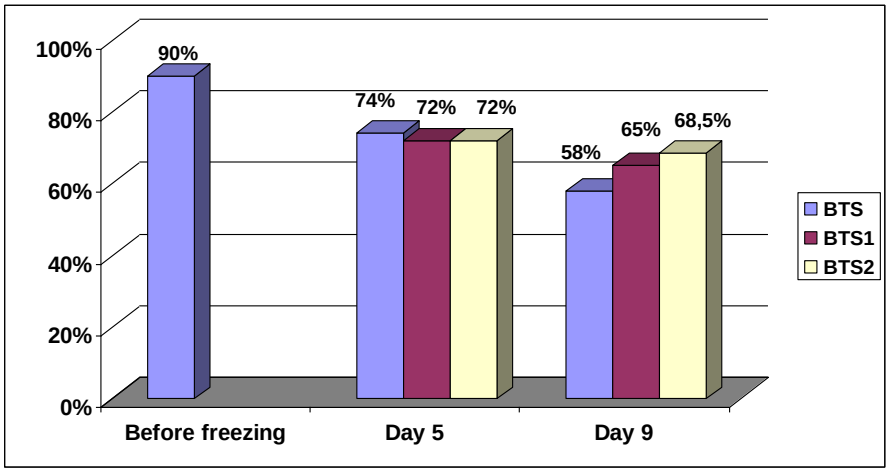

Fig. 3. Changes in mobility for Androhep® Plus, A1 and A2 extenders

Sperm viability percentages obtained on the fifth day of the protocol were 80% for the commercial extender, 79% for the first experimental variant and 81% for the second. On day nine, when the commercial extender was used we noticed a decrease by about 14 percent of the parameter analyzed (56%). Extenders A1 and A2 showed a viability of 60% and 66.50% (Fig. 4).

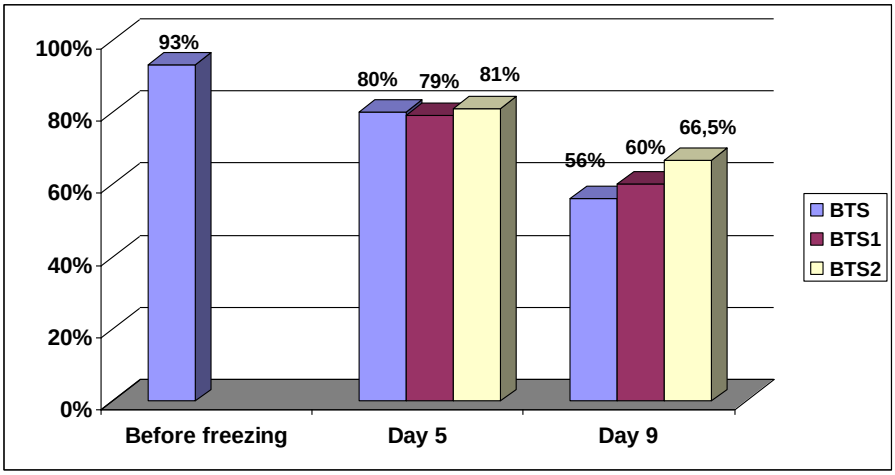

Fig. 4. Changes in sperm viability for Androhep® Plus, A1 and A2 extenders

On the fifth day of the experiment, the mean percentages of dead sperm had an upward trajectory. The use of commercial extender resulted in 20% dead sperm while the experimental extenders yielded 21% and 19% dead sperm. On the ninth day, there was a considerable increase in the average percentage of dead sperm, up to 44% for the commercial extender. The original extenders showed an average percentage of dead sperm of 40% and 33.50%.

Conclusions and recommendations

After conducting the experiments on freezing boar semen, collected between 2007-2010, we formulated the following conclusions and recommendations:

1. the original short term extenders based on BTS were able to maintain the mobility of semen for a period of two days (85%);
2. after thawing the straws on the second day of the protocol, we found satisfactory percentages of live sperm in both proposed experimental extenders (86%);
3. the use long-term freezing extenders based on ANDROHEP ® Plus were able to maintain semen properties for a period of 9 days for all extenders tested;
4. on the ninth day, the mobility was of 58% for the commercial extender 65% for A1 and 68.50% for A2;
5. the viability data, recorded on day nine, are in favor of the original media (60% and 66.50% viable sperm) compared with the A extender (56%);
6. preservation of boar semen for more than two days allows its transportation on considerable distances for artificial insemination thus facilitating the genetic progress in all breeds.
7. we recommend the extender to be supplemented with bovine serum albumin (BSA), a key factor in maintaining sperm viability and mobility.

Table 3. Quality testing for short term extenders (BTS, BTS1, BTS2

INITIAL						Day 1 (average values)									Day 2 (average values)								
						Mobility %			Viability %			Dead sperm %			Mobility %			Viability %			Dead sperm %		
No.	Reg. no.	Vol.	Mobil. %	Viabilit %	Norm. sp. %	BTS	BTS 1	BTS 2	BTS	BTS 1	BTS 2	BTS	BTS 1	BTS 2	BTS	BTS 1	BTS 2	BTS	BTS 1	BTS 2	BTS	BTS 1	BTS 2
1	3287	160	80	90	90																		
2	5689	127	76	90	90																		
3	3314	184	87	90	95																		
4	2587	210	90	95	95																		
5	2244	198	85	90	94																		
6	2581	97	75	78	90																		
7	2489	230	95	95	95																		
8	2411	200	90	95	92	86	86	86	88	89	90	12	11	10	77.5	82.5	82.5	70	85.5	86	30	14.5	14
9	6889	95	80	83	92																		
10	6741	250	95	95	95																		
11	6541	220	90	93	95																		
12	6698	231	95	95	94																		
13	6321	187	95	95	94																		
Average		183,77	87,15	91,07	93,15																		

Table 4 Quality testing for long term extenders (ANDROHEP® Plus, A1, A2)

INITIAL						Day 5 (average values)									Day 9 (average values)								
						Mobility %			Viability %			Mobility %			Viability %			Viabilitate %			Spz.morti %		
Nr. crt.	Nr.matr	Vol.	Mobil. %	Viabilit %	Spz. norm. %	A	A1	A2	A	A1	A2	A	A1	A2	A	A1	A2	A	A1	A2	A	A1	A2
1	3287	157	76	90	90																		
2	3314	176	85	90	95																		
3	2587	187	84	94	90																		
4	2244	180	82	82	89																		
5	2489	200	95	95	95																		
6	2411	182	87	90	90	74	72	72	80	79	81	20	31	19	58	65	68.5	56	60	66.5	44	40	33.5
7	6889	74	75	78	88																		
8	6741	208	90	95	95																		
9	6541	196	88	90	92																		
10	6698	207	90	90	90																		
11	6321	165	90	93	92																		

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CORRELATION BETWEEN THE BIOLOGICAL VALUE OF RAM SEMEN AND FERTILITY IN SHEEP

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Abstract
Semen characteristics were analyzed as follows: volume, mobility, concentration, percentage of dead spermatozoa, abnormal and immature cells. To apreciate the ability of semen fertilized capacity, we analyzed the number of sheeps who became pregnant in estrus, fertility and prolificacy. Semen concentration is one of the main characteristics of sperm. The number of spermatozoa can be appreciated by subjective determination - method used in units not equipped with an optical microscope. Semen sample can be directly analyzed in the collector glass, observing the consistency and waves motions. If the ejaculate has milky or watery aspect, the ram is not used for reproduction and for artificial insemination.The aim of this paper is to find the correlation between biological value of ram semen and fertility in sheep.

Key words: ram semen, semen concentration, semen abnormalities, fertility

Materials And Methods

The study was conducted on a 3 year period, between 2007 and 2011,in the Research Unit in Karakul Sheep Breeding from Popăuţi, Botoşani.

In 2007, the study was conducted on a total of 32 rams, 18 coming in the spring calving, and the rest were from previous year. In 2008, the three experimental groups were made up of 24 rams, in 2009 of 21 rams and in 2010 were taken into study 18 rams. The data obtained was processed statistically, making an average of each annual group, then on the entire experimental period. Statistical processing was performed by known equations and methods, calculating the arithmetic mean and dispersion indexes: variance (S2), standard deviation (S), standard error of the mean (± Sx) and coefficient of variation (V%).

In the autumn, rams semen was thick. During the off season the semen quality depended on the individual and the month the semen has been harvested, so the ejaculates were assessed as being rare, medium or thick.

Anomalies recorded in collected semen from adult rams were represented by the spermatozoa with flattened head and spermatozoa with their tail twisted.

Results and discussions

The group from which semen was collected for the research was comprised of a total of 46 rams. Sexual reflexes were well expressed in the natural breeding season (September, October and November) and less in the months outward of this season (June, July, August).

11 rams with an operating period of four years were harvested by 396 times the normal breeding season, and 138 times during out of this natural breeding season. Average volume per ejaculate was 1.2 ± 0.03 ml, range between 0.5 and 2.6 ml in natural season, and 0.63 ± 0.02 ml, range between 0.2 and 1,5 ml, out of this season.

We studied 7 rams with a service life of 5 years. A total of 324 of ejaculate were collected in natural breeding season, whose volume varied between 0.5 and 2.4 ml/ sample. Calculated average volume was 1.21 ± 0.08 ml. During out of the natural breeding season, from these rams were obtained 382 samples, with volume between 0.3 and 1.2 ml, with an average of 0.78 ± 0.09 ml/ samples.

In the below images are shown semen characteristics, depending on age and experimental group.

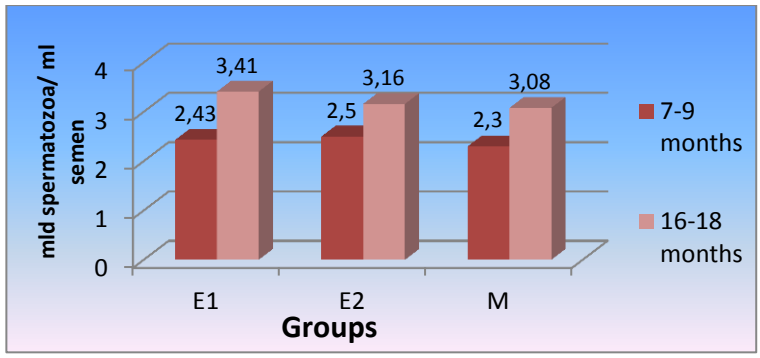

Fig. 1. Concentration of semen collected from ram lambs of 7-18 months, observed by age and groups

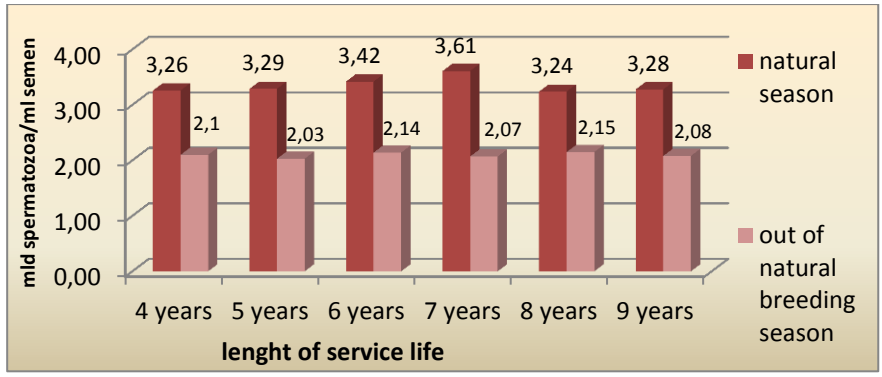

Fig. 2. Concentration of semen collected from adult rams, depending on season and duration of operation

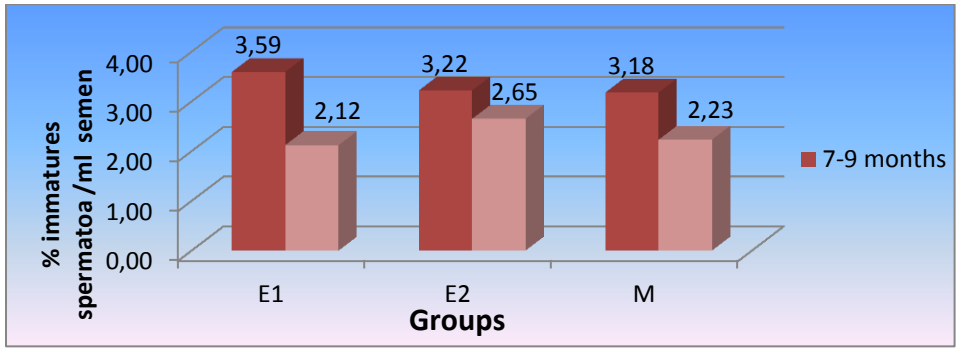

Fig. 3. The percentage of immature spermatozoa in semen of ram lambs, observed by age and groups

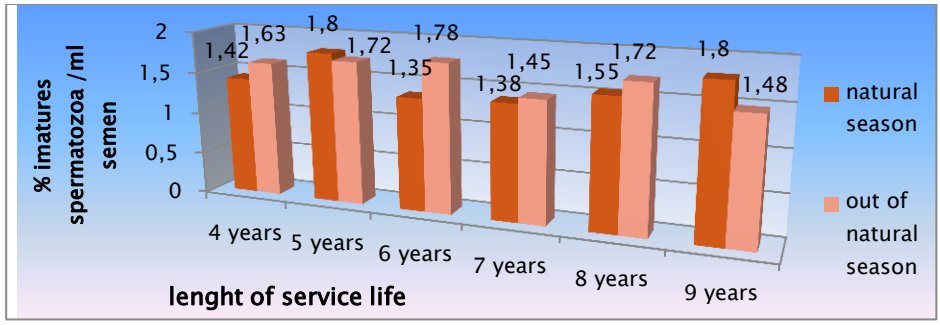

Fig. 4. The percentage of immature spermatozoa in the semen collected from adult rams, depending on season and duration of operation

The percentage of dead spermatozoa per sample varies greatly depending on the season when harvesting was performed and on the individual.

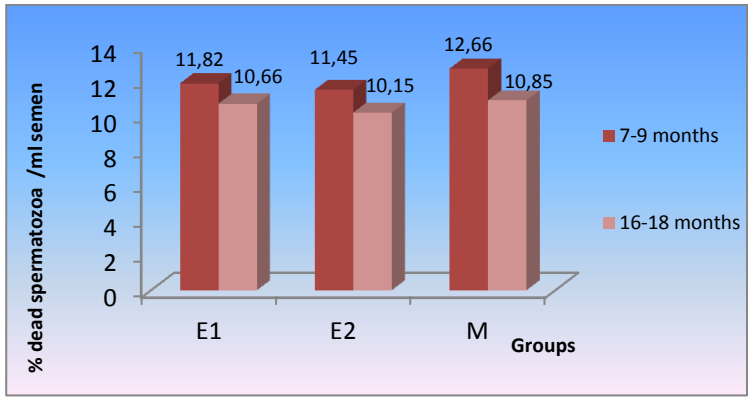

Fig. 5. The percentage of dead spermatozoa in the ram lambs semen, observed by age and groups

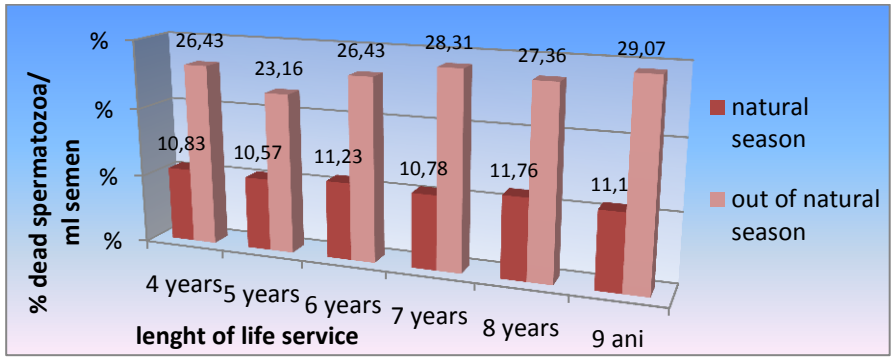

Fig. 6. The percentage of dead spermatozoa in the semen collected from adult rams, depending on season and duration of operation

Anomalies recorded in collected semen from adult rams were represented by the spermatozoa with flattened head and spermatozoa with their tail twisted.

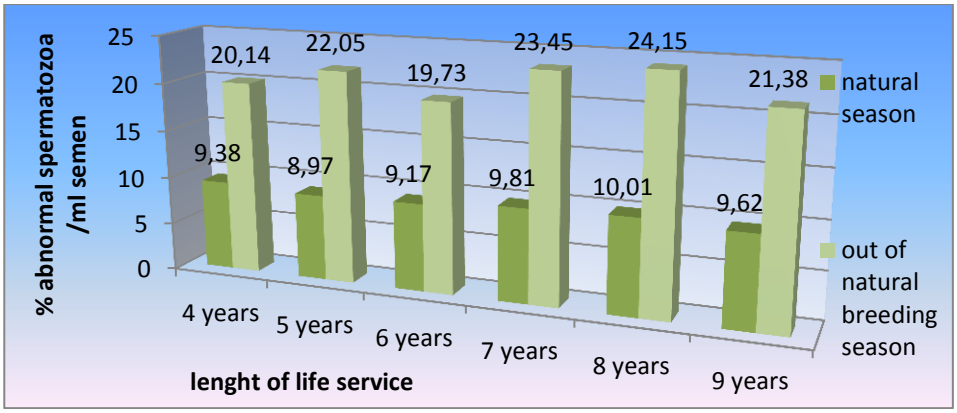

Fig. 7. The percentage of abnormal spermatozoa in semen collected from adult rams, depending on season and duration of operation

Rams with a service life of 4 years were used for natural or artificial breeding for a total of 1234 females. The percentage of females that were pregnant after breeding was 86.95 ± 1.75%, with limits of 69.83 and 98%, corresponding to a number of 1073 females.

For a period of 5 years, 7 rams have mated a number of 872 females, of which 767 remained pregnant, the percentage being 87.95 ± 0.37%, and the annual limits were 71.51 and 99.6%.

In table 1 are presented values of the fecundity percentage, obtained after mating these studied rams, during all experimental study.

Table 1. Average values for fecundity (F%) obtained from adult rams, during the natural breeding season

Lengh of service years	No. rams	No. mated sheeps	No. sheeps who gave birth	Fecundity %	Limits %	S_x	V%
4	11	1234	1039	84,20	70,04-97,21	0,73	3,48
5	7	872	744	85,32	73,40-98,13	0,64	1,61
6	9	1028	887	86,28	72,62-97,13	0,55	2,43
7	10	1103	965	87,48	74,58-100	0,53	1,62
8	6	1732	1485	85,73	76,21-96,83	0,28	1,50
9	3	1466	1290	87,99	72,04-94,01	1,34	265

Conclusions

1. High temperatures and long duration of the day - light have a negative effect on the neuroendocrine system of rams, resulting in decreased intensity of sexual reflexes, the ability of spermatozoa and their fertilizing capacity.
2. In the autumn, rams sperm was thick; during out of the natural breeding season, depending on the individual and the month when samples were harvested, samples were assessed as being rare, medium or thick.
3. PH value had a variation similar to concentration, tending towards acidity in thick semen to alkalinity in medium and rare semen, depending on season and individual values ranging between 6.5 and 7.1.
4. Percentage of fertility for rams with the service life of four years, calculated on 1234 female mated was 84.20 ± 0.73%, meaning 1039 females that have calved.

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REPRODUCTIONS INDICATORS OBTAINED BY ADULT RAMS OUT OF THE BREEDING SEASON

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Abstract
Karakul rams can be succesfully used for natural breeding all year long. There are some variations regarding the intensity of their sexual reflexes and the semen characteristics. Sheeps from this breed manifest estrus only during the natural period of breeding, starting August till the end of December, having a peak in October. In order to intensify the breeding process and obtain two calvings per year, it is necessary to induce and syncronise estrus out of the normal breeding season, using hormone therapy. The aim of this paper is to find the correlation between hormone therapy in sheeps and fertility out of the breeding normal season.
Key words: hormone therapy, rams, semen characteristics

Materials and methods

The study was processed during three years, starting 2007, till the end of 2011, in the Research Unit in Karakul Sheep Breeding from Popăuţi, Botoşani.

In 2007, the study was conducted on a total of 32 rams, 18 coming in the spring calvings, and the rest were from previous year. They were maintained on field, and at the age of 5-7 months and 16-18 months, formed two experimental groups and one witness: group E1, group E2 and group M. The factors that could influence semen quality and sheep's breeding were monitorised: alimentation and when entering for service - for young males, and mating season and the length of service for adult rams.

Semen characteristics were analyzed as follows: volume, mobility, concentration, percentage of dead spermatozoa, abnormal and immature cells. To apreciate the ability of semen fertilized capacity, we analyzed the number of sheeps who became pregnant in estrus, fertility and prolificacy.

Results and discussions

In order to accustom the, males breeding, they were used as testing rams at the beginning of mating, for a period of 10 days, 30 minutes / day.

To examine semen, we harvested the semen from ram lambs using the artificial vagina and we analyzed parameters coming from an average of 10 ejaculate / ram lamb. In Table. 1 are represented values of the volumes obtained from semen samples from ram lambs of 7-18 months, in 2010, depending on the groups and age groups.

For the experimental group E1, age 7-9 months, two ram lambs were harvested, from which were examined 18 semen samples. Their volume ranged from 0.2 to 1.5 ml with a mean of 0.79 ± 0.06 ml calculated. In the same group was a number of 3 MIORI , from which 33 samples were examined. Their volumes were between 0.5 and 2 ml, with an average of 1.05 ± 0.01 ml.

Group E2 was composed of 7 ram lambs. From 4 weaned males were harvested 40 samples. The calculated average volume was 0.87 ± 1, 01 ml, with limits ranging between 0.3 and 1.5 ml. In group 2 were 3 ram lambs, and the number of samples collected from them was 31. Average volume per ejaculate was 1.03 ± 0.04, individual values ranging between 0.5 and 2 ml / sample.

Table 1. Average values of the volumes obtained from semen samples from ram lambs of 7-18 months, in 2010

Group	Age (months)	No. animals	No. ejaculates	Limits (ml)	X (ml)	S_x	V%
E1	7-9	2	18	0,2-1,5	0,79	0,06	3,37
	16-18	3	33	0,5-2,0	1,05	0,01	5,9
E2	7-9	4	40	0,3-1,5	0,87	0,01	7,6
	16-18	3	31	0,5-2,0	1,03	0,04	2,1
M	7-6	3	28	0,3-1,5	0,90	0,05	2,8
	16-18	3	33	0,5-2,0	1,08	0,03	1,5

Number of semen samples collected from ram lambs aged 7 and 9 months, integrated in the control group was 28, and the average volume calculated was 0.9 ± 0.05 ml, individual range between 0.3 and 1.5 ml. The three Miori of this group recorded values of the semen volume between 0.2 and 2 ml per sample, with an average of 1.08 ± 0.03 ml.

Analyzing the data presented, it appears that there are considerable differences in semen volume depending on the group, therefore nutrition has not played a decisive role. The differences concern only age groups within each experimental year. Mean semen volume of young male sheep, and the age groups are given in Table 2.

Table 2. Average values of the volumes of ram lambs semen, observed on groups and the significance of differences between age groups

Group	Age (month)	No. animals	No. semen samples	Limits ml	X ml	S_x	V%	$\frac{d}{sd}$	P %	semnificatio n
E1	7-9	17	151	0,2-1,7	0,84	0,06	8,69	$\frac{0,20}{0,09}$	0,1	Very
	16-18	13	139	0,4-2	1,04	0,03	3,36			
E2	7-9	19	177	0,2-1,8	0,9	0,04	2,78	$\frac{0,13}{0,07}$	0,1	Very
	16-18	18	184	0,4-2	1,04	0,06	7,86			
M	7-9	16	131	0,2-1,8	0,87	0,04	5,17	$\frac{0,22}{0,04}$	1	Distinct
	16-18	15	164	0,5-2	1,09	0,04	4,67			

In experimental period of 2010, sperm mobility values were assessed by examining the samples collected from 86 weaned rams and 97 taken from Miori ejaculate. The values obtained are shown in Table 3.

For the age group 16-18 months, the mean of sperm mobility was 87 ± 0.57% in the experimental group E1, 87.5 ± 0.58% in the experimental group E2, and 85.57 ± 1.1% for the control group. Limit values recorded for the 33 samples analysed in group E1 are 85 and 90%, same as for the 31 samples corresponding group E2. The control group, on which were examined 33 samples, showed the mobility limits of 81 and 90%.

Table 3. Values for the mean sperm mobility for ram lambs of 7-18 months, in groups, in 2010

Group	Age (months)	No. animals	No. samples	Limits %	X %	S_x	V%
E1	7-9	2	18	60-90	81,3	0,92	4,79
	16-18	3	33	85-90	87,0	0,57	3,75
E2	7-9	4	40	65-90	81,6	0,64	4,95
	16-18	3	31	85-90	87,5	0,58	3,68
M	7-6	3	28	60-90	81,65	0,85	5,49
	16-18	3	33	81-90	85,57	1,1	7,37

We also studied three groups of sheeps, treated with different hormonal regimen. Since it was intended to obtain lambs skins, and their sacrifising is early, we did not keep track of fatherhood, which is important when products are to be obtained for breeding.

Group no. 1 was composed of 124 Karakul sheeps, to whom were administrated progesterone treatment, parenterally, and Folligon. Lot No. 2 was composed of 163 females. In order to induce and synchronize estrus were used Folligon and Veramix sponges. Group no. 3 was composed of 126 females treated with progesterone and SIG scheme. All sheeps that came into oestrus were naturally mated with the 50 studied males, with different operating periods for 5 consecutive days.

Table 4. The results of treatment regimens used for induction and synchronization of oestrus

Group	Treatment	No of treated sheeps	Sheeps in oestus		Sheeps with lambs		No of lambs obtained	P%
			Nr	%	Nr	%		
1	Progesteron+ Folligon	124	103	83	62	50	102	164,50
2	Veramix+ Folligon	163	120	73,61	59	36,19	66	112,0
3	Progesteron+ SIG	126	53	42,06	35	27,77	41	117,14

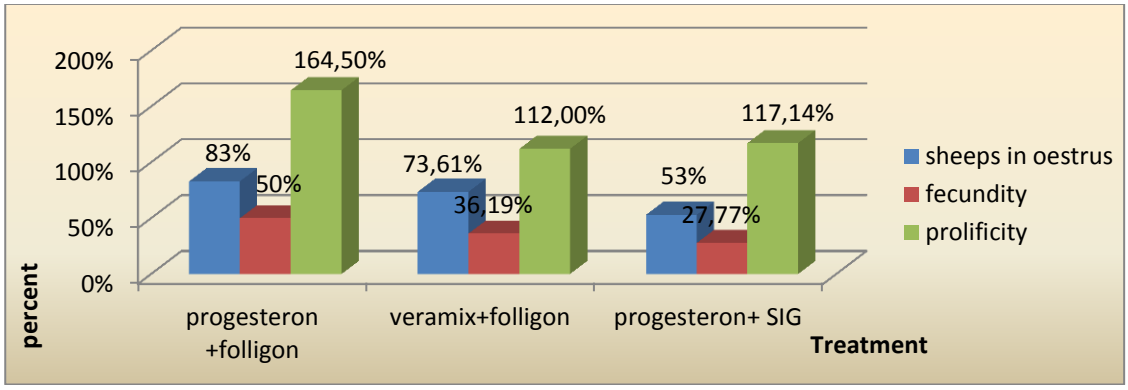

Fig.1. Fertility rates and prolificacy obtained out of the natural mating season, depending on the used treatment

For group no. 1, the percentage of sheep in the heat after using hormonal therapy was 83%, meaning 103 out of 124 females. Of these, 62 have given birth, meaning 50% of the herd, resulting in 102 products. Prolificacy percentage was 164.5%.

In group no. 2, 120 females manifested heat, representing 73.61% of those treated with sponges after Veramix and Folligon scheme. From all sheeps who manifested heat, 59 sheeps gave birth after mating, meaning a percentage of 36.19, resulting in 66 products. Percentage of prolificacy was calculated of 112%.

On group 3 of 126 women treated with progesterone and SIG, came in heat 53 sheeps, meaning 42.06%. Of these, 35 have given birth, representing 27.77% of the sheeps treated by this scheme. The number of lambs produced was 41, corresponding to a percentage of 117.14% prolificacy.

Percentage of fertility obtained out of the natural breeding season is low, ranging between 27.77 and 50%, depending on the applied treatment, compared with the fecundity average realized during the natural season, which ranged between 84.20 and 87.99 %.

Percentage of prolificacy achieved out of the normal breeding season was between 164.5 and 117.40% higher than the one obtained in natural season, which is between 101.46 and 102.31%. This is due to the hormonal therapy effect on ovulation rate out of the normal breeding season.

Conclusions

1. For group E1 at the age 7-9 months were examined a 18 samples, which registered a 81.3 average mobility ± 0.92%, range 60 to 90%. At the same age in group E2 were examined 40 samples, which recorded a mean of 81.6 ± 0.64% spermatozoa, limits ranging between 65 and 90%.
2. Semen collected from ram lambs aged between 7 and 18 months, during the entire experimental period, had the parameters of sperm quality and quantity of adult rams.
3. Karakul breed females show estrus spontaneously only during the natural breeding season; out of this season, breeding is possible only by using hormonal therapy to induce and synchronize estrus.

4. Hormonal treatment used for induction and synchronization of oestrus, which gave the best results (highest fertility and prolificacy rate) was based on the use of progesterone administered parenterally, combined with Folligon.
5. The low rate of births outside the natural breeding season is not just the result of female reproductive function characteristic for Karakul breed, but also because of the rams of the breed. The high prolificacy outside the natural breeding season, compared with that obtained in natural season, is the result of the hormonal therapy on ovulation rate.

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SEASONAL ALTERATIONS OF THE BLOOD STATUS IN TIGAIE SHEEP IN THE BRAN AREA

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Abstract
The large number of sheep in a herd and frequency of conditions require finding markers that can be used in health surveillance. Blood can be considered an important marker of the health status of sheep because it provides data on the diagnosis of diseases of the blood tissue, hematopoietic organs, of other organs and systems or metabolic disturbances. This paper aims to identify whether the biochemical examination of blood can be used as a marker of health surveillance in sheep.
Keywords: Sheep, biochemical markers, blood tissue disease

Material and method

The biological material used was represented by an estimated 8 sheep, male and female, aged 1-5 years, of Bran, Brasov county which has approx. 400 heads of sheep of the breed Tigaie Belle with white heads.

Biological material was sampled before drinking, the sheep grazing. Blood was collected in sterile vacutainers. Heparinized vacutainers were used for blood counts and vacutainers without anticoagulant were used for biochemical determinations. Biological material was collected in November 2010 and March 2011.

For hematological and biochemical analyzes we have used: automatic veterinary hematology analyzer. The veterinary hematology analyzer is a fully automatic device for measuring cells for in vitro diagnosis, manufactured by Diatron LTD., USA.

Results and discussions

Of the total of 400 sheep we randomly selected a number of 8 cases. Presentation of results will be offered by case study, based on the analysis report prepared following the biochemical examination of blood.

Blood samples were collected on November 10, 2010. Analyses were performed in the Clinical Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca (Table 1).

Table 1. Analysis bulletin-biochemical examination

Parameters/ Cases	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Ref.**
Ca^{2+} (mg/dl)	14,3	12,1	12,0	11,75	13,4	12,0	11,1	14,3	9,2-12,6
Mg^{2+} (mg/dl)	2,9	2,89	3,8	3,69	3,51	3,0	2,98	3,12	1,9-3,65
P (mg/dl)	6,4	5,4	5,45	5,1	6,0	5,56	5,0	6,3	4,0-6,5
Na^{+} (mg/dl)	140	158	129	154	143	154	152	160	149-160
K^{+} (mg/dl)	4,74	5,3	5,8	5,2	5,5	2,9	4,58	4,73	3,5-5,5

Prot.tot. (g/dl)	5,68	6,2	6,55	6,33	5,8	5,6	5,38	5,3	6,0-8,0
Alb. (g/dl)	3,39	4,0	4,21	4,11	3,44	4,0	3,88	3,52	2,5-2,92
PAL (U/l)	385,8	451,7	301,7	213	404,3	464,3	265,7	284,8	40-200
GGT (U/l)	53,7	38,2	49,5	45	34	23	36	59	10-30
ALAT (U/l)	32	34	30	27	25,5	24,5	18,4	25	<14
ASAT (U/l)	148	133	135	118	105	62	112	107	<65
α – amylase (U/I)	155,9	28,4	18,0	120,1	51,8	11,1	79,2	29,1	-
Urea (mg/dl)	38,2	28,2	31,0	26,8	23,5	38,3	27,5	30,1	24-40
Creatinin(mg/dl)	1,05	0,98	1,06	0,93	0,89	1,0	0,96	0,95	0,6-1,4

Observations. Out of the eight cases sampled:

- cases 1, 5 and 8 show a slight increase in calcium due to hypervitaminosis D and an increased intake, sheep are grazed all summer. Hypercalcemia may be seen in the case of neoplasms, which causes release of hypercalcemia producing substances (pseudo-hyperparathyroidism), which resulted in the presence of calcium values exceeding several times the normal value, not met in these cases.
- cases 3 and 4 show a slight increase of magnesium due to an increased intake, sheep are grazed all summer.
- case 3 shows a slight increase in potassium due to diarrhea syndrome, and in case 6 a slight decrease due to an insufficient intake.
- cases 1, 5, 6, 7, 8 show a decrease of one unit of total protein due to subclinical parasitic infections.
- All cases have a relative increase in albumin due to a dehydration process, samples were collected at noon, sheep not being watered.
- all cases show an increase in alkaline phosphatase, correlated with elevated transaminases. These changes can be found in liver deficiencies, which led us to recommend performing a stool examination for diagnosis of liver parasites.

Blood samples were collected on March 30, 2011. Analyses were performed at the Laboratory of Veterinary Medical Analysis ERIVET Cluj-Napoca and documented in the analysis reports 1-8 (Table 2).

Table 2. Analysis bulletin- biochemical examinations

Parametres / Cases	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Unit**
TOTAL CA	8,83	8,90	8,81	8,86	9,1	11,3	9,0	8,85	mg/dl
K.	2,35	1,89	1,88	1,93	1,94	2,3	2,1	2,0	mg/dl
ALP	153	164	162	165	160	44,0	180,0	23,0	U/I
GAMMA GT	28	31	29	41	28	27,0	40,0	42,0	U/I
UREA	23,8	24	20,2	16	14	26,0	12,0	17,0	mg/dl
CREA	1,45	1,90	1,1	1,74	0,90	1,3	1,2	1,81	mg/dl
GOT	124	96	94	51	127	68,0	71,0	120,0	U/l

GPT	17	31	18	17	53	47,0	40,0	49,0	U/l
ISE-Na	144	156	151	146	152	158,0	150,0	142,0	mEq/1
ISE-K	4,5	5,1	5,2	4,7	4,6	4,3	4,5	4,4	mEq/1
TOT PROT	7,0	6,1	6,2	6,3	6,0	7,5	7,4	6,6	g/dl
ALBUMIN	2,74	3,2	3,0	2,9	2,65	6,9	3,4	2,7	g/dl
AMYLASES	240	250	145	201	190	260,0	185,0	242,0	U/l
TOT BILIRUB	0,34	0,25	0,44	0,36	0,23	0,49	0,33	0,14	mg/dl
P	4,1	3,98	4,3	5,2	3,97	5,7	5,4	4,5	mg/dl

In case no. 1 there was a slight decrease in serum calcium due to an insufficient intake during the winter and the increase with a unit of GOT is put on behalf of subclinical liver failure.

In case no. 2 there was a slight decrease in serum calcium and magnesium due to an insufficient intake during the winter and the increased alkaline phosphatase is put on the account of metabolic bone disorder which is directly correlated with lower calcium and magnesium.

In *case no. 3*, similar to case 2 there was a slight decrease in serum calcium and magnesium due to an insufficient intake during the winter, and increased alkaline phosphatase is put on the account of metabolic bone disorder which is directly correlated with lower calcium and magnesium. Increased alkaline phosphatase can be found in the liver, which lead to high transaminases, which was not found in this case.

In case no. 4, similar to case 2 and 3 there was a slight decrease in serum calcium and magnesium due to an insufficient intake during winter, and increased alkaline phosphatase to put on the account of metabolic bone disorders, and it is directly correlated with lower calcium and magnesium.

In case no. 5, similar to case 2, 3, 4 there was a slight decrease in serum calcium and magnesium due to an insufficient intake during winter, and increased alkaline phosphatase put on the account of metabolic bone disorder which is directly correlated with lower calcium and magnesium.

In case no. 6 increased transaminase (GPT) is due to liver problems, which led us to recommend examination of the stool for diagnosis of parasitic liver. A relative increase in albumin was attributed to a drying process; samples were collected at noon, sheep not being watered. Case no. 6 being male, maintains the normal concentration of calcium, magnesium and alkaline phosphatase which were modified in studied females.

Case no. 7 shows an increase in alkaline phosphatase attributed to metabolic bone disorders. Increased alkaline phosphatase can be found in the liver but it must be correlated with changes in transaminase increase, something that was not found in this case.

In case no. 8 there was a slight decrease in serum calcium due to an insufficient intake of winter, and the increase of a unit's of GOT is put it on behalf of subclinical liver failure. Decreased alkaline phosphatase is probably due to a disorder associated with malnutrition because other parameters do not change.

Conclusions

Based on these results several conclusions can be drawn.

1. Blood can be considered an important marker of health status of sheep because it provides data review on the diagnosis of diseases of the blood tissue, hematopoietic organs, other organs and systems or metabolic disturbances.
2. Blood biochemical examination revealed changes in the type of nutrition, physiological status and time of harvest - fall, spring.
3. During the grazing period there was a slight increase in serum calcium, phosphate and magnesemia and during these calves periods suffer a slight decrease. The changes were attributed to substance intake and not due to a disease, which is close to the upper, respectively lower limit.
4. In all cases the relative increase of albumin is due to a drying process, forages samples were collected at noon, sheep were not watered.
5. All cases show an increase in alkaline phosphatase, correlated with elevated transaminases. These changes can be found in liver, which led us to recommend performing a stool examination for diagnosis of liver parasites, knowing that increased transaminase activity appears long before clinical signs.
6. Decreased total protein, suggest a liver disease, dehydration and can provide data on gastrointestinal tract and adnexal glands function in case of malabsorption.
7. Increased alkaline phosphatase without changes correlated with increased transaminases put it on account of metabolic bone disorder that is directly correlated with lower calcium and magnesium.
8. Based on the results and interpretations, we believe that the laboratory determination we have used can be used as para-clinical methods, in sheep, for diagnosis of parasitic disease etiology. For the sheep's that we have studied, we recommended deworming and a closer monitoring of pastures to reduce the risk of infestation.

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INTRODUCTION OF SOME THERAPIES TO IMPROVE THE REPRODUCTIVE PERFORMANCE OF POSTPARTUM EGYPTIAN BUFFALOES

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Abstract

The current study was designed to investigate the effect of different postpartum (PP) treatments on uterine involution, initiation of ovarian activity and the subsequent reproductive performance in buffaloes. 167 parturient buffaloes (2nd- 5th calving) were allotted into 15 groups according to the applied regime. All treatments were started at 0 h PP except for levamisole (LV) which was given on Day 0, 2, 4 d PP. Group1 (n=11), group 2 (n=12) and group 3 (n=11) received I.M injection of prostaglandin (PG), oxytocin (Ox) and methergin (MT), respectively. Group 4 (n=12) received S.C injection of levamisole (LV) only. Group 5 (n=15) and group 6 (n=10) received intrauterine infusion (IUI) of oxytetracycline (OXt) and Betadine (BT), respectively. Group 7 (n=9) and group 8 (n=12) received PG plus IUI of OXt of and PG plus IUI of BT, respectively. Group 9 (n=11) received PG plus S/C of LV. Group 10 (n=11) received both OX plus IUI of OXt. Group 11 (n=10) received OX plus IUI of BT. Group 12 (n=14) received OX plus S.C LV. Group 13 (n=7) received MT plus IUI of OXt. Group 14 (n=9) received MT plus IUI of BT. Finally, Group 15 (n=13) received MT plus S.C of LV. The current results revealed that the shortest time to placental dropping (3.400 ± 0.221 h), complete uterine involution (28.00 ± 0.41 d), onset first PP estrus (55.18 ± 2.51 d) and days open period (59.44 ± 2.68 d) was recorded in group 7 (OX+BT), group 13 (MT+LV) and group 4 (PG+LV), group 2 (PG+OXt), respectively. The number of service/conception was minimal (1.11±0.11) and the conception rate was the highest (88.89 ± 11.11%) in group 2 treated with PG+OXt. We could conclude that, treatment with prostaglandin beside intrauterine infusion with oxytetracycline is beneficial in improving the reproductive performance of postpartum buffaloes in terms of short days open, decease the number of services per conception and obtaining the highest conception rate.

Key words: Buffalo, Days open, Postpartum, Uterine involution.

Introduction:

Buffaloes represent the majority of animal wealth in Egypt because of its importance in agricultural system and its great importance as a dairy animal. Nevertheless, 30% of buffaloes and cattle were recorded to suffer from reproductive disorders which constitute a major waste in the national income. Methergin (MT); a derivative of ergot alkaloid; increases the tone and amplitude of uterine contractions through stimulation of uterine $PGF_{2\alpha}$ release (1). Methylergometrin immediately injected after calving decreases the incidence of retained placenta and shortens the service period in cases with a history of retained placenta (2).

Roles of prostaglandin (PG) in reproduction process were assumed as a uterine luteolytic hormone in some species and sensitize the uterus to the action of oxytocin (3). PG causes vasoconstriction and increase motility of uterus and fallopian tube (4), induces lyses of the corpus luteum and onset of estrus, and reduces the interval between parturition and conception (5). An injection of PG early PP was found to increase the conception rate (70% vs. 44% in control) (6).

Oxytocin (OX) has specific receptors in the myometrium, that reaches its maximum concentration during pregnancy and early PP. Exogenous administration of OX in the diestrus phase has a luteolytic effect due to stimulation of the release of PG (7). The biological effect of OX and its analogues depends on two factors: how quickly it is removed

from the circulation and whether there are enough receptors capable of binding the drug (8). It has been suggested that the uterus is still sensitive to the action of OX between 14-16 h after calving and its uterotonic effect starts within approximately 10 minutes after treatment and lasted over the next two hours (9).

Antibiotics treatment is recommended as a part of prophylactic measures during PP period to reduce the incidence of retained fetal membranes. An early recovery from metritis in buffaloes was noted with the use of oxytetracycline (OXt) and the conception rate after insemination at 1st estrus post-treatment was higher than that after lugol's iodine treatment (10). Lugol's solution containing iodine stimulates uterine tone and mobilizes neutrophils; this in turn helps in preventing placental retention and endometritis (11).

Levamisole (LV); a synthetic broad spectrum anthelmintic; has been recognized as an immune-stimulating drug which augmented the protective effect of brucella vaccine in mice (12). In general, it was accepted that LV increased phagocytosis by polymorph nuclear leucocytes and macrophages, acute phase of proteins and leukocyte interferon production (13). LV administration on days 0, 2, 6, 10, 14 and 18 of the estrous cycle appeared to have good therapeutic effects in treating endometritis and improve the pregnancy rate (14).

The present study was designed to study the efficiency of different attempts to improve the fertility of buffalo herd through the use of some drugs like prostaglandin, oxytocin, methergine, betadine, tetracycline and levamisole at an early stage of the PP period.

Materials and Methods

***Animals**:*

The present study was carried out on a total number of 167 parturient Egyptian buffaloes belonged to the research station, faculty of agriculture, Menoufia University and some of small private farms. Animals under experiment were fed concentrates plus clover and straw during green season and in dry season they were fed concentrates plus hay and straw, they were vaccinated against infections discuses and were free from any parasitic diseases.

Experimental design

Animals under experimental condition were randomly divided according to type of treatment into 15 groups (table 1&2) according to the type of post parturient therapies. All treatments started at 0 h postpartum (PP).

For levamisole (LV) which was given at the day 0, 2, 4 d PP. Routine examination of the genitalia per rectum was conducted once every 2 days to judge the uterine involution and ovarian cyclicty, beside a twice daily observation for checking the onset of heat. Animals observed in heat were naturally mated by a proven fertile bull. Efficacy of treatment was judged through the following reproductive parameters: *i. Uterine involution*: Uterine involution was considered complete when both horns were nearly symmetrical and no further change took place between two consecutive examinations (15). *ii. Ovarian rebound and heat detection* depending on the presence of ovarian structures e.g. Graffian follicles or Corpora lutea. *iii. Conception rate*: the non-return animals after 21 days post servicing (conceived) were confirmed by rectal examination 50 to 60 days later for pregnancy diagnosis. *iv.* Days to first PP breeding. *v.* Number of services per conception and date of fertile service (indicated by pregnancy diagnosis) were registered in the herd records. *vi.* Days open interval from calving to the date of fertile service confirmed by pregnancy check (16).

Table 1 Drugs used and route of administration

Drug	Active principle	Abbrev.	Dose	Route	Company
Lutalyse®	Dinoprost (Prostaglandin $F_{2\alpha}$)	PG	25 mg (5mg/ml)	i.m.	Pharmacia NV/SA-Belgium
Oxytocin®	Oxytocin	OX	20 IU (5IU/ml)	i.m.	ADWIA Co. for Pharmaceuticals and Chemical Industries, Cairo.
Methergine®	Methylergometrine hydrogen maleate,	MT	1 mg (200µg/ml)	i.m.	Novartis Pharma AG.
Ucimisole®	Levamisole	LV	200 mg (10mg /ml)	s.c.	Amoun Pharmaceutical Co., , Cairo.
Uvomycin®	Oxytetracycline hydrochloride	OXt	20 ml (5mg/ml)	IUI	Intervet Egypt for Animal Health, Cairo.
Betadine® (Vaginal - 5%)	(Povidone Iodine)	BT	100 ml of 3% prepared soultion	IUI	Nile Co. for Pharmaceuticals and Chemical Industries, Cairo.

i.m=intramuscular, s.c. =subcutaneous and IUI=intrauterine infusion.

Table 2 Distribution of buffalo groups and line of treatment postpartum

Animal group	**n**	**abbreviation**	**Line of treatment**
Group 1	11	PG	i.m injection of PG.
Group 2	9	PG+OXt	i.m of PG and IUI of OXt.
Group 3	12	PG+BT	i.m of PG and IUI of BT.
Group 4	11	PG+LV	i.m of PG and S.C of LV.
Group 5	12	OX	i.m injection of OX.
Group 6	11	OX+OXt	i.m of OX and IUI of OXt.
Group 7	10	OX+BT	i.m of OX and IUI of BT.
Group 8	14	OX+LV	i.m of OX and S.C of LV.
Group 9	11	MT	i.m injection of MT.
Group 10	7	MT+OXt	i.m of MT and IUI of OXt.
Group 11	9	MT+BT	i.m of MT and IUI of BT.
Group 12	13	MT+LV	i.m of MT and S.C of LV.
Group 13	12	LV	s.c. injection of LV.
Group 14	15	OXt	IUI of OXt.
Group 15	10	BT	IUI of povidone iodine (BT).

Statistical analysis

Data were tabulated and statistically analyzed by one-way analysis of variance (ANOVA) using SPSS (ver. 14). Significant differences between means were determined by Duncan's multiple range tests. P-Values <0.05 were regarded as significant.

Results

1. Time needed for placental dropping (hours):

The average time of placental dropping after calving in buffaloes under experimentation was 3.71 ± 0.05 h. In relation to the effect of treatment, the lowest time of placental dropping appeared with the group of animals treated by OX and BT (3.40 ± 0.22 h), while the highest time appeared with the group of animals treated by PG (4.09 ± 0.25 h). Analysis of variance did not show any significant difference in the time of placental dropping between the different treated groups (Fig, 1A).

2. Time allowed for uterine involution (days):

In buffaloes under observation, the average time needed for uterine involution was 29.25 ± 0.12 d. The lowest time for uterine involution appeared with the MT group (28.00 ± 0.41 d), while the longest time appeared with the group of animals treated by MT and LV (30.57 ± 1.45 d). Statistical analysis revealed a significant ($P<0.05$) effect of treatment on the time for uterine involution (Fig, 1B).

3. Time from calving to first estrus (days):

The average time elapsed from calving to the first estrus recorded in the current work was 62.23 ± 0.98 d. The lowest time from calving to the first estrus appeared with the group of animals treated by PG and LV (55.18 ± 2.51 d), while the longest time was recorded in PG group (71.55 ± 4.59 d). Analysis of variance showed a highly significant ($P<0.01$) difference in time elapsed from calving to the first service between the different treated groups (Fig, 1C).

4. Days open period (days):

The average days open period in the present work was 71.98±1.40 d. The shortest days open period appeared with the PG + OXt group (59.44±2.68 d), while the longest period appeared with the group of animals treated by PG only (83.82±4.16 d). There was no significant difference in the days open period between the different treated groups (Fig, 1D).

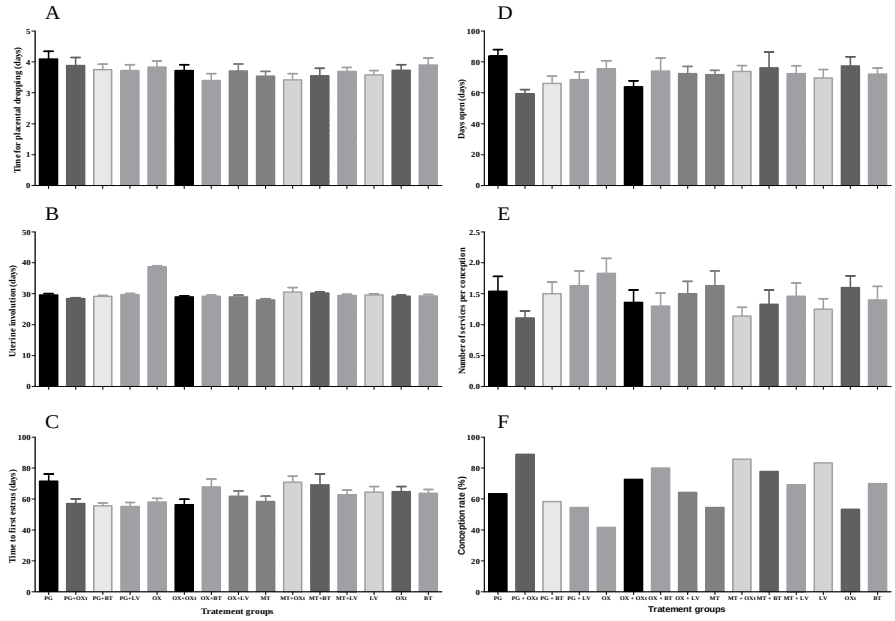

Fig 1. Changes in the reproductive parameters of Egyptian buffaloes in response to different therapies allowed after calving

5- Number of service per conception:

The average number of service per conception recorded in the present work was 1.46 ± 0.05. The lowest number of service/conception appeared with the group of animals treated by PG and OXt (1.11 ± 0.11), while the highest number appeared with the group of animals treated by OX (1.83 ± 0.24). Nevertheless, there was no significant difference between the different treated groups in terms of number of service per conception (Fig, 1E).

6- Conception rate (%):

The overall mean of conception rate recorded in animals in this work was 66 %. The lowest conception rate appeared with the group of animals treated by OX (41 %), while the highest rate appeared with the group of animals treated by PG and OXt (89 %). The conception rate did not show significant differences between the different treated groups (Fig, 1F).

Discussion

Buffalo have been characterized with its low reproductive efficiency as expressed by the calving interval exceeding 500 days (17) and this constitutes a major impediment for its productive performance (18). The reproductive efficiency largely depends on the changes occurring during the puerperal period and the subsequent fertility. Early identification and diagnosis of infertility problems are essential steps of successful dairy management.

The time for placental dropping was the shortest in the animals treated with OX and BT, and was the longest after PG administration. OX is known to act through an increase the intracellular calcium levels and cause contraction of the myometrium (19), and this in turn aid in placental descend and arrest the potential hemorrhage (20). On the other hand, iodine stimulates uterine tone and mobilizes neutrophils, these in turn helps in preventing placental retention and preventing the incidence of endometritis (11).

The time for uterine involution was observed to be the shortest in buffaloes treated with MT, while being the highest time in MT+LV group. Ergometrine (ergot alkaloid) is uterotonic or uterine ecobolics like drugs (9). MT is a vasoconstrictor, results in rapid disappearance of the carunuclar stalk and stimulates myometrial involution (21).

The calving to the first estrus interval was observed to be the shortest in the group of animals treated with PG and LV, while the highest period was noticed within the animals treated by PG. The immune-stimulant LV when injected on days 0, 2, 6, 10, 14 and 18 of the estrus cycle appeared to have good therapeutic effects on endometritis. Such effect is improved by the synergism with antibiotic (14). This might be attributed to the action of LV which increases the phagocytosis by polymorph nuclear leucocytes and macrophages, lymphokynes complement acute phase of proteins and leukocyte interferon production (13).

The days open period was the shortest in PG + OXt treated buffaloes, while being the longest within the group of animals treated by PG. The potential effect of PG may be due to the induced lyses of the corpus luteum, induction of estrus (5). Besides, OXt neutralizes the bacterial growth and aid in curing the endometritis (22). OXt penetrates well the uterine wall and endometrium after the IUI and sufficient therapeutic levels reached all uterine layers with 2 h and maintained for 24 h, where it reduces the severity of metritis (23). Animals treated with tetracycline after placental shedding had a more rapid recovery from infections with *A. pyogenes* and *F. necropharum* and shorten the uterine infection (24).

The lowest number of service/conception was observed in the group of animals treated with PG + OXt, while the highest period noticed within the group of animals treated

by OX. Khasatiya et al. (25) found that an injection of PG decreases the number of services per conception. PG acts as a mediator between luteinizing hormone and cyclic AMP during ovulation, facilitates the release of anterior pituitary hormones, and sensitizes the uterus to the action of the oxytocin (3). While the enhancing effect of OXt might be due to the early recovery rate from metritis that increased the conception rate after insemination at first estrus (10).

The conception rate was recorded the lowest OX treated animals, while it was the highest in the group of animals treated by PG and OXt. Similarly, Archbald et al. (26) found that the Conception rate to first insemination PP was improved in cases treated with PG.

Taken together, administration of drugs and treatment regimes PP has a positive impact on buffalo reproductive performance. Some of administered drugs do this synergistically and results in better response. A given example was clear in the groups of animals treated with prostaglandin and oxytetracycline (intrauterine). This improvement obviously exemplified by a decrease in the days open and number of services per conception and the increase in the conception rate.

Acknowledgement

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SUSCEPTIBILITY TO ANTIMICROBIALS OF BACTERIAL STRAINS ISOLATED FROM OTITIS IN DOGS

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Abstract
Otitis is a common disease, met especially in certain breeds of dogs, which can be complicated in terms of evolution if they are not treated or improperly treated in time. The aim of this study was to determine the bacterial etiology of ear diseases by identifying the main microorganisms isolated from ear pathological products and to evaluate the microbial susceptibility to antibiotics in order to apply etiologic treatment. From the sampled pathological products, there were isolated Staphylococcus, Proteus, Pseudomonas and Escherichia bacteria, and Malassezia pachhydermatis yeasts. The obtained results revealed a higher frequency of monomicrobial otitis compared to polymicrobial otitis. Antibiogram of the isolated bacterial pathogens was considered necessary in order to establish an appropriate therapeutic protocol. The performed tests revealed that bacterial susceptibility was observed mainly to quinolones, especially ciprofloxacin.

Key words: otitis, bacteria, dog, antibioresistance, quinolones

External otitis is a common disease in pets that can cause complications difficult to treat (1, 6). Diagnosis is relatively difficult due to the multiple primary and predisposing factors involved in otitis pathogenesis (5).

Individual therapeutic needs of each patient must be identified based on a solid interpretation of the data provided by history and clinical examination, but also on complementary examinations including cytology, bacteriology, antibiogram, radiography and biopsy. A poor diagnosis and an inadequate treatment may complicate the development of otitis (3, 4).

In order to apply an appropriate treatment correlated with the results of microbiological laboratory analysis, it is necessary to determine the susceptibility of identified pathogens to antimicrobial substances (2).

The aim of this study was to determine the bacterial etiology of ear diseases by identifying the main microorganisms isolated from ear pathological products and to evaluate the microbial susceptibility to antibiotics in order to apply etiologic treatment.

Materials and methods

The study comprised 25 dogs presented at the consultation with different clinical and evolutive forms of otitis. Diagnostic protocol included microbiological examination of ear secretions in all cases.

The cases taken under study revealed a higher frequency of monomicrobial otitis compared with polymicrobial otitis. From the collected secretions, there were isolated bacteria of the genera *Staphylococcus*, *Streptococcus*, *Proteus*, *Pseudomonas*, *Escherichia*, which have been tested in terms of susceptibility/resistance to antibacterial substances.

Antibiogram was performed by Kirby-Bauer disc diffusion method, usually from pure bacterial culture, and less commonly, from mixed primary culture. Following materials were necessary: Mueller-Hinton agar, sterile saline solution, tubes, pipettes, antibiotic microtablets, dispenser for antibiotics, dental forceps, graded ruler, standard for interpreting the results.

Results and discussions

Antibiogram testing for the isolated bacterial strains revealed a series of information, as shown in Table 1.

Table 1. The results of antibiograms for the tested bacterial strains

Antibacterial substance	*Staphylococcus sp.*				*Streptococcus sp.*				*Pseudomonas aeruginosa*				*Proteus mirabilis*				*Escherichia coli*			
	N	S	MS	R	N	S	MS	R	N	S	MS	R	N	S	MS	R	N	S	MS	R
Amoxicillin	5	1	2	2	1	1	0	0	-	-	-	-	-	-	-	-	2	0	0	2
Amoxicillin-clavulanic acid	9	4	1	4	1	1	0	0	1	0	0	1	-	-	-	-	1	0	0	1
Ampicillin	1	0	0	1	1	1	0	0	2	0	0	2	1	0	0	1	-	-	-	-
Ampicillin-sulbactam	1	1	0	0	1	1	0	0	1	0	0	1	1	0	0	1	1	0	0	1
Oxacillin	2	1	0	1	-	-	-	-	1	0	0	1	-	-	-	-	-	-	-	-
Cloxacillin	3	3	0	0	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
Cephalexin	7	6	0	1	1	1	0	0	2	0	0	2	1	0	0	1	1	0	1	0
Cefsulodin	1	0	0	1	-	-	-	-	1	0	0	1	-	-	-	-	1	0	0	1
Cefoperazone	3	1	1	1	-	-	-	-	2	2	0	0	-	-	-	-	1	0	0	1
Cefaclor	1	1	0	0	1	1	0	0	1	0	0	1	1	1	0	0				
Cefuroxime	1	1	0	0	-	-	-	-	1	0	0	1	-	-	-	-	-	-	-	-
Ceftiofur	-	-	-	-	-	-	-	-	2	2	0	0	-	-	-	-	-	-	-	-
Cefquinome	-	-	-	-	-	-	-	-	2	2	0	0	-	-	-	-	-	-	-	-
Gentamicin	7	4	1	2	-	-	-	-	5	3	1	1	1	1	0	0	2	2	0	0
Kanamycin	1	1	0	0	-	-	-	-	1	0	0	1	-	-	-	-	1	1	0	0
Streptomycin	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	-	-	-	-
Amikacin	-	-	-	-	-	-	-	-	4	3	0	1	-	-	-	-	-	-	-	-
Rifampicin	5	4	0	1	-	-	-	-	1	0	0	1	-	-	-	-	-	-	-	-
Florfenicol	4	4	0	0	1	1	0	0	4	0	1	3	1	1	0	0	-	-	-	-
Chloramphenicol	3	2	0	1	1	1	0	0	1	1	0	0	-	-	-	-	1	1	0	0
Tetracycline	1	1	0	0	-	-	-	-	1	0	0	1	-	-	-	-	1	0	0	1
Oxytetracycline	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	-	-	-	-
Doxycycline	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	-	-	-	-
Colistin	-	-	-	-	-	-	-	-	2	2	0	0	1	0	0	1	2	0	1	1
Polymyxin B	-	-	-	-	-	-	-	-	4	4	0	0	-	-	-	-	-	-	-	-
Bacitracin	1	0	0	1	-	-	-	-	2	0	0	2	1	0	0	1	-	-	-	-
Lincomycin	4	1	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spectinomycin	-	-	-	-	-	-	-	-	4	2	0	2	-	-	-	-	-	-	-	-
Lincomycin-spectinomycin	2	1	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Novobiocin	1	1	0	0	1	0	1	0	2	0	0	2	1	0	0	1	-	-	-	-
Enrofloxacin	10	9	0	1	2	1	1	0	6	3	3	0	1	1	0	0	2	1	0	1
Ciprofloxacin	5	5	0	0	1	1	0	0	6	6	0	0	1	1	0	0	2	1	0	1
Norfloxacin	1	1	0	0	-	-	-	-	1	1	0	0	-	-	-	-	1	1	0	0
Flumequine	1	1	0	0	1	0	0	1	3	0	0	3	1	1	0	0	-	-	-	-

Legend: N – number of tested strains; S – susceptible; MS – moderately susceptible; R – resistant.

After analyzing the antibiograms of *Staphylococcus* strains, the results revealed that its susceptibility to antibiotic substances is high (Fig. 1).

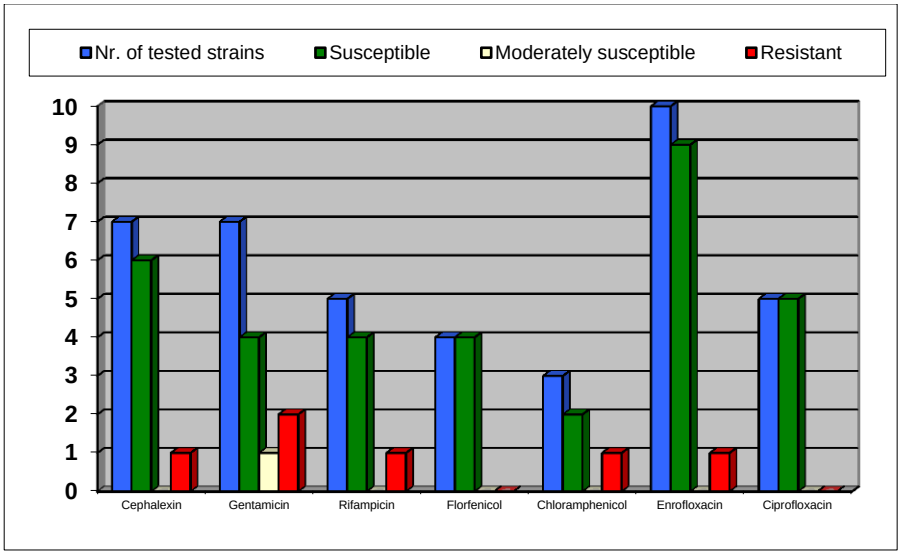

Fig. 1. Susceptibility of *Staphylococcus sp.* to different antibacterial substances

For example, to cefalexin, six of the seven tested strains were susceptible; to rifampicin, four of the five tested strains were susceptible; to florfenicol and ciprofloxacin, all strains were susceptible and to enrofloxacin, nine of the ten tested strains were susceptible. Resistance was recorded mainly to some penicillins: amoxicillin (combined or not with clavulanic acid), ampicillin, oxacillin, but also to bacitracin.

Due to the small number of analyzed *Streptococcus* strains, the results can not be considered conclusive. However, we noted that only for enrofloxacin, flumequine, novobiocin and oxytetracycline it was recorded moderate susceptibility or resistance.

In case of *Pseudomonas*, bacteria known to have a pronounced variability to antimicrobial substances and, in particular, an obvious resistance to many antibiotics, we found that, depending on the category of antibacterial substances, the susceptibility degree varies considerably. For example, in case of beta-lactams, we found an evident resistance, excepting some cephalosporins, like ceftiofur, cefoperazone, cefquinome, to which all strains were susceptible. Although the action of aminoglycosides is recognized to be effective for most strains of *Pseudomonas aeruginosa*, we found a marked variability in the behavior of these bacteria to the antibiotics in this class. Thus, for gentamicin, only three of five tested strains were susceptible; to spectinomycin, of the four tested strains, two were susceptible and two were resistant; to amikacin, three strains were susceptible and one strain was resistant, while kanamycin and streptomycin were not active (Fig. 2).

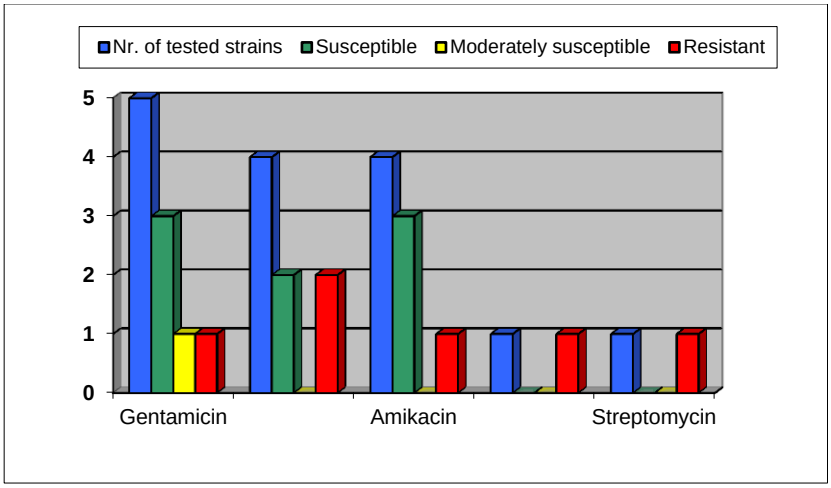

Fig. 2. Susceptibility of *Pseudomonas aeruginosa* to aminoglycoside antibiotics

Among polypeptide antibiotics, there were analyzed colistin and polymyxin B, both showing obvious antibacterial activity against analyzed *Pseudomonas* strains. Moreover, these antibiotics are found in the composition of many therapeutic formulas recommended in the topical treatment of otitis.

Florfenicol was found to be ineffective against *Pseudomonas* bacteria, three of the four strains being resistant.

Chemotherapeutic drugs from quinolones category have shown a good antibacterial effect: ciprofloxacin was effective against all six strains tested, while in case of enrofloxacin, only three strains were susceptible and three were moderately susceptible (resistance to enrofloxacin was not recorded); in case of flumequine, all three *Pseudomonas* strains tested were found to be resistant (Fig. 3).

In conclusion, we subscribe to the recommendation that, in infections with *Pseudomonas aeruginosa*, antibiogram should be performed, although some quinolones are permanently effective against these bacteria.

It is known that the involvement of *Enterobacteriaceae* bacteria in the etiology of ear infections corresponds to obvious deficits of local and/or systemic immunity. In this context, the efficiency of antibiotic treatments is much reduced and effective therapeutic solutions against *Enterobacteriaceae* bacteria should be found. In this case, we refer to ear infections involving especially *Proteus* and *Escherichia* bacteria.

We noticed that, in case of, the behavior of antibiotics and chemotherapeutic agents known to be effective for this bacterial category has not always been demonstrated. The results revealed that gentamicin, ciprofloxacin, enrofloxacin, and some cephalosporins were effective against *Proteus mirabilis*.

Similar to the situation of *Proteus mirabilis*, in case of *Escherichia coli* we found a solid efficiency of gentamicin, kanamycin and colistin, while for ciprofloxacin and enrofloxacin there were also recorded resistance situations.

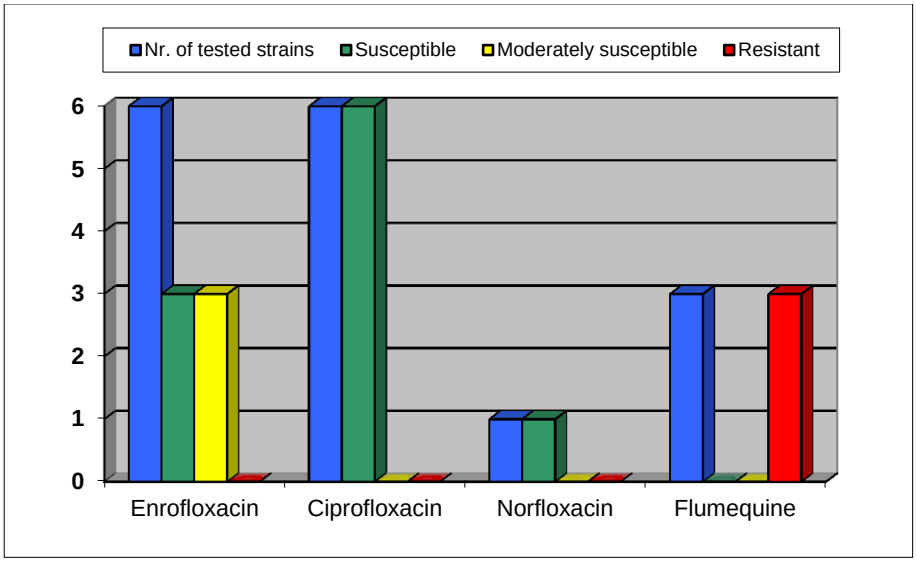

Fig. 3. Susceptibility of *Pseudomonas aeruginosa* to fluoroquinolones

Conclusions

1. After analyzing the antibiograms of *Staphylococcus* strains, the results revealed that the susceptibility of these bacteria to antibacterial substances is high.
2. *Pseudomonas*, bacteria known to have a pronounced variability to antimicrobial substances, showed susceptibility mainly to some cephalosporins, aminoglycosides and quinolones; also, polypeptide antibiotics were very effective.
3. *Enterobacteriaceae* bacteria showed resistance to many antibacterial substances.
4. Overall, bacterial susceptibility was observed especially to quinolones, particularly ciprofloxacin.
5. In order to apply an adequate therapeutical conduct, microorganisms isolated from otitis must be tested in terms of behavior to antibacterial substances, by performing antibiograms.

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STUDIES ON OTITIS FREQUENCY IN DOG AND ESTABLISHMENT OF THERAPEUTIC PROTOCOLS

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Abstract
In dogs, otitis are complex entities, with evolution often occult, multicausal, comprising long-term treatments and frequently frustrating results and, on this basis, subject to numerous interventions by practitioners. General objectives of otitis treatment in dogs are to remove primary factors, to reduce inflammation, to treat bacterial / fungal infections and to clean and maintain the ear dry. All these points can finally be achieved by using topical and systemic therapy. Following clinical investigations, supplemented by microbiological analysis, resulted valuable data on the seasonality of ear diseases in dog, affected breeds, clinical and evolutionary nature of the otitis, the microorganisms most commonly found in ear secretions as well as some particularities of the sampled pathological products. Thus, otitis were found in a variety of dogs breeds, the highest frequency being recorded in German Shepherd and Cocker Spaniel breeds, especially during spring-summer. In terms of age, the highest frequency of otitis was found in dogs aged 5-10 years. After conducting laboratory analysis (microbiological tests and antibiograms), antimicrobial treatments were mainly based on the use of antibacterial substances in quinolones group.

Key words: dog, otitis, treatment, frequency, breed

In dogs, external otitis are complex entities, with evolution often occult, multicausal, comprising long-term treatments and frequently frustrating results and, on this basis, subject to numerous interventions by practitioners (1, 3).

General objectives of otitis treatment in dogs are to remove primary factors, to reduce inflammation, to treat bacterial / fungal infections and to clean and maintain the ear dry (5, 6). All these points can finally be achieved by using topical and systemic therapy (2).

Currently there are many drugs for the treatment of ear disorders, containing a variety of active substances. Application of appropriate techniques and therapeutical management is very important, as the conditions of the disease can change during its evolution. Of a high importance is to fully assess primary, predisposing and perpetuating factors that can contribute to the worsening of the disease (4).

The purpose of this study was to obtain data on the seasonality of ear diseases in dog, affected breeds, clinical and evolutionary nature of the otitis, the microorganisms most commonly found in ear secretions as well as some particularities of pathological products.

Materials and methods

Studies were performed on 25 dogs of various breeds presented to consultation with infectious ear diseases, during 2011.

The patients with these conditions were analyzed depending on race, age, sex, in order to have a clearer picture of the frequency of otitis in relation to these factors. In addition, we tried to establish some correlations between the aspect of pathological material sampled and isolated germs.

The patients were clinically examined, pathological material was collected, microbiological investigations were performed, and by corroborating the obtained data, it was established the diagnosis.

Results and discussions

The results obtained after the investigations on the season, race, age, sex, clinical features and the results of microbiological exams are presented in table 1.

Table 1. Some characteristics of dogs with diagnosed external otitis

Nr.	Season	Rasa	Age / Sex	Clinical features	Isolated microorganism
1.	Spring	German Shepherd Dog	2 years/M	Recurrent bilateral otitis	*Staphylococcus sp*
2.		Doberman	8 years /M	Bilateral otitis	*Malassezia pachydermatis*
3.		Carpathian Shepherd Dog	5 years /M	Bilateral otitis	*Malassezia pachydermatis*
4.		German Shepherd Dog	9 months/M	Unilateral suppurative otitis, acute, recurrent	*Staphylococcus sp.*
5.		Cocker Spaniel	6 years /M	Unilateral otitis, chronic, recurrent	*Proteus mirabilis*
6.		German Shepherd Dog	10 months/M	Bilateral otitis, chronic, painful, dry, oedematous	*Malassezia pachydermatis*
7.		Shar Pei	1 year/M	Subacute bilateral otitis	*Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus sp.*
8.		Doberman	5 years /M	Chronic bilateral otitis	*Staphylococcus sp.*
9.		Shar Pei	3 years /M	Chronic cerumen otitis, bilateral	*Staphylococcus sp., Malassezia pachydermatis*
10.		Pit Bull	1 year/M	Chronic allergic otitis	*Malassezia pachydermatis*
11.		Caucasian Shepherd Dog	2 years /M	Chronic otitis	*Staphylococcus sp., Pseudomonas aeruginosa*
12.		Cocker Spaniel	3 years /F	Chronic unilateral otitis	*Malassezia pachydermatis*
13.		German Bracke	11 years /F	Recurrent bilateral otitis	*Staphylococcus sp, Proteus mirabilis*
14.		German Shepherd Dog	10 years /M	Unilateral otitis, recent, untreated	*Staphylococcus sp.*
15.		Cocker Spaniel	9 years /M	Bilateral otitis, chronic, rebellious to treatment	*Staphylococcus sp., Corynebacterium sp., Pseudomonas aeruginosa*
16.	Summer	Boxer	8 years /M	Purulent otitis, unilateral, subacute, due to a bite	*Pseudomonas aeruginosa*
17.		Mixed Breed Dog	4 years /F	Bilateral otitis, chronic, rebellious to treatment	*Staphylococcus sp., Streptococcus sp.*
18.		Caniche	10 years /F	Recurrent unilateral otitis, chronic	*Staphylococcus sp, Escherichia coli, Corynebacterium sp., Malassezia*

					pachydermatis
19.		German Dog	10 years /M	Bilateral chronic otitis	*Pseudomonas aeruginosa*
20.		German Shepherd Dog	7 years /M	Exacerbated chronic unilateral otitis	*Escherichia coli, Proteus mirabilis*
21.		Shih Tzu	7 years /M	Unilateral allergic otitis	*Staphylococcus sp., Klebsiella sp.*
22.	Autumn	Caniche	9 years /M	Bilateral otitis	*Staphylococcus sp.*
23.		German Shepherd Dog	1 year/M	Chronic otitis	*Pseudomonas aeruginosa*
24.		German Shepherd Dog	7 years /M	Chronic bilateral otitis, recurrent	*Malassezia pachydermatis*
25.		German Bracke	6 years /F	Bilateral otitis	*Malassezia pachydermatis*

The information presented in Table 1 show that most cases of ear diseases were found in shepherd dogs (9 cases out of 25), German shepherd breed being often affected (28% of total cases with otitis). However, we noticed that ear diseases are found in a wide range of races, both with short hair and long hair, with straight or dropped ears, and with different sizes.

For a better statistical representation of data on the frequency of otitis depending on dog breed, Fig. 1 is presented for analysis.

Another purpose of the microbiological exams was to correlate the characteristics of the sampled pathological products with the presence of certain microorganisms, isolated and then identified in ear secretions. These findings are presented in Table 2.

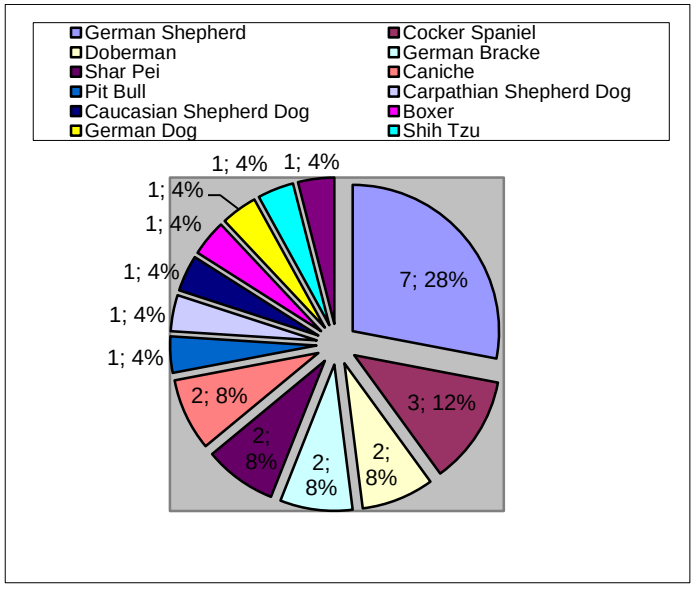

Fig. 1. The incidence of otitis externa in dogs, depending on race

Table 2. Correlation between the aspect of ear secretion and identified microorganism

Microbial species	The aspect of ear secretion
Pseudomonas aeruginosa	Purulent exudate, yellow greenish gray, low consistency
Staphylococcus sp.	Yellow purulent thick secretion
Malassezia pachydermatis	Dark brown secretion, in large quantities
Proteus mirabilis, Escherichia coli	Brown secretion, then yellow
Corynebacterium sp.	Purulent secretion, slightly hemorrhagic

From the presented data results, in particular, that ear infections with *Pseudomonas aeruginosa* correlates with a purulent, suppurative and low consistency aspect of the ear secretion. Also, in *Malassezia pachydermatis* yeast involvement, ear secretion was abundant and brown, even to black. However, not always close correlation could be established between certain aspects of ear secretions and microbial flora found in pathological products.

As for otitis incidence depending on age of the dogs, we found that more than half of cases (56%) corresponded to the age range 5-10 years; on the second place as incidence were situated young animals (1-5 years) and very few cases were recorded in puppies less than 1 year old (8%) and in old dogs over 10 years (4%) (Fig. 2). However, at advanced ages, ear disease severity was inversely proportional to the frequency with which we diagnosed the disease in those cases.

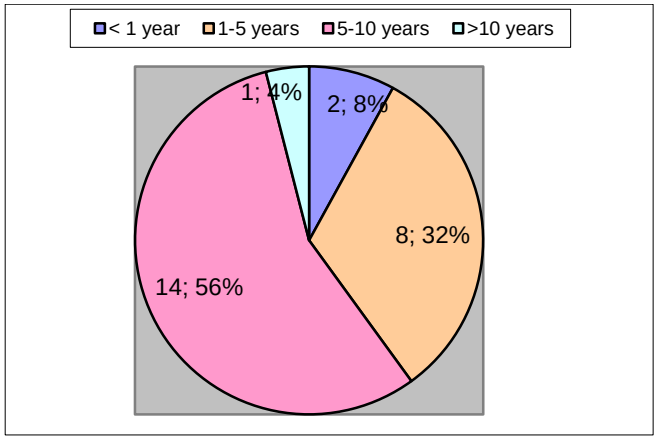

Fig. 2. The incidence of external otitis in dog, depending on age

Depending on sex, most pathological cases that we studied, respectively 80%, were found in males, aspect that is considered not to match the idea that skin is thicker in males than in females (Fig. 3). Other predisposing factors probably justify the higher incidence of otitis in males.

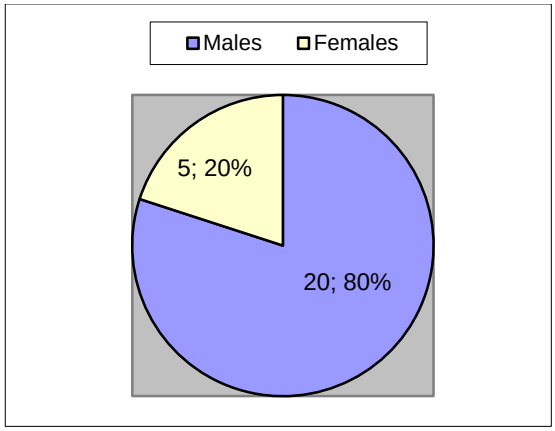

Fig. 3. The incidence of external otitis in dog, depending on sex

The results of established therapy were different depending on the stage of the disease, the bacterial and fungal complications identified by microbiological exams and the seriousness with which local and general treatment has been applied.

Conclusions

1. In this study, otitis occurred in a variety of breeds of dogs, increased frequency being registered in German shepherd (28%) and Cocker Spaniel (12%).
2. In terms of age, of the total number of cases, the highest frequency was found in dogs aged between 5-10 years.
3. In terms of season, the maximum frequency of external otitis with infectious nature in dogs has been registered in spring.
4. Microscopic characteristics of the ear secretion are related to clinical features of the otitis and to isolated microbial flora.
5. Fungal otitis have been diagnosed with a high frequency, probably because in otitis is often used topical treatment containing broad-spectrum antibiotics, which creates a dismicrobism, stimulating the proliferation of yeasts.
6. Early diagnosis and appropriate treatment setting and compliance helps in faster healing of otitis.
7. Applying a topical treatment in combination with a general treatment is indispensable for healing infectious otitis in dogs.

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THE HEMATOLOGICAL PROFILE AS INDICATOR OF SHEEP STRESS DURING TRANSPORT

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Abstract

Sheep welfare during transport is directly influenced by the loading, transport and unloading conditions which must minimize the animals' coping efforts to physiological and psychological stressors.There have been determined hematological parameters values on two batches of 15 lambs from Ţigaie breed, approximately 4-5 month old, such as: red blood cell count (RBC), hemoglobin, hematocrit (packed cell volume - PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), segmented neutrophils count, immature neutrophils, eosinophils, basophils, lymphocytes, monocytes and platelet count (PLT). Blood samples were collected in three stages: before loading into vehicle, after unloading and after six days of resting in the sheep collecting center. There were used the following devices and methods: Beckman Coulter ACT 5 Diff CP Hematology analyzer, DIFF simultaneous methodology, WBC/BASO methodology and Leica DML S2 microscope for cytomorphological examination. The results interpretation was made according to the reference values from the literature. Following investigations, it was found that, concerning the red blood cells, there were not registered significant changes between the two batches in the three stages. Regarding the white blood cells, there were noticed some transport stress related changes: leukocytosis, neutrophilia, lymphocytopenia, eosinopenia, basopenia, especially in the samples collected after unloading.

Key words: hematological parameters, lambs, transport, reference values, batches

Up to present, the public concern for sheep welfare was insignificant, sheep being regularly described as "physically resistant" (3). Thus, these animals are able to survive during poor transport conditions, which in other species might generate high dead on arrival levels (D.O.A.). Every handling procedure (marking, collecting, and transportation) is associated with stressors: mixing with unfamiliar animals, crowding, thermal stress, fear, food and water deprivation, with a negative impact on sheep welfare.

The assessment of animals' welfare during transport is based on establishing the compliance with optimum conditions, which minimize animals' efforts to cope with physiological and psychological stressors (2, 4, 5).

In order to identify sheep transport issues, there are used some parameters including the hematological ones.

Materials and methods

The paraclinical tests (hematological exam) were conducted on two batches of 15 lambs each from Țigaie breed, approximately 4-5 month old.

Animals handling was different in the two batches: animals from batch A were properly handled at loading into vehicle, while animals from batch B were brutally handled.

The hematological examinations were performed on a total of 15 samples from each batch in three stages: before loading, after unloading and after six days of resting in the sheep collecting center.

From the collected blood samples, the following hematological parameters were determined: red blood cell count (RBC), hemoglobin, hematocrit (packed cell volume - PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), segmented neutrophils count, immature neutrophils, eosinophils, basophils, lymphocytes, monocytes and platelet count (PLT).

The determination of the red blood cell count, hemoglobin, hematocrit, white blood cell count, platelet count and erythrocyte constants were made using Beckman Coulter AcT 5 Diff CP Hematology analyzer. The monocytes, neutrophils, eosinophils, lymphocytes and basophils were determined by DIFF simultaneous methodology (the principle of absorbance cytochemistry and focused flow impedance) and WBC/BASO methodology (the Coulter principle – by impedance and differential thresholds). The cytomorphological examination was made with Leica DML S2 microscope on May Grunwald – Giemsa stained blood smears.

The results interpretation was made by comparison with reference values from the literature (1).

Results and discussions

The average comparative values of the hematological parameters (erythrocytic series) are presented in table no. 1.

According to the data in the table, it can be noticed that the red blood cell count for all the samples, regardless the sampling stage, recorded values slightly lower than the reference values.

Table 1. Average comparative values of the erythrocyte constants in the two lambs' batches

No.	Determined parameter	M.U.	Determined values						Reference values
			Before loading		After unloading		After six days of resting		
			Batch A	Batch B	Batch A	Batch B	Batch A	Batch B	
1	RBC	10^9 /cm	8.5± 2.4	8.6±3. 0	8.2±2. 6	8.6±2. 2	8.3±2. 3	7.0±2. 3	12±4
2	HGB	g/dl	10.7± 1.3	11.3± 1.8	10.4± 1.2	10.3± 2.3	11.3± 1.2	10.4± 1.2	12±2
3	HCT	%	34.6± 3.0	35.8± 0.5	34.3± 2.6	36.5± 3.0	33.8± 2.5	34.6± 3.2	36±4
4	MCV	μm^3	40.8± 3.5	38.7± 3.2	42.3± 3.4	36.4± 2.5	32.6± 2.5	36.3± 3.4	34±6
5	MCH	pg	13.3± 1.4	13.1± 1.6	12±1.1	14.8± 1.1	13.8± 1.3	15.4± 0.7	10±2
6	MCHC	g/dl	29.8± 2.5	31.6± 1.9	30.4± 2.8	39.1± 2.3	33.1± 2.5	42.4± 1.3	32±3

The hemoglobin, determined from samples collected in all three stages, had similar values for both sheep batches, slightly lower than the reference values.

Like the previous parameters, the hematocrit recorded similar values in most of the samples and in all stages, maintaining close below the reference value.

The MCV recorded different values in the two batches. Thus, the figures for the samples collected from batch A were higher than the reference values before loading and after unloading, and lower (compared with the previous stages) for the samples collected after six days of resting. In batch B the values were slightly higher than the reference, being noticed a descendent trend from the loading stage to the last two stages.

In the samples from batch A, the MCH value was slightly higher than the reference before loading and after six days of resting, and slightly lower after unloading in relation with the above mentioned stages. In batch B, in all samples the values increased from a transport stage to another, being clearly higher than the reference.

MCHC in the samples from batch A was slightly lower than the reference value before loading and after unloading, and close to the reference in the last stage. In the samples collected from batch B, MCHC figures were slightly lower than the reference before loading and higher after unloading and after six days of resting.

The MCV, MCH and MCHC figures for both sheep batches in the three stages varied, but didn't have a significant diagnosis value. The reactive changes in the erythrocyte count, hemoglobin and erythrocyte constants in the two batches during the three investigation stages were within the physiological ranges and cannot be related to the transportation stress.

The centralized results recorded for leucocytic series are presented in table no. 2.

According to the data in the table, the white blood cell count in the samples collected from sheep in batch A before loading and after six days of resting in the center recorded values close to the reference ones, but after unloading the values were significantly higher, being noticed leukocytosis. In the samples collected from sheep in batch B, the leukocyte count was slightly higher than the reference values before loading and significantly higher after unloading and in the center (after six days of resting).

Table 2. Average comparative values of white blood cells in the two lambs' batches

No.	Determined parameter	M.U.	Determined values						Reference values
			Before loading		After unloading		After six days of resting		
			Batch A	Batch B	Batch A	Batch B	Batch A	Batch B	
1	WBC	10^3 /cm	10.9± 0.2	11.1± 2.2	15.7± 1.2	21.8± 1.9	11.5± 0.4	16.5± 2.3	9±3
2	Segmented neutrophils	%	30.4± 5.0	31.6± 6.4	45.3± 5.5	64.9± 19.0	33.8± 4.9	53.5± 0.7	32±14
3	Immature neutrophils	%	3.8±1.5	4.6±2.1	4.4±1.8	7.3±2.3	4.1±1.5	4.3±0.3	3±3
4	Eosinophils	%	3.6±1.4	3.3±0.6	3.1±0.8	2.4±1.3	3.0±1.4	2.7±0.2	2±2
5	Basophils	%	1.0±0.8	1.3±0.6	0.8±0.2	0.7±0.2	1.5±0.4	1.9±0.3	1.5±1.5
6	Lymphocytes	%	58.2±6	56.3± 15.0	43.2± 4.1	21.0± 3.4	53.8± 5.4	34.1± 0.7	58±10
7	Monocytes	%	2.8±1.6	2.9±2.1	3.2±1.7	3.1±1.9	3.8±0.8	3.3±0.6	3±3

The segmented neutrophils recorded higher values than the reference for batch A in the samples collected after unloading and for batch B in the samples from the second and the third transport stage, being observed neutrophilia.

Immature neutrophils had values higher than the reference in all the samples, the highest values being recorded in the samples collected from batch B after unloading.

Eosinophils have exceeded the reference values in all the samples, a slight eosinopenia being recorded in sheep from batch B after loading.

The basophils had the same evolution as the eosinophils, basopenia being recorded in the samples collected from sheep in batch B after unloading.

The lymphocyte percentage was in normal ranges for most of the samples except those collected from batch A and B after unloading and those from batch B after six days in the center – in which values were lower (lymphocytopenia).

Regarding the monocytes percentage, no significant changes were recorded in any sample or transport stage.

Centralizing and analyzing the data regarding the white blood cells, it was noticed a significant increase of the leukocytes count, of the percentages for segmented and immature neutrophils, eosinophils, basophils, and lymphocytopenia.

The data show leukocytosis associated with neutrophilia and lymphopenia, as result of corticoid hormones action. These hematologic changes depict the evolution of a stress reaction caused by handling.

Neutrophilia is due to a decrease of the neutrophils' ability to adhere at the vascular endothelium, resulting in a prolonged cell bloodstream circulation time, also the neutrophil discharge from the bone marrow is stimulated.

Lymphocytopenia can appear by redistribution or by lymphocytes lysis as a result of stressors' action.

In table no. 3 are shown the platelet counts.

Table 3. Average comparative values of thrombocytes (platelet count) in the two lambs' batches

No.	Determined parameter	U/M	Determined values						Ref. values
			Before loading		After unloading		After six days of resting		
			Batch A	Batch B	Batch A	Batch B	Batch A	Batch B	
1	PLT	10^3 /mmc	813± 29.6	804± 26.0	828± 22.9	659± 49.2	703.6± 3.2	814±2.9	250 – 750

Analyzing the results, there weren't noticed significant changes related with transportation stress in the PLT in any batch or investigation stage.

Conclusions

1. Following the researches, it could be concluded that for both batches in the three transport stages there are not recorded significant changes for erythrocytic series: WBC, hemoglobin, hematocrit, MCV, MCH and MCHC.

2. The major changes affecting leucocytic series were: leukocytosis with neutrophilia and lymphocytopenia, eosinopenia, basopenia. These reactive modifications were statistically highly significant after unloading, after six days of resting in the center in batch A the values going back to the initial level.
3. In relation with batch A, the changes observed in batch B were more intense, severe, persisting in the six days of resting at the destination.

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THE DYNAMICS OF CYATHOSTOME (NEMATODA: *CYATHOSTOMINAE*) LARVAE ON PASTURES FROM TIMIŞ AND CARAŞ-SEVERIN COUNTIES

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Abstract
The most important and widespread parasites of horses are cyathostomes or small strongyles. This paper describes the dynamics of pre-parasitic elements, mainly infesting larvae of cyathostomes, on 10 pastures from Timiş and Caraş-Severin counties during the months of October, November and December 2011, and March and April 2012, respectively. Grass samples (about 200 g) were collected every month, and their weight in the moment of being collected and after 25-30 days of drying at a temperature of 27°C (dry weight) was taken into consideration. Baermann method was used to collect larvae from grass samples. The following species of nematodes were identified: cyathostomes, Strongylus equinus, Strongylus edentatus, Strongylus vulgaris, digestive strongyles (from other species), and free-living nematodes, respectively. The most abundant horse parasites on pastures were cyathostomes, a low number of them surviving over winter.

Key words: horses, cyathostomes, pastures, Timiş and Caraş-Severin counties

In the last few decades, cyathostomes or small strongyles represent the most important parasites of horses with a high prevalence worldwide. This kind of parasitism can cause the decrease of exercise capacity, diarrhea, excessive weight loss, and death.

The economical influence of subclinical parasitism can't be properly appreciated only taking into consideration factors relating to pasture contamination and animal susceptibility.

All over the world horses are exposed to different order or genera of parasites, sometimes with high morbidity and mortality [Ogbourne, 1976; Gawor, 1995; Silva et al., 1999; Hodgkinson, 2006]. Nevertheless, cyathostome infestations are the most significant [Becher et al., 2010; Kornas et al., 2011; Stratford et al., 2011; Morariu et al., 2012]. The properly identification of parasites is very important to understanding these infestations and diseases caused by them.

The objective of this study was to define the seasonal dynamics of small strongyles (mainly cyathostomes) transmission patterns. The L3 stage levels were monitored from October 2011 to April 2012.

Materials and methods

The grass samples were collected every month, from October 2011 up to April 2012, excepting the months of January and February from the pastures of Timiş (Dumbrăviţa, Pişchia, Fibiş, Variaş şi Lovrin localities) and Caraş-Severin (Măureni, Bocşa, Reşiţa, Constantin Daicoviciu şi Caransebeş localities) counties (Figures 1 and 2).

The samples were collected from surfaces describing a circle with a one meter in diameter, around the faeces samples. Each time, 200 g of grass were collected. There was taken into consideration the weight of grass in the moment of being collected and after 25-30 days of drying at a temperature of 27°C (dry weight). Up to the moment of being processed, the grass samples were packed in plastic bags.

In the Parasitic Diseases Department, the collected grass was well washed. The liquid obtained after washing was examined by Baermann method. At the same time, the liquid obtained after washing the plastic bags was centrifuged for 5 minutes, at 3000 rpm. The sediment was filtered through a very fine sieve (with 25 μm mesh) and washed with tap water. The larvae remained on sieve were suspended in 30 ml of tap water.

1/5 of the sample (6 ml) was examined in order to count the larvae. The first 100 larvae were identified.

The calculation formula was the following:

No. of L3/kg of dry grass = [no. of larvae x 1000/dry weight of the grass (grams)] x 5

The pasture field was used by horses, sheep and cattle. Due to a long winter, when the pasture was covered by snow, no grass was collected in January and February.

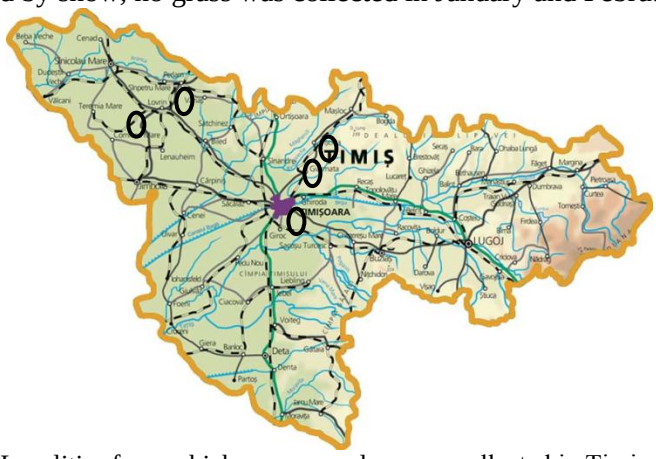

Fig. 1. Localities from which grass samples were collected in Timiș County

Fig. 2. Localities from which grass samples were collected in Caraș-Severin County

Results and discussions

Tables 1 and 2 show the results obtained after the examination of the grass samples collected from the 10 locations of Timiș and Caraș-Severin counties (average number of larvae from all investigated pastures).

Table 1. Average number of nematode larvae collected from pastures of Timiș County during the study

Species	No. of L3 per month (%):				
	Oct.	**Nov.**	**Dec.**	**Mar.**	**Apr.**
Cyathostomes	14,460 (44.91%)	5,643 (36.00%)	1,195 (27.27%)	14 (4.29%)	1,966 (27.52%)
Strongylus equinus	336 (1.04%)	226 (1.44%)	59 (1.34%)	0	101 (1.41%)
Strongylus edentatus	471 (1.46%)	263 (1.67%)	67 (1.52%)	1 (0.30%)	124 (1.73%)
Strongylus vulgaris	322 (1.00%)	115 (0.73%)	10 (0.23%)	0	21 (0.29%)
Digestive stongyles (other species)	6,514 (20.23%)	3,244 (20.69%)	1,078 (24.60%)	47 (14.41%)	1,705 (23.86%)
Free-living nematodes	10,089 (31.34%)	6,187 (39.46%)	1,973 (44.41%)	264 (80.98%)	3,227 (45.17%)
TOTAL	**32,192**	**15,678**	**4,382**	**326**	**7,144**

Table 2. Average number of nematode larvae collected from pastures of Caraș-Severin County during the study

Specii	Nr. L3 în luna (%):				
	Oct.	**Nov.**	**Dec.**	**Mar.**	**Apr.**
Ciathostomine	14.104 (42,36%)	6.721 (34,57%)	2.302 (30,17%)	14 (4,73%)	1.401 (26,01%)
Strongylus equinus	443 (1,33%)	294 (1,51%)	81 (1,06%)	0	71 (1,31%)
Strongylus edentatus	504 (1,51%)	372 (1,91%)	89 (1,16%)	0	109 (2,02%)
Strongylus vulgaris	349 (1,04%)	207 (1,06%)	37 (0,48%)	0	25 (0,46%)
Strongili digestivi (alte specii)	7.188 (21,58%)	3.955 (20,34%)	1.632 (21,39%)	42 (14,19%)	1.278 (23,72%)
Nematode libere	10.706 (32,15%)	7.893 (40,59%)	3.487 (45,71%)	240 (81,08%)	2.503 (46,46%)
TOTAL	**33.294**	**19.442**	**7.628**	**296**	**5.387**

Free-living nematodes were the most representative in both counties, the lowest prevalence being recorded in October 2011 (31.34%) in Timiș County and the highest one in March 2012 (81.08%) in Caraș-Severin County.

During the monitoring process the digestive strongyle larvae have had a relatively constant proportion, with insignificant variations. The lowest presence was noticed in March 2012 (14.19%) in Caraș-Severin County and the most important one in December 2011 (24.60%) in Timiș County.

The migrating (large) strongyle species (*S. equinus, S. edentatus* and *S. vulgaris*) have had a "significant" presence in Caraș-Severin County, even this is insignificant inside the investigated parasites infrapopulation structure. The population peak (2.02%) was registered in April 2012 for *S. edentatus*. In March 2012 no larvae of these species were identified excepting the species mentioned above, which shows the low resistance of these larvae in conditions of severe winter.

Cyathostomes have had a higher prevalence during October 2011 (44.91% in Timiș and 42.36% in Caraș-Severin) and November 2011 (36.00% in Timiș and 34.57% in Caraș-Severin), but lower in the other investigated months. The lowest prevalence (4.29% in Timiș and 4.73% in Caraș-Severin, respectively) was noticed in March 2012. Nevertheless, cyathostomes were ranked second.

As far as the dynamics of cyathostomes population concerns (Figure 3) we can notice a seasonable distribution of L3, on pasture. On October and November 2011, when there was enough humidity and the day-time temperatures varied between 4 - 28°C, the L3 of cyathostomes were abundant. On winter months, when the temperature went down to 0°C and more, the larvae did not survive in such a manner; this was proved by the fact that, on March 2012, only a small number of L3 (about 4%) were recovered.

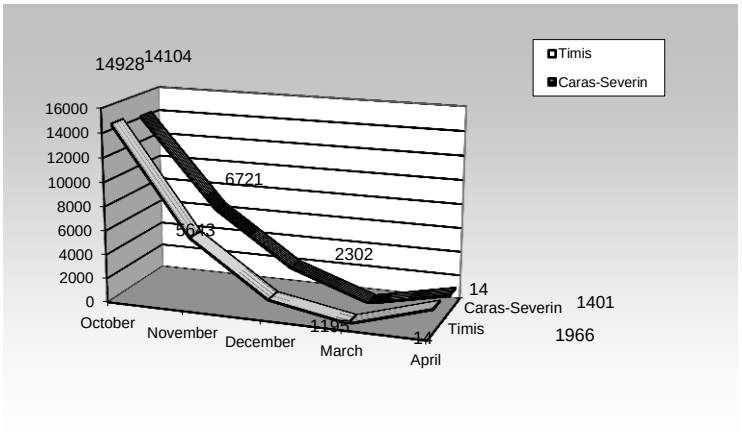

Fig. 3. Cyathostome L3 dynamics on pastures between October 2011 and April 2012

It was also established in a previous work that cyathostome larvae from horse faeces did not develop to the infective stage when faecal humidity levels dropped below 23%, even if solitary preinfective larvae were still recovered after 151 days at a humidity level of 19.5%

[Langrova et al., 2008; Morariu, 2012]. On the contrary, during autumn, L3 of cyathostomes developed correspondingly, reaching population peaks. As a matter of fact, it was reported that these L3 develop optimally when the temperature varies between 10-33°C and the excessive humidity negatively influence their development, although it is necessary for evolution [Courtney, 1999; Baudena et al., 2000].

Conclusions

1. In the grasses samples collected from the pastures of western Arad and Șiria localities the following species of nematodes: cyathostomes, *Strongylus equinus, S. edentatus, S. vulgaris*, digestive strongyles, and free-living nematodes were identified, respectively.
2. L3 of cyathostomes do not survive efficiently during winter on pastures from both counties, with a remark for migrating strongyles whose larvae survived better in Timiș County.
3. The population peaks of L3 of cyathostomes on investigated pastures were recorded in autumn months.

Acknowledgments

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MAIN THREATS OF POLLUTION IN SWINE FARMS

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Abstract
After the disposal of animal wastes in soil it is accomplished an increased soil nutrient concentration and a potential water pollution. Manure has the potential to cause degradation in the quality of subsurface waters. Waste transport to distant locations for disposal is uneconomical and that's why animal wastes are disposed close to the farms. Soil chemistry is affected due to a several years waste application to the same soils. Present review paper presents the main threats of swine breeding industry.
Keywords: animal waste, soil, pollution

Because soil is an important component of the bioshere, the capacity of soil to sustain productivity, water quality and animal health has a major role. After applying pig slurry it is revealed the content in nitrogen perturbing the dynamic equilibrium of nitrogen in soil. The effects of spreading pig slurry are important for the investigation of ammonia oxidizers in soil (Doran 2000, Ceccherini 1998).

The impact of swine manure management (table 1) on bacterial contamination in subsurface drainage is difficult to assess in the field. Bacteria from manure can pollute water, soil and vegetation threatening the environment and human health. In addition to flowing water, pathogenic bacteria from manure my be transmited by wind, insects, rodents (Morrison and Martin, 1977).

Animal manure doesn't always correspond to plant's growth needs in micronutrients (Evers 1996, Pierzynski 2000) and a movement of P from soil to surface waters can lead to eutrophic pollution and loss of environmental quality (Evers 1996, Pierzynski 2000, Sharpley 1999).

Swine manure (figure 1) is disposed daily by washing shelters into anaerobic holding lagoons and then sprayed repeatedly in soil for many years (King 1990). Long-term application of swine waste to soil leads to high levels of P, K, Mg which imbalances the soil nutrient profile beeing able to affect population levels and activity (King 1990, Pierzynski 2000).

Animal wastes in soil increases: microbial biomass C, N, P; microbial enzymes activity, population levels and observable structures (Acosta-Martinez and Harmel 2006, Perez-de-Mora 2006). An increased activity or microbial biomass is connected to an increased mineral N, organic C, cellulose degradation and soil organic matter (Schnurer 1985, Perez-de-Mora 2006, Ros 2003, Lovell and Jarvis 1996, Entry 1997, Pratt 2008).

Pig slurry improves soil structure due to it's high value in nutrients: nitrogen and potassium and a disadvantage would be it's content in heavy metals and sodium as a pottential contaminant (Diez 1999, Ham 1998).

Swine manure has the potential to affect air quality through the discarge of N_2O, NH_3, CH_4, H_2S and volatile organic compounds (Harper 1997, Asman 1995, Eklund 1995, Sharpe 1998, Safley 1992, VanWicklen 1997).

Although soil has three forms of nitrogen (figure 2), plants can use only two: ammonium and nitrate form of nitrogen. Plants can use organic nitrogen only after an intervention of soil microbes.

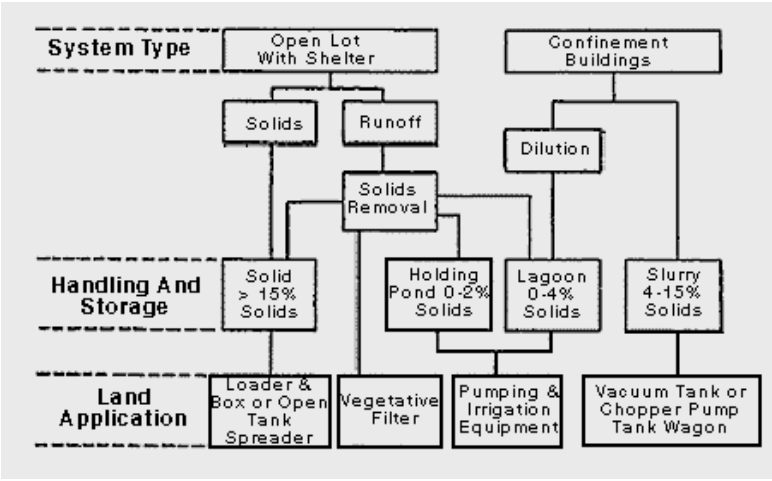

Fig. 1. Swine manure flow chart (After Dickey 1996)

When liquid manure is applied, the two forms of nitrogen penetrate a short depth of soil and after that ammonium nitrogen is converted to nitrate nitrogen.

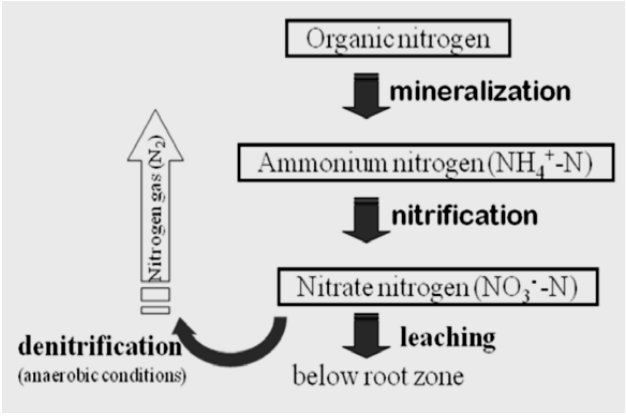

Fig. 2. Soil nitrogen transformation (After Frate 2008)

High nitrate concentration has destructive effects in groundwater and it is influenced by rainfall. It tends to have a high concentration in areas where the amounts of rainfall are low because the diluting effect is reduced.

Table 1. Advantages and disadvantages in swine manure management strategies (After Dickey 1996)

Unit	Advantages	Disadvantages
Below floor slurry	• Easy collection and storage. • Minimum volume. • Maximum fertilizer value.	• Odors and gases. • Solids accumulation. • Solids agitation and removal problems.
Outside storage slurry	• Manure gases in building minimized. • Adaptable to liquid/solid separation and methane production. • Maximum fertilizer value.	• Extra cost for storage and transfer. • Dependence on transfer system. • Solids removal.
Mechanical scraper	• Positive removal. • Handle in slurry form.	• Higher cost. • Equipment and time dependency. • Cold weather, ice. • Possible disease and drug transmission in open gutter. • Maintenance. • Ammonia in building.
Flushing-open gutter	• Lower construction cost. • Quick manure removal. • Lower odors within building. • Manure movement aided by animal access. • Animals attracted to gutter, good dunging patterns.	• Cleanliness dependent on proper design. • Possible disease and drug transmission. • Lagoon requirement. • Equipment dependency.
Flushing-below slat	• Retrofit to existing buildings. • Low odor and ventilation requirements. • Minimized possible disease and drug transmission.	• Higher cost than open gutter. • Cleanliness dependent on design. • Lagoon requirement. • Equipment and time dependency.
Anaerobic lagoons	• Storage and application flexibility. • Low solids liquid for simple irrigation and recycle for flushing system. • Low cost, low labor.	• Land requirement. • Odor potential. • Nitrogen loss. • Sludge buildup. • Recycle salt problems.
Open lot	• Low cost, management. • Nutrient retention in solids. • Easily constructed.	• High labor. • Liquid and solids handling equipment. • Cold weather effects on pigs and producer.

Soils with higher infiltration rates will absorb water and the accompanying dissolved nutrients and pesticides faster than soils with low infiltration rates.Temperature is an important factor, because the warmer the soil is, the faster ammonium nitrogen will be converted to nitrate nitrogen (Frate 2008).

An excess of nitrate in water might accelerate algae and plant growth in lakes and streams leading to oxygen depletion. Drinking water nitrate concentrations can injure young animals or human infants. High nitrate in drinking water has been documented to cause methemoglobinemia, or blue baby syndrome, in infants under 6 months of age (Johnson 1987).

Furthermore, Spalding and Exner (1993) note that consumption of nitrate contaminated drinking water may be linked to hypertension, birth defects, cancers, and infant mortality.

Pig slurry and manure are used as fertilisers because they are important sources of organic matter. Pig slurry is submissive to biological aerobic treatment and subsequent nitrogen removal by nitrification and denitrification as an alternative to nitrogen surplus (Beline 1998, Gitton 1999, Daumer 2001).

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RESEARCH AND OBSERVATION IN THERAPEUTIC CONDUIT OF SUBMANDIBULAR SIALOADENITIS IN THE DOG

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Abstract

Sialoadenis is inflammation of the salivary glands affecting mainly submandibular glands especially in medium and large breeds dog(1,2). Submandibular sialoadenitis appearance in dogs is variable but depends on several factors. The main etiology is traumatic factors induced by the action of external factors and occasionally mechanical trauma from the mouth (consumption of bones, hard objects) that traumatizes the gland, the salivary duct (channel) and their opening orifice. Is important to mention the implications of septic processes (periodontal disease, abscesses) or viral infections at this process(5). The intensity of etiological factors induce the development of these inflammatory conditions, subacute evolution is mentioned frequently, resulting in a long evolution with tendency to become chronic. Because in chronic progressive forms the treatment is long-term is not always the results are the expected ones, in the present study we wanted to investigate the surgical treatment options so to get the functionality of affected submandibular gland(3). This study was performed on a total of 9 dogs with chronic submandibular Sialoadenitis. Surgical treatment was applied by opening glands, evacuation, and on 5 of them to replace the duct. Duct replacement was necessary because it was completely obliterated due fibroconjunctive proliferation. Were used Dacron vascular prostheses with small mesh used in vascular surgery. Replacement was performed from the origin by termino-terminal anastomosis using nonresorbable suture. The postoperative evolution was favorable in all 5 cases achieving healing from 15-22 days.

Key words: sialoadenitis, dog, surgical treatment

Materials and methods

Research and our observations were made at the Faculty of Veterinary Medicine Cluj-Napoca in 2007-2009 on a group of nine dog, by different breeds, ages and sex. We also mention that the group consisted of medium to large dogs presented to the clinic for diagnosis and treatment protocol.

In terms of clinical findings , animals taken in observation had chronic form of evolution, presenting in the submandibular area a round formation (fig. 1) or oval shape (fig. 2) by different sizes. On palpation, the formations presented was under tension, smooth skin, without tenderness or pain. Three of the examined dogs they had difficulty in mastication.

Fig.1. Submandibular sialoadenitis round shape

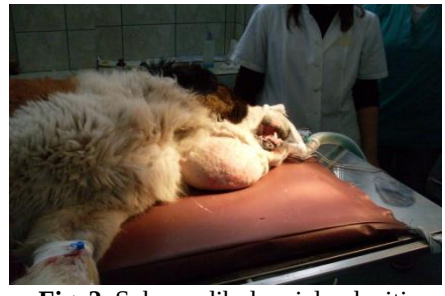

Fig. 2. Submandibular sialoadenitis oval shape

In terms of evolution, the owners have indicated that they have not noticed a particular change in behavior or general evolution of animals, probably due to the region less exposed and because of hairyness of region. Also, all dogs were treated with antibiotic medication (gentavet, lino-spectin, spectam) and anti-inflammatory (dexamethasone), along with a local rubefiant (camphorated alcohol). In these clinical cases taken in the observation, treatment results were inadequate (6).

Methods used

Because of inadequate results after treatment with antibiotics and antiinflammatory we proceeded to evacuation puncture of these submandibular structures with sampling content and perform a bacteriological examination. Content looks serosanguinous, thick, viscous (fig. 3.), the amount varies from 50-500 ml.

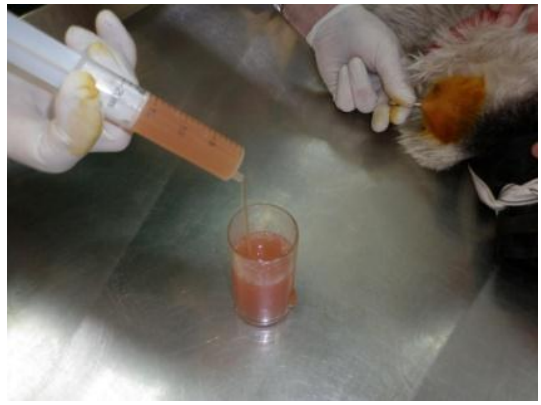

Fig. 3. Evacuating puncture and content aspects

After complete evacuation of the contents we performed a lavage within the gland properly by introducing sterile saline solution + ampicillin and making repeated until the liquid used was clear. To reduce the inflammatory process eventually we introduced 2 g hydrocortisone+ enrofloxacin solution. + spectam solution , completed by a slight massage to disperse the solution over the surface. Bacteriological examination of the contents from the submandibular gland revealed two cases the presence of streptococci and staphylococci, similar situation with determinations of other authors(4).

Evacuation puncture was performed and then repeated every 4-6 days to drain the gland content wich accumulate gradually.

This protocol did not give satisfactory results, and we decided surgical opening. Surgery was performed in 5 cases. After opening the cavity was found gland reaction manifested by walls thickening, thickening of the ducts (fig. 4.), presence of fibrin deposits (fig. 5.) and excretory duct obliteration. The latter we proceeded to substitute ducts by Dacron prostheses used for vascular prostheses.

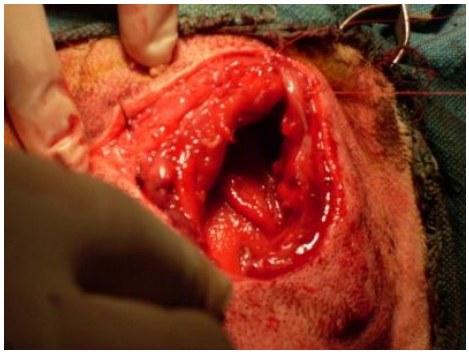

Fig.4. Thickening of the ducts

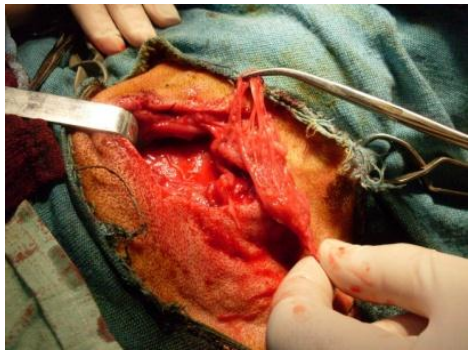

Fig.5. Fibrin deposits

Results and discussion

Surgery is justified because in the cavity is organized during the evolution fibrin deposits that interfere the movement of saliva. Furthermore, fibrin is formed in the gland cavity and inside the ducts and particular the main duct is completely blocked (fig. 6.). In our study fibrin deposits were significant amount in the form of deposits (fig.7.) and as a network which disturb irreversible proper functioning of the gland.

Because the long persistence of fibrin generated fibroconjunctive reaction with the lumen narrowing and then obliteration gland duct, we opted for duct replacement. In all 5 cases submandibular gland duct was replaced with Dacron prosthesis of appropriate diameter, prostheses used in vascular surgery in human medicine.

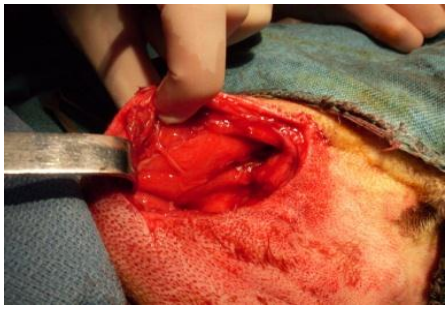

Fig.6. Complete duct obstruction

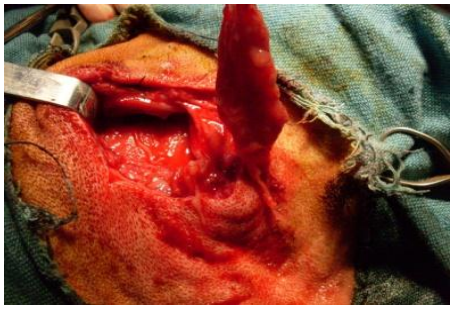

Fig.7. Fibrine deposit

The surgical replacement was performed by termino-terminal anastomosis with Ethicon nonresorbabil, performed from duct origin to the pappila . Duct prosthesis followed the original route. On site cavity gland incision we lasts for 7 days an oriffice of 1 cm daily for drug administration and drainage. Postoperatively for three days dogs received parenteral antibiotics and were fed parenteral for 5 days. After this period, food was administered in liquid form for two weeks. Postoperative evolution was favorable in all patients operated on.

Conclusions

1. In cases of compromising with functional submandibular gland duct failure (adhesions, fibrosis of the walls, obstructions, tumors) substituting in good condition can be achieved by synthetic prostheses.
2. Chronic submandibular sialoadenitis with saliva retention must be addressed to surgical treatment to ensure drainage.
3. In chronic forms of sialoadenitis structural changes occur (deposits of fibrin, fibroconjunctive reaction) of the wallsglands and excretory ducts(7).
4. Secretion of affected submandibular gland fram monitored clinical cases was free of pathogens with the exception of two cases which reveal staphylococci and streptococci.

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MAGNESIUM DYNAMIC BESIDE OTHER PARAMETERS IN EQUINE ACUTE RHABDOMYOLYSIS

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Abstract
Deterioration of energizing metabolism establishes an acute myopathy (Acute rhabdomyolysis syndrome : A.R.) wich affect also sport horses and work horses by diminution of sports and work performances and work capacity.the triggers of A.R. etiopathogenesis is effort due to (amid) a high- carbohydrate nutrition and muscles inactivity. The biological material taken in study were represented by 24 heavy breed horses and their crossbreed from farmsted fall into two groups according to clinical expression and evolution. Group A was formed by 15 horses with moderate acute rhabdomyolysis symptoms wich delivered in therapy. Ggroup B was formed by 9 horses with serious symptoms and 4 of them died being group C.at horses with acute rhabdomyolysis taken in own study we doesn't talk about a magnesium deficiency, rather about a magnesium deplation or a dismagnesemia: the serum level of magnesium is grew, but erythrocyte magnesium (intracellular) is low. Serum hypermagnesemia is largely a result of leaving the cell by magnesium result of acidosis, muscle contraction (muscle damage), and therefore a degree of haemoconcentration through water loss (heavy sweating). The behavior of magnesium in acute rhabdomyolysis is like with there of potassium, with mention that its depletion induced important biochemical alters, normalize of its concentration favors the cell activity by maintaining the membrane gradient. The calcium and potassium are linked with magnesium metabolism among the three elements being some interdependences.

Keywords:.rhabdomiolysis, serum magnesium, erythrocyte magnesium

Introduction

Acute rhabdomyolysis is in essence an acute myopathy as result of deterioration of energizing metabolism with disturbances in membrane and sarcoplasmic exchange, mitochondrial function inhibition and sarcolema, muscle fibres, muscle fiber cytoskeleton damage (Mircean, 2003; Art and coll., 1990; Beech and coll., 1993). In muscle magnesium decreases the neuromuscular fiber excitability (corrugated fiber- muscle relaxation effect and smooth fiber- muscle contraction) and increases muscle performance through participation in the action molecules formation. The neuromuscular effects of magnesium depend on its action of membrane with maintain the acetylcholine and potassium ions in the nerve cell. Consedering its importance in neuromuscle physiology this study follows in dynamic the plasma and erythrocyte magnesium concentration, beside other parameters and points the correlation between concentrations and the gravity of clinical symptoms at horses with acute rhabdomyolysis diagnosis.

Material and methods

The animals taken in study were represented by 24 horses from farmstead. From all of them: 19 horses were treated and consulted in Medical Pathology Clinic and 5 horses were counsulted and treated in Aschileu Veterinary Sanitar Circumscription. Patients were grouped into two groups: group A was formed by 15 horses with moderate acute rhabdomyolysis symptoms which delivered in therapy. Group B was formed by 9 horses with grave symptoms and 4 of them passed away forming C group. The history showed carbohydrate hypernutrition in all horses correlated with muscle inactivity (concentrated feed: oats, corn, barley, hay). In all cases the trigger was the effort of variable intensity. Tabel 1 is illustrative in terms of clinical signs.

Tabel 1. Clinical signs at horses from our study

	Clinical signs
Group A	*Clinical signs*: mers rigid, tremuraturi musculare, accelerarea marilor functii (tahicardie, tahisfigmie, tahipnee), hipertermie, efidroza, redoare musculara, dificultati in mictiune, mioglobinurie (urina cu aspectul zatului de cafea).
Group B	*Clinical signs*: atitudine de lumbago, tumefactia asimetrica a musculaturii crupei, coapsei si a trenului anterior, decubit lateral, agitatie, marile functii accelerate cu tulburari de ritm cardiac, mioglobinurie masiva, complicatii decubitale: sindrom de strivire, plagi decubitale, congestie pulmonara pasiva, edem pulmonar hipostatic

The biochemical blood investigations: serum magnesium (SMg), erythrocyte magnesium (EMg), calcium (Ca), Potassium (K) inorganic phosphate (P), glycemia, lactic acid, BUN, creatinine, muscle enzimes: creatinphosphokinase (CPK), aspartataminotransferase (ASAT) determination were conducted on blood samples taken during the jugular vein puncture in vacuum tubes with and without anticoagulants substantes (Li-heparin) for the whole blood and serum. The dosages were based on molecular absorption spectrophotometry methods recorded on SCREEN MASTER PLUS analyzer. The calculating of erythrocytic magnesium (EMg) has been made indirectly using a relation the value of total magnesium (TMg) obtained from whole blood prelevated on Li-heparin , the hematocrit value (H) and serum magnesium value (SMg) in according with the following formula :

$$EMg = \frac{(TMg - SMg) \times 100}{H} + SMg$$

The results are expressed in mg/dl, mEq/l or mmol/l.

Results and discusions

At horses with A.R. taken in owen study analysis of the results noted different evolution of the biochemical and mineral constituents, but in relation to the intensity of clinical manifestations.. The values recorded for glycemia, lactic acid, BUN, creatinine and muscle enzimes: CPK and ASAT are expected considered by any authors as being very important for diagnosis (Ghergariu and coll., 1997; Miu and Drăgotoiu, 2000; Mircean, 2003) (graph. 1, 2).

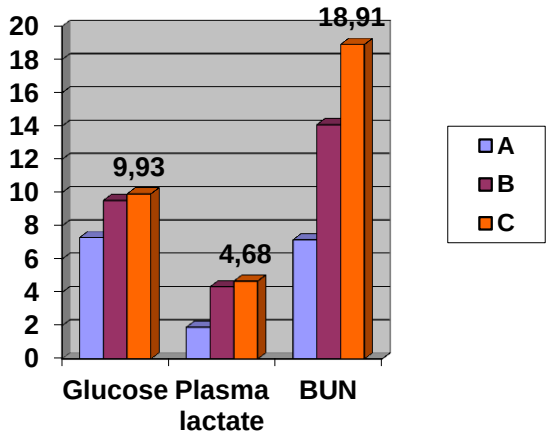

Fig. 1. Blood levels of the biochemical constituents in horses with A.R. taken in study

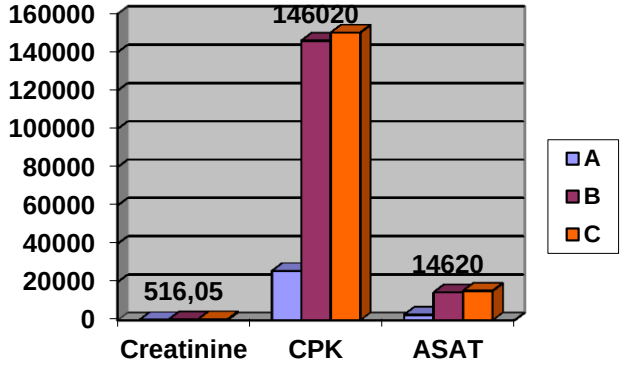

Fig. 2. Blood level of the muscular enzymes in horses with A.R. taken in study

About macrominerals in special for magnesium in acute rhabdomyolysis the data are parcimony excepting the main intacellular cation: potassium. He showed it increased the average value of horses in groups B and C (6,41±0,52 mmol/l and 7,43±0,43 mmol/l) compared with group A (5,65±0,32 mmol/l) whose values were within the reference limits (2,4-4,7 mmol/l after Simesen, quote by Kaneko, 1980; 3,32-5,62 mmol/l after Ghergariu and coll., 2000).

Increased serum magnesium level is the result of muscle tissue lesion extrusion due to acidosis and myoglobinuria nephrosis (Ghergariu and coll., 1997; Miu and Drăgutoiu, 2000; Mircean, 2003).

The question of importance arises magnesium in a condition such as A.R. It is almost to an intracellular ion, second only to potassium. The plasma magnesium level is only about 1% of magnesian fund, therefore in the presence of magnesium deficiency-related diseases is compulsory to dosage of plasma or serum magnesium and intracellulary, respectively

erythrocyte mangnesium. Serum magnesium showed significant increases in the average value of horses in group B and C (1,09±0,09 mmol/l şi 1,28±0,01 mmol/l) compared with group A (0,83±0,11 mmol/l) and the reference values (0,80±0,10 mmol/l after Simesen, quote by Kaneko, 1980; 0,65 mmol/l after Walser, quote by Miu, 2000; 0,53-1,02 mmol/l after Ghergariu and coll., 2000; 0,76±0,09 mmol/l after Gherghiceanu Daniela Mihaela, 2009). Instead erythrocyte magnesium recorded significant decreases in the average value to horses in group A (1,89±0,09 mmol/l), B and C (1,73±0,10 mmol/l ; 1,67±0,07 mmol/l) compared with the reference values (3,19 mmol/l after WALSER, quote by MIU, 2000; 2,26±0,27 mmol/l after Gherghiceanu Daniela Mihaela, 2009) (graph 3).

At horses with acute rhabdomyolysis taken in own study we doesn't talk about a magnesium deficiency, rather about a magnesium depletion or a dismagnesemia: the plasmatic levelof magnesium is grew, with statistical signficant differences among groups ($p < 0,001$) (A group :0,83±0,1mmol/l, B group: 1,09±0,09mmol/l, C group: 1,28±0,01mmol/l) but erythrocyte magnesium (intracellular) is low, also with significant statistical differences among groups ($p < 0,001$) (A group: 1,89±0,09mmol/l, B group: 1,71±0,1mmol/l, C group: 1,63±0,07mmol/l), see graph 3.

Serum hypermagnesemia is largely the consequence of magnesium cell leaving as a result of acidosis, muscle contraction (muscle lesions) and a lesser degree of haemoconcentration through loss of water (heavy sweating). JOBORN and coll. (1985) suggests the magnesium exit of cells (muscle fibers, erythrocytes) that certainly contribute to plasma or serum level increased, so well known to potassium.

Although no mechanism to control the magnesium placement during metabolic disorders is not clearly described, will take into account the magnesium effect of membrane stabilizing. Magnesium maintains potassium in the cell by Na/K pump activated, ATP-ase activated with ATP- Mg complex thereby controlling memebrane exchanges at maintaining the electrolyte transcllular gradient (Miu and Drăgotoiu, 2000).

In magnesium depletion the permeability of plasma membranes grow, and the muscle cell as so erythrocyte an able to stop potassium. Deterioration of cell membranes functions is in corelation with deterioration of intracellular biocemical activity. The cells are charged with calcium and sodium ions, losing phosphate, magnesium and potassium ions (graph 4). According to some authors these direct effects of magnesium on the membranes structure can be enhanced by parallel effects on dependent- ATP pumps that regulate the sodium (Na), potassium (K) and calcium (Ca) ions transport (Miu and Drăgotoiu, 2000). The uncontrolled and dangerous penetration of sodium and calcium ions, in special that of calcium, in cells is resulted in its premature destruction, the appearance is obvious at myocardic fibre level, where that charge with calcium, associated with magnesium depletion induces its pass away and implicitly major myocardic lesions incompatibely with surviver (Fleckenstein and Spah, quote by Zeană, 1994).

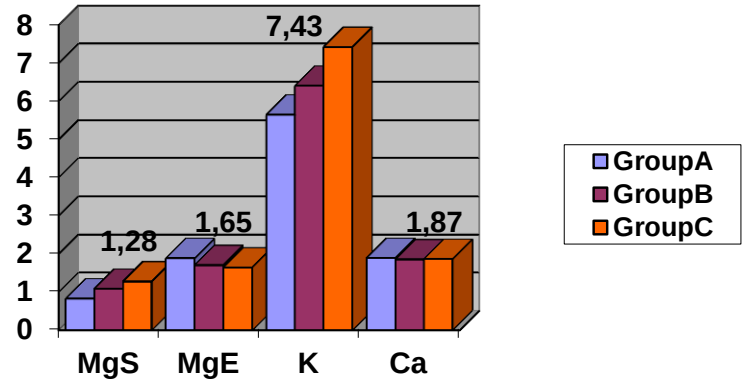

Fig. 3. Magnesium (SMg; EMg)-potassium (K)-calcium (Ca) relationship compared to the three lots of horses with A.R

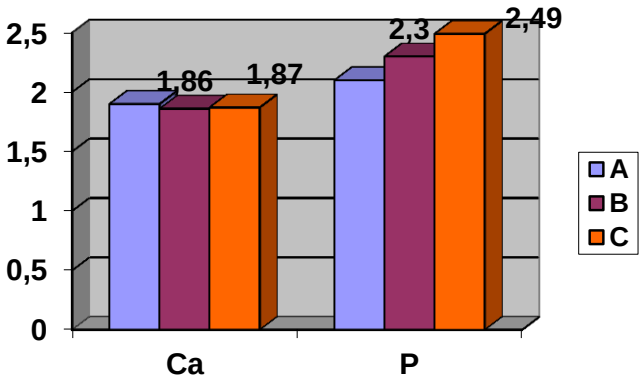

Fig. 4. Calcium (Ca) and inorganic phosphate (P) blood level, compared to the three lots of horses with A.R

Hyperphosphatemia found at all horses with acute rhabdomyolysis [group A=2,10±0,08 mmol/l; group B=2,30±0,08 mmol/l; groupl C=2,49±0,01 mmol/l, references values from literature: 1,50-6,00 mg/dl (0,48-1,93 mmol/l) after Ghergariu and coll., 2000] is due to cells extrusion, as follow of membrane permeability deterioration in relation with renal excretion deterioration (myoglobinuric nephrosis) and due to activity intensification of healthy muscle for the supply the disfunction of affected muscle (Art and coll., 1990; Beech and coll., 1993; Mircean, 2003). BUN and phosphates holding back established the metabolic acidosis and the diminution of calcium. That explain the low values of calcium at horses with acute rhabdomyolysis from the three groups (special at B and C groups: 1,86±0,03mmol/l; 1,87±0,03mmol/l from group A: 1,90±0,04 mmol/l and compared with reference values from literature: [2,78-3,34 mmol/l after Simesen, quote by Kaneko, 1980; 2,75 mmol/l after Walser, quote by Miu, 2000; 8-13 mg/dl (1,96-3,19 mmol/l) Ghergariu and coll., 2000].

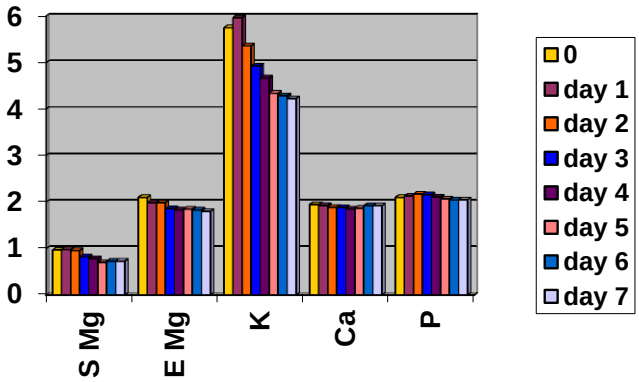

Fig. 5 . Mineral constituents screening in horses with A.R. from lot A during hospitalization

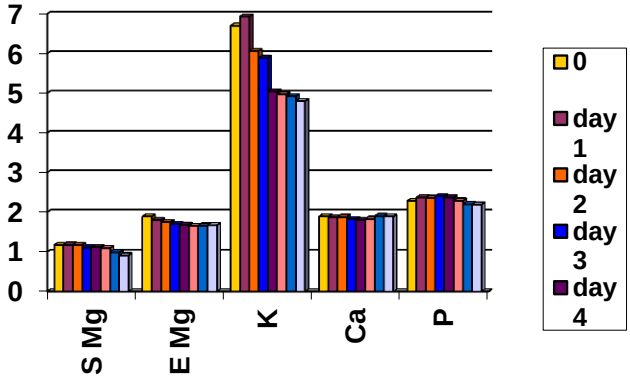

Fig. 6. Mineral constituents screening in horses with A.R. from lot B during hospitalization

The evolution in time of serum magnesium concentrations (on days of treatment) at the three groups of horses sustains the diminution to normal values at horses with midle evolution (A group) and a slow diminution but with values superior to reference one at the horses with grave evolution (B group) (graph 5,6). The explantion of serum magnesium concentration decreased i srepresented on the one hand the electrolyte solutins therapy and on the other side of hyperkalemia. The electrolyte solution therapy applied to maintain the kidney cleansing help to renal elimination of magnesium and potassium.

The proximity of plasma magnesium and potassium values to referance values doesn't exclude the variation of K and mg of cells. There aspects inform the monitoring and the evaluation of K and Mg balance in acute rhabdomyolysis given being a low level for erythrocyte mangesium concentration at both groups (A group: 1,89±0,09mmol/l; B group: 1,73±0,1mmol/l). The recovery of the erythrocyte magnesium level (intracellulary level) is to slow compared with the serum level.

The behaviour of magnesium in acute rhabdomyolysis is alike with there of potassium, with mention that its depletion induced important biochemical alters which brings

it in attention. The normalising of concentration encouraging cell activity by maintainance of membrane gradient. Calcium and potassium are linked with magnesium methabolism among the three elements being some interdependences.

Conclusions

1. Transient plasma hypermagnesemia of horses for groups A and B is the consequence of leaving acidosis, muscle contraction and reduce renal removals.
2. Persistance of erythrocyte magnesium low level in horses with acute rhabdomyolysis studied along side together transient plasma hypermagnesemia pleds for installing magnesium depletion.
3. Magnesium depletion induced the cell membrane functions and the intracellular biochemical activity altering with uncontrolled and dangerous calcium and sodium entrance in the cells and a concomitent removal fo potassium, magnesium and phosphate.
4. The hyperphosphatemia persent in all horses with rhabdomyolysis taken the study, is attributed to removal of cells by altering membrane permeability and enhancing muscle contraction substitute.
5. The magnesium behaviour in the acute rhabdomyolysis syndrom is similar to potassium thus its structure role and membrane stabilizing it bring to the first plan of biochemical changes
6. Calcium and potassium are closely related to the magnesium metabolism, between the three elements exist a number of interdependencies.

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SOME ADVANTAGES OF FIELD CASTRATION IN HORSES USING HENDERSON EQUINE CASTRATING INSTRUMENT

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Abstract
Potential complications of horse castration include postoperative swelling, haemorrhage, eventration, funiculitis, peritonitis, hydrocele, penile damage. Techniques for castration in horses include open, closed and half-closed techniques. This study investigated an efficient method of closed castration using Henderson Equine Castrating Instrument in 20 mature stallions. Mature stallions (n = 20) were castrated under general anaesthesia in dorsal recumbency using a scrotal approach. After scrotal incisions, the two cords were isolated using digital dissection, Henderson Instrument was applied and the testis were totally removed after about five turns. Three of 20 (15%) of the horses had a postoperative local swelling, which resolved without treatment and no other complications were noticed. All horses made a full recovery between 3-4 weeks postoperatively. This technique of castration with Henderson tool via scrotal approach had a low incidence of local and general complications compared with other methods.

Key words: Henderson Instrument, horse, castration, complication

Introduction

Castration is one of the most common equine surgical procedures and is usually performed to sterilize horses unsuitable for contributing to the genetic pool and to eliminate masculine behaviour *(Auer, 2012).* The emasculator models most commonly used are the improved White's, the Reimer, and the Serra emasculators *(Moll et al., 1995).*

A range of open and closed surgical techniques, sedation and anaesthetic protocols are described for equine castration *(Colahan et al., 1999; Adams and Fessler, 2000).* Complications associated with castration are a common cause of malpractice claims against North American equine practitioners *(Searle et al., 1999).*The new Henderson Equine Castrating Instrument is a redesigned version of the Henderson Castrating Tool that was introduced in 1994 for castrating bulls. The instrument is easy to use and faster than many other forms of castration *(STONE Equine Castrating Brochure)* and it has proven to be a safe, fast, and cost effective method of castration in stallions.

Materials and methods

The present study was performed on 20 clinically healthy stallions, aged 1,5-3 years and weighing 400-600 kg, with two normally descended scrotal testes. Food was withheld for 12 hours before surgery.

A general physical examination of the horses preceded castration. Drugs used for anesthesia were xylazine HCl (Xylazin Bio, *Bioveta*) 0,5-0,7 mg/kc, butorphanol tartrate (Butomidor, *Richter Pharma*) 0,044 mg/kc in combination with ketamine (Ketaminol, *Intervet*) 2,2 mg/kc given intravenously 5 minute after xylazine. The horses were positioned in left lateral recumbency (right-handed surgeon) with its upper pelvic limb pulled forward and secured with a rope around neck (Fig. 1). The scrotum was inspected for inguinal

herniation and for the presence of both testes. Two parallel 10 cm incisions 1 cm distant from the raphe were made, while compressing the testes against the bottom of the scrotum (Fig. 2). The cords were isolated using digital dissection. After the parietal tunic was separated from the surrounding fascia, the cord contents were prepared for the emasculator.

The Henderson Equine Castrating Instrument used is a tool designed for castration in horses (Fig. 3). With slight tension on the drill and with the instrument held parallel to the cord, the testis was rotated slowly for about five turns (Fig. 4). The speed of the rotations was gradually increased while keeping slight tension on the cord. After 20 to 25 rotations, the cord separated about 8 to 10 cm proximal to the instrument. Twisting of vascular elements of the testicular cords assures a safe hemostatis (Fig. 5). Portions of scrotal fascia protruding from the scrotal opening were trimmed with scissors. An anti-bacterial, anti-fungal, anti-inflammatory and anti-pruritic skin gel and spray (Charmil, *Ayurvet Limited*, *India*) was applied into the scrotal pouches. The scrotal wounds were left unsutured.

Fig. 1. Left lateral recumbency

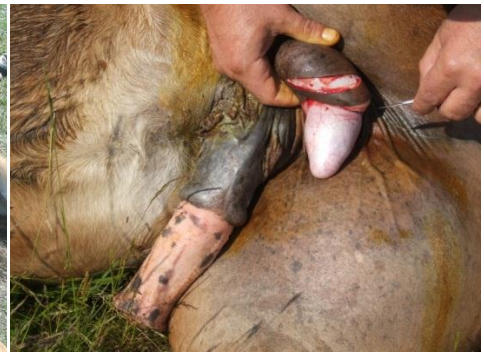

Fig. 2. Scrotal incisions and isolation of the cords

Fig. 3. The Henderson Equine Castrating Instrument

Fig. 4. Testis rotation and ablation

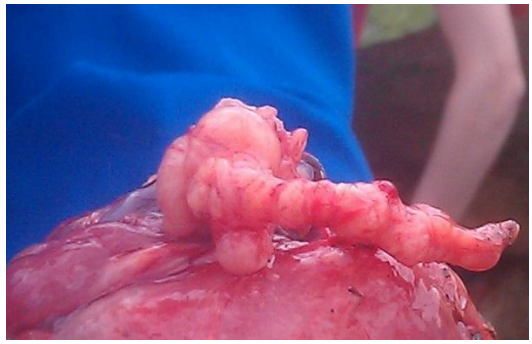

Fig. 5. Vascular elements of the cords twisted

All horses received tetanus antitoxin after castration. The horse's efforts were restricted for the first 48 hours after castration to prevent haemorrhage. After this period, the horses were walked daily to prevent excessive preputial and scrotal edema.

Results and disscutions

Scrotal and preputial swelling occurred in 3 patients, reaching their maximum extent on day 3-4 after surgeries. Complete resorbtion and evacuation was observed in all three horses after 7 days postoperative. No other surgical or postoperative complications were noted in patients from our study.

Charmil *(Ayurvet Limited, India)* skin gel and spray protected the wounds against infections and flies. The castration wounds were totally healed in our patients in the interval by 3-4 weeks postoperatively.

Conventional castration techniques require a more invasive procedure and a longer time and occasionally result in serious complications *(Howaida et al., 2012).* The anesthetic protocol consisting in combination xylazine/butorphanol/ketamine is adequate for this type of castration. The addition of botorphanol tartrate to the ketamine hydrochloride enhances muscular relaxation and analgesia and prolongs anesthesia for several minutes *(Tranquilli et al., 1983).*

The open technique of castration is probably the most commonly used technique *(Moll et al., 1995),* but the majority of complications are associated with this technique.

As a result of closed castration by using Henderson Equine Castrating Instrument, the subsequent complications (eventration, peritonitis, evisceration) that can usually occur in case of open technique were prevented. One handle of this plierslike instrument is attached to a variable speed drill. With one hand holding the testis, the instrument is clamped across the entire cord, just proximal to the testis. The twisting of the cord seals the severed vessels *(Auer, 2012).*

The transfixation ligature placed around the entire spermatic cord is not necessary when Henderson Equine Castrating Instrument is used. This is another advantage of this technique, because, the ligatures may increase the risk of postoperative infections *(Moll et al., 1995; Walker and Vaughan, 1980).*

A large, grass-covered field is an ideal environment for postoperative recuperation, but the owner should be cautioned that turning a horse into a field does not ensure that it will receive adequate exercise *(Auer, 2012).* Thus, we recommend for the owners to walk the

horses, 1,5 hours in the morning and 1,5 hours in the evening, during 2 weeks postoperative.

We agree the idea that an antimicrobial treatment is unnecessary if clean surroundings are provided.

Conclusions

1. In 20 patients castrated using Henderson Equine Castrating Instrument no major complications (hemmorhage, evisceration, swelling, infection and trauma) were observed.
2. Short-acting anesthesia resulted after combination of xylazine-butorphanol-ketamine and lateral recumbency are the best conditions for the Henderson Technique.
3. Castration with the Henderson method is one of the safest and easiest method of castration in horses in field conditions.

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THE RELEVANCE OF THE ULTRASOUND INVESTIGATIONS OF THE ABDOMINAL CAVITY IN ADULTS HORSES

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Abstract
Abdominal ultrasound allows evaluation of the abdominal cavity organs, vascular structures, diaphragm, abdominal formations, peritoneal effusion, and possible topographical changes. The study was conducted on 6 horses (aged 2 years to 6 years) with various diseases which interested the abdominal cavity organs/structures. The accuracy and specificity for the gastrointestinal parietal changes and accumulations in peritoneal space is recommended that an imaging technique with high relevance, easy and non invasive.
Key words: ultrasonography, horses, abdominal cavity

Ultrasound examination structures aiming to evaluate the abdominal cavity: parenchimatous organs, cavitary organs, vascular structures, diaphragm, peritoneal accumulation, changes in tone, motility and possible topographical changes of these cavitary organs (2, 7).

Material and methods

The study was performed on horses (n = 6) with different ages (2 years and 6 years), with various problems that interested organs/structures from the abdominal cavity, especially in cavitary organs: stomach, intestine, bladder (n = 1) and accumulation in the peritoneal space (n = 5).

Ultrasound examinations were performed with portable ultrasound (Mindray and Esaote Pie Medical) with linear and convex probes with different frequencies between 3,5 and 10 MHz.

In the diagnosis of the cavitary organs` (stomach, small and large intestine, and urinary bladder) diseases, correlated with obvious clinical signs, the ultrasound examination can easily confirm the clinical hypothesis of the clinical suspected affections (3).

The ultrasound examination of the cavitary organs was performed using linear or convex probes, of normal or high frequency (3,5 ,5, 6; 6,5; 7,5; 8 or 10 MHz), obtaining valuable information about their wall structure and thickness (normal 3-5 mm). The ultrasound evaluations were performed using different probes (according to the age of the foal). The clinical changes were registered and correlated with the results of the paraclinical investigations, especially with the ultrasound investigation results.

In such cases, for ensuring a maximum accuracy of the ultrasound investigations we`ve followed and respected strictly the steps for obtaining the most relevant images, without or with minimum artifacts images, in order to diminish the relevance of the obtained ultrasound images of the cavitary organs (4, 6).

The main causes of the artifacts inducing images are in most cases attributed to the gas accumulation, the lack of content (in case of urinary bladder evaluation).

On behalf of the tendency of maximum reducing of the improper artifactual situations, was very important to recommend prior the ultrasound examination the food diet and the proper oral fluid intake (2, 9).

Results and discussions

The most important objective of our study it was to achieve and to correlate the clinical expression of the abdominal cavity and cavitary organs` diseases with the ultrasound changes, in order to find strictly correspondences between these, in confirming the diagnosis.

The normal patterns of the stomach and intestine can be easily appreciated and revealed in the ultrasonographyc image, with the compounds of the wall architecture (Figure 1 and Figure 2).

After the clinical and ultrasound investigation of the cavitary organs (stomach, small and large intestine and urinary bladder), the most easily identified and confirmed were the diseases with high degree of specificity, as the inflammatory changes, dominated by the uniform thickening of the wall, especially of the superficial components and the maintaining of the parietal tonus (in acute inflammations)/or diminishing the parietal tonus (for the chronic inflammations).

From ultrasonographyc point of the view, the most important and facile too is to identify the changes of the parietal architecture and wall thickening, specific for each cavitary organ.

In this frame, for the investigated cavitary organs, the stasis of the content can be specific clinical correlated with the ultrasound changes, as in gastric or intestinal inflammations (Figure 3, 4, 5 and 6), with obvious digestive specific simptomatology, in accordance with the degree of parietal injuries (1, 5).

In case of urinary bladder, the distension is more easily observed by ultrasound scanning, less correlated with the clinical signs, with minimum influence to the layers architecture or thickness, but any changes in normal anechoic content of the normal bladders can be observed and evaluate (Figure 7 and Figure 8).

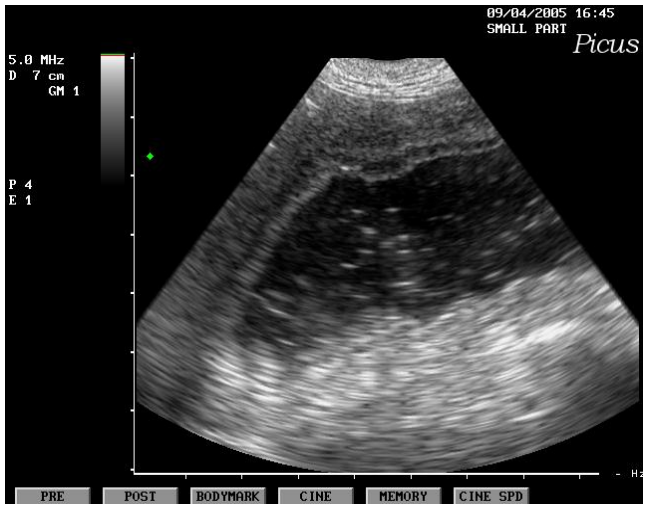

Fig. 1. The normal ultrasonographyc aspect of the stomach.
Can be easily identified the parietal components,
the typeand the aspect of the gastric content

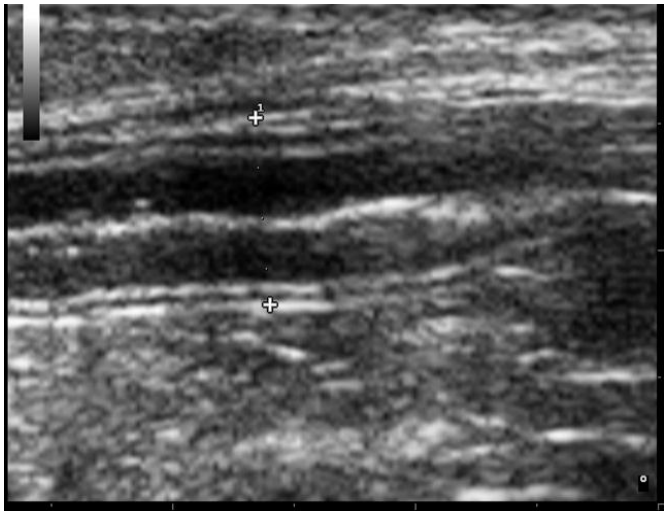

Fig. 2. The normal view of the parietal compounds
of the intestinal wallof the small intestine –
(lumen, mucosa, submucosa, musculosa and serosal compund)

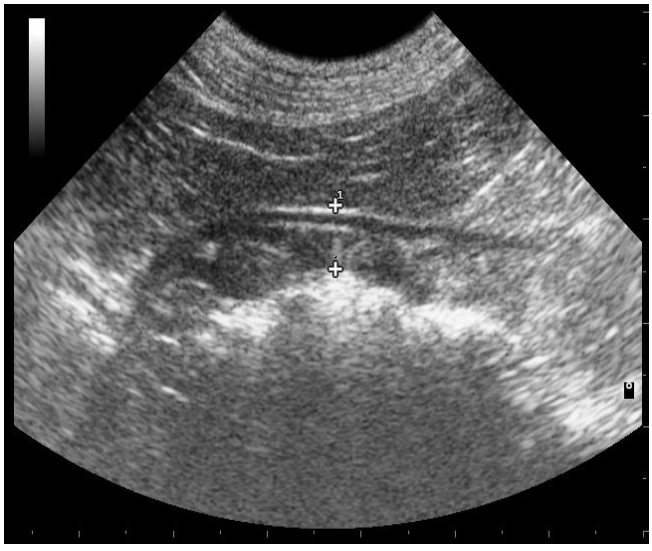

Fig. 3. Stomach with an obvious thickening of the wall – gastritis
(reaction of the superfficial compound),
with keeping unaltered the parietal architecture

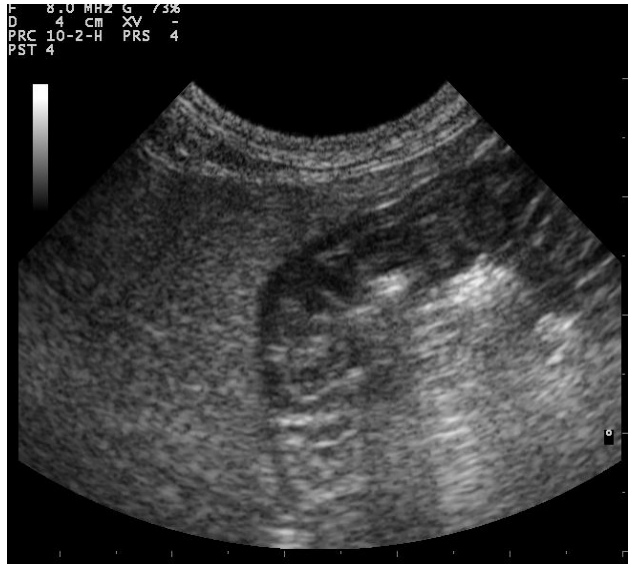

Fig. 4. Acute gastritis. Obvious parietal reaction
(uniforme thickening) with important reaction
of parietal affected components (hypertrophy of gastric mucosa)

From clinical point of view, in most cases the quantity changes of the cavitary organs indicates alterations of the morphology and functions of these (especially parietal injuries). Changes in the quality of the content of these cavitary organs (stomach, intestine, urinary bladder) are usually suggestive regarding the type of the registered morphologic alterations (10).

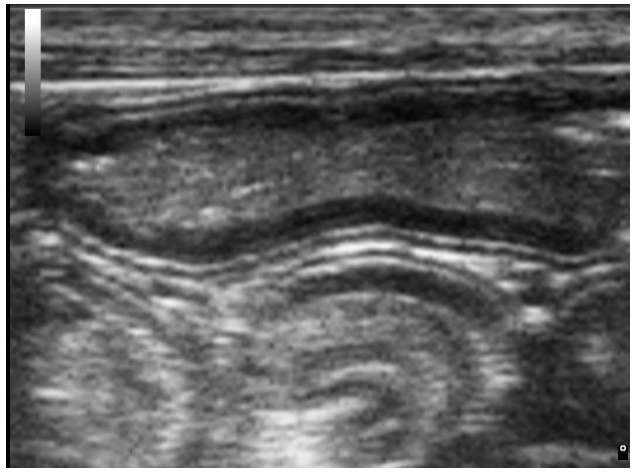

Fig. 5. Acute enteritis. Aspect of intestinal stasis
(distended by hypoechoic content – with visible lumen)
and important parietal reaction (uniform thickening of the wall)

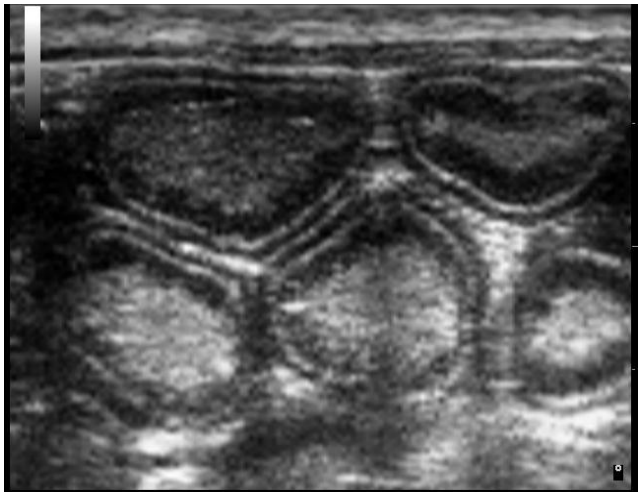

Fig. 6. Characteristic aspect of acute enteritis.
Intestinal stasis (intestin distended by hypoechoic content – with highlighting of the lumen) and important parietal reaction (uniform thickening)

Peritoneal fluids can be easily identified and can appreciate: the quantity, nature, echogenicity, homogeneity, particles in suspension.

Ultrasound technique is extremely useful in achieving abdominal puncture. With the ultrasound technique can be assessed the amount of free liquid peritoneal space and its character (Figure 7 and Figure 8).

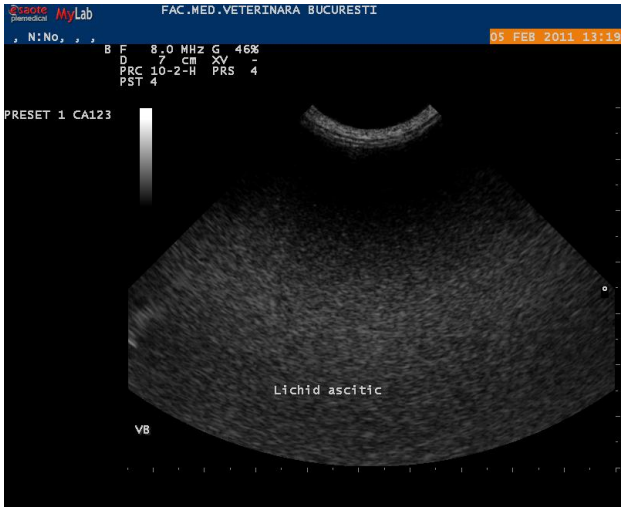

Fig. 7. Ascitic fluid (anechoic aspect) with important cellularity (numerous corpuscular elements in suspension)

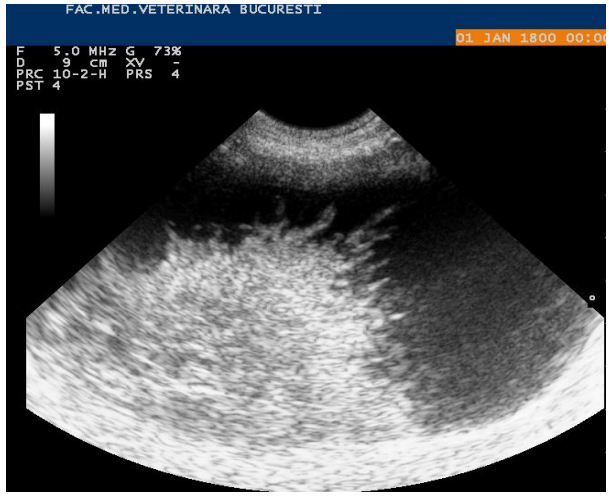

Fig. 8. Severe peritoneal effusion, with particles in suspension – Vortex aspect (tornado) of the corpuscular elements in suspension (uroperitoneum)

Conclusions

The studies described in this paper were aimed for highlighting the main quantification and corroboration of clinical matters and ultrasound (ultrasound) changes in some internal diseases, in order to assess the relevance of ultrasound technique in the diagnosis of organs `diseases and/or systemic diseases in horses. The analysis of the results obtained correlated with those described in the literature allowed the separation following conclusions:

1. Ultrasound examination of abdomen generally allows identification and evaluation of the abdominal cavity organs, vascular structures, formations of intra-abdominal free fluid in the peritoneal space and / or at the hollow, and any topographic changes.
2. Due to peculiarities of sonography, peritoneal fluid can be identified most easily (because of transonic - being anechoic), considering the quantity, nature, echogenicity, homogeneity, presence or absence of particles (corpuscular elements) in suspension, fibrin or hematoma.
   In the cavitary organs (stomach, intestine, and bladder) can be assessed the constituents' parietal (parietal components), parietal thickness, retention/loss of architecture specific parietal and parietal any changes.
   At gastric, intestinal and bladder easiest and specificity are valued highly inflammatory changes (characterized by uniform thickening of the parietal type), with parietal tonus and keeping the interest components superficial reaction.
3. Quantitative changes/stagnation/stasis contents (gastric, intestinal, urinary retention, respectively) reveal a lesional substrate (responsible for these functional disorders) that can be easily evaluated by ultrasound.
   The presence of gas in the gut (cecum, colon) can significantly limit the evaluation of these organs, and because of artifacts induced, prevents assessment of adjacent structures.

4. Non-invasive nature, high degree of specificity and accuracy of results obtained from carrying out ultrasound, recommended this technique in the diagnosis of parenchymatous organs diseases and/or cavitary organs - in young horses.

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THE IMPACT OF SOME STRESSORS ON THE FUNCTIONAL STATUS, RESISTANCE AND ADAPTIVE CAPACITIES OF THE CALVES' ORGANISM DURING THEIR POSTNATAL EARLY ONTOGENESIS

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Rezumat

În studii experimentale a fost evidenţiată şi analizată dinamica metabolismului, rezistenţei şi capacităţilor adaptive la viţei în ontogeneza postnatală timpurie la acţiunea factorului termic stresogen de menajare. A fost stabilit că modificările parametrilor fiziologici testaţi aveau un caracter fazic în dependenţă de factorul stresogen termic şi parazitar, determinat de periodicitatea proceselor de dezvoltare şi creştere, maturizarea organelor şi sistemelor de organe, caracterul metabolismului în diverse perioade şi a nivelului de infestare. S-au observat două ascensiuni de schimbări ale acestor indici: la a 7-8 a zi şi 25-30 a zi de la naştere, ce coincid cu perioadele critice de dezvoltare în ontogeneza postnatală timpurie – imunodeficitar, depresie a reacţiei stresogene, dominaţie şi retardaţie. S-a evidenţiat un efect pozitiv al acţiunii asupra stresrezistenţei, capacităţilor adaptive şi ritmul de creştere a organismului.

The increase of productivity of animals and obtaining healthy offsprings is possible only while taking into accountant the influence of the environmental factors and the particularities of organisms' reaction to such [2, 3, 10, 12]. That is why the issue of adaptation of animate beings towards environmental factors is considered as one of the most important problem of the nowadays science and practice [6,11]. Due to non-observance of microclimatic parameters the productivity of different species of animals can be reduced from 10% till 35%, reproduction capacity may be reduced from 15% till 30%, the mortality of young animals could be increased up to 10-30% and costs of forage used for one animal could be increased from 15 up to 100% [5, 7, 15].

Among all abiotic factors of physical environment, the temperature plays an important role since it influences the processes of thermal energy exchange happen during all basic biochemical reactions in the organism [1,13]. It influences the organism to keep ready its adaptive mechanisms that contributes to preserving of the constant internal environment related to temperature variations [9]. It have been proved that the more is producing capacity the more sensitive is organism towards the thermal factor [4,14]. The temperatures that exceed the limits of the comfort zone are perceived by organism as overwork. The relationships of the organism with environment will depend on the extent and duration of thermal influence.

The aim of the research was to establish the influence of the thermal stressor on the functional status, resistance and adaptation capacities of the calves organism during their early postnatal ontogenesis.

Materials and methods

The experiences have been organized in controlled conditions of a climacteric room in vivarium of the Institute of Physiology and Sanocreatology of A.S.M. As stressor factors were chosen impulsive temperature and parasitic influence. The research objects were 10 calves of Black-and-White Holland race aged 3-30 days. As soon as animals have been introduced in the climacteric room and they adapted to its conditions during 40-60 minutes, the temperature have been gradually reduced up to 5^{o}C. The level of infestation in calves have been controlled in dynamics, while taking 5g of coprological materials. The influence of the thermal stressor on the animal have been realized and researched during the 3-rd, 8-th,15-th, 20-th, 25-th and 30-th days at birth. The following indexes have been studied: the total proteins and protein particles, urea, macro elements such as Ca, P, Na, K, the content of glucose, bactericide activity, concentration of cortisone. There were applied the traditional research methods. The collection of the specimen of blood from animals have been undertaken 30 minutes before applying stressor factor and immediately after the finalizing the experiments. Additionally, the body weight of calves have been observed during all experimental timeframe.

The results obtained and its analytical review

The undertaken research allowed to study the influence of the impulsive stressor thermal factor on the functional status of calves during different stages of their early postnatal ontogenesis. Several indexes of protein metabolism have been researched in calves affected by stressor thermal factor: the content of the total proteins, protein particles and urea in blood plasma. The data indicating its changes in blood before and after influence of stressor factor are presented in Table nr.1.

As presented in Table 1 the tendency of increase of the total proteins level in the blood serum in early postnatal ontogenesis of calves have been observed upon application of stressor factor. The considerable changes have been observed at the 15-day at birth that have been marked with a highest its concentration (up to 10,8% increase). At the 30-th day of postnatal development a 8,2% decrease of the level of total proteins have been observed compared to level observed before the application of stressor stimuli. The pronounced changes have been observed upon determination of the urea concentration in blood serum of animals that have been affected by stressor temperature factor. In this way, at the 15-th and 30-th day at birth at the influence of stressor factor the urea concentration have been reduced by 37,4 and 25,1% correspondingly. In all other research periods the changes have been oscillating in comparatively small limits.

The influence of the stressor factor caused the phasic changes of protein particles indices during different stages of early postnatal ontogenesis. Under the influence of decreased temperature the protein concentration, compared to the level before application of stimuli at the 3-rd day at birth, have been increased at the 15-th and 30-th days. The α-globulins particles at the 3-rd day revealed the decreased concentration (2,4 times) at the influence of the stressor temperature factor, at the 15-th day – 1,3 times decrease and at the 25-th day it have been increased by 1,4 times. The concentration of β-globulins after influence of the stress factor have been changed starting with 8-th day at birth; it increases at the 15-th day by 25% followed by a certain decrease especially at the 25-th day - by 30,3%. Similar evolution is observed in regard to the content of γ-globulins in blood serum: at the

15-th day at birth upon application of stressor it reveals 30,5% decrease, and at the 25-th day a tendency of its increase have been already documented.

Table 1. The dynamics of the protein metabolism indices in calves affected by impulsive stressor factor during their early postnatal period (n=10 animals)

Age (days)	Total proteins g/l	Protein particles, %				Urea, mmol/l
		albumins	α-globulins	β-globulins	γ-globulins	
Before the influence of stressor factor						
3	78,0±2,75	31,8±2,28	7,6±0,18	20,3±0,47	40,2±0,59	3,00±0,13
8	78,3±2,77	41,1±2,29	3,8±0,14	17,9±0,46	37,2±0,57	2,58±0,11
15	70,0±2,72	44,2±2,31	6,8±0,17	15,2±0,40	33,8±0,52	2,30±0,10
20	68,3±2,73	45,3±2,35	6,7±0,16	18,4±0,48	29,6±0,43	2,94±0,11
25	65,9±2,71	46,7±2,41	6,6±0,14	22,4±0,49	24,3±0,41	2,44±0,11
30	64,1±2,60	45,0±2,38	11,5±0,39	16,4±0,41	26,2±0,42	2,87±0,12
After the influence of stressor factor						
3	78,6±2,78	38,5±2,11	3,2±0,12	18,6±0,34	39,7±1,37	3,13±0,14
8	78,3±2,71	40,3±2,17	7,5±0,21	13,4±0,21	38,8±1,36	2,73±0,13
15	75,8±2,70	50,0±2,88	5,1±0,17	19,0±0,51	25,9±1,23	1,44±0,09
20	73,3±2,65	49,9±2,33	6,9±0,19	17,3±0,47	25,7±1,22	2,26±0,10
25	70,1±2,67	50,0±2,39	9,0±0,24	15,6±0,28	25,4±1,20	3,01±0,14
30	62,5±2,48	54,1±2,94	13,5±0,39	11,2±0,20	21,2±1,18	2,15±0,09

$P \leq 0,05$

In this way, the following phasic changes in the indices of protein metabolism have been observed while applying the stressor temperature factor on the organism in its early postnatal period: a tendency towards increased level of the total proteins with pronounced its decrease at the up to 30-th day at birth and a tendency of decrease of urea concentration in the blood serum. Taken into consideration the above mentioned, it could be admitted that during the early postnatal ontogenesis at the influence of the stressor factor the catabolic and anabolic processes at certain levels are intensified. The obtained data allow to confirm that this index characterizing functional status of the organism was relatively stable and fluctuated in small limits.

One of the important physiologic index that characterizes the level of saline metabolism and demonstrates the functional status of the organism during its early postnatal ontogenesis is the concentration of macro elements Ca, P, Na, K in blood serum at the various conditions of the environment as well as the correlation Ca:P and Na:K. The dynamics of the concentration of these elements in blood serum of calves during their early postnatal ontogenesis before and after the influence of the thermal stressor have been researched (Table 2).

Table 2. The dynamics of the saline metabolism indices in calves during their early postnatal period at influence of impulsive thermal stressor
(n=10 animals)

Age, days	Ca, mmol/l	P, mmol/l	Ca:P	Na, mmol/l	K, mmol/l	Na:K
Before the influence of stressor factor						
3	3,00±0,13	1,8±0,10	1,67±0,12	156,40±4,95	5,90±0,28	26,51±1,28
8	2,42±0,11	1,94±0,11	1,25±0,08	153,40±5,11	6,70±0,34	22,90±1,1
15	2,62±0,12	1,81±0,10	1,45±0,10	156,50±1,96	6,20±0,32	25,24±1,14
20	2,53±0,11	1,62±0,09	1,56±0,11	155,20±4,87	6,10±0,30	25,44±1,19
25	2,41±0,11	1,55±0,07	1,55±0,11	153,60±4,89	6,10±0,31	25,18±1,13
30	2,26±0,10	1,63±0,08	1,39±0,10	150,00±4,71	5,80±0,29	25,86±1,10
After the influence of stressor factor						
3	3,04±0,13	1,90±0,11	1,60±0,13	168,20±5,21	6,10±0,36	27,57±1,25
8	3,00±0,12	2,33±0,14	1,29±0,06	162,40±5,18	6,20±0,21	26,19±1,23
15	2,50±0,11	1,45±0,07	1,73±0,14	156,10±4,94	6,30±0,34	24,78±1,15
20	2,50±0,11	1,56±0,08	1,60±0,13	154,80±5,14	6,50±0,48	23,82±1,13
25	2,51±0,11	1,62±0,09	1,55±0,11	153,20±5,12	6,70±0,29	22,87±1,10
30	2,49±0,10	1,68±0,09	1,48±0,11	149,30±5,08	5,40±0,30	27,65±1,25

$P \leq 0,05$

The data from Table 2 indicate that after the influence of thermal stressor the values of Ca in blood serum of calves have been changed compared to those registered before its influence. These changes had a phasic character. The analysis of the obtained data show that in the dynamics of the maximum concentration of Ca in serum of animals after influence of the stressor have been observed at the 8-th and 25-th day at birth. The concentration of Ca have been slightly decreased yet generally corresponded to the norm.

The moderate influence of the stressor factor induced the unessential decrease of P level in blood serum compared to that before the influence of thermal stressor. The analysis of the dynamics of P concentration at 15-th day have been reduced by 20% and this tendency have been preserved during the corresponding periods of study. Moreover, during all period of study its parameters (1,45-1,90 mmol/l) at the influence of the thermal stressor have fluctuated unessentially in the limits of the norm (1,29-1,94 mmol/l).

It should be mentioned that the correlation Ca:P in calves affected by stressor have been observed in the limits of the norm during all study periods and fluctuated between 1,29±0,06 and 1,73±0,14.

The data in Table 2 indicate that the level of K in animals blood affected by thermal stressor almost have not been affected. There was a slight tendency of increase till the 25-th day at birth, while as at the 30-th day its concentration dropped out considerably (by 19,4%). The study of concentration of Na in serum during first periods of study (3 and 8 days at birth) upon influencing the stressor has revealed its increase compared to indices before application of stressor factor; yet during ontogenesis including up to 30-th day it gradually decreased reaching the indices exceeding the norm limits (139,0-148,0 mmol/l). The increased level of Na concentration in animals serum have been observed also before the application of the thermal stressor. Correspondingly, the correlation Na:K have been characterized by the

similar tendency as well as each separate element. This correlation indices have been observed higher at the 3-rd, 8-th and a 30-th days after birth.

Taken into accountant the above one can conclude that the concentration of chemical elements in animal blood being influenced by impulsive thermal stressors are subjected to phasic changes and varies in the different timeframe period of postnatal ontogenesis. It should be mentioned that cations of Na^+ prevail numerically. More quantity of Na and K in blood plasma could be found only upon ions analysis. Among bivalent ions those Ca^{++} in normal blood reaction, partially (40%) are bounded with proteins and 20% are presented in the forms of complex compounds. With aging the quantity of calcium, nondissociated compounds is decreasing from 60% up to 35%, and as of ionized one is increasing from 16% up to 30 %. During the first month the quantity of Mg^{++} is quite high, especially of the bound one, and its ionized form remains almost unchanged [3]. Yet the correlations Ca:P, Na:K indicate that these changes were mostly varying in the limits of norm except the 3-rd, 8-th and the 30-th days when the correlation Na:K have been marked by more higher values.

The other indices studied during the conducted experiences that revealed the organism functional status and development of the stress related reaction were the changes in the concentration of glucose and acid reserve in the blood of calves during their early postnatal ontogenesis while affected by moderate thermal stressor. The obtained data are presented in Figure 1 and 2.

The analysis of the obtained in the experimental way data (Fig. 1) indicates that the concentration of glucose varies in the animal blood during various stages of their early postnatal ontogenesis. Its maximum level (5,57 mmol/l) before applying the thermal stressor have been registered at the 15-th day at birth; it gradually decreased and at the 30-th day it reached the index of 4,75 mmol/l. Being influenced by the stress factor the concentration of glucose in blood serum have been decreasing at all stages of study and these changes had the phasic character. Compared to the situation before applying the thermal stressor the tendency of decrease of the glucose concentration (by 21%) have been observed at the 15-th day, and was more pronounced (by 32%) at the 25-th day. The analysis of the dynamics of the concentration of glucose in blood of animals subjected to thermal stressor reveals two picks of its changing at 8-th day (5,00±0,16 mmol/l) – its increase, and at the 25-th day (13,4±0,13 mmol/l) – a decrease compared to all other periods of experimental research.

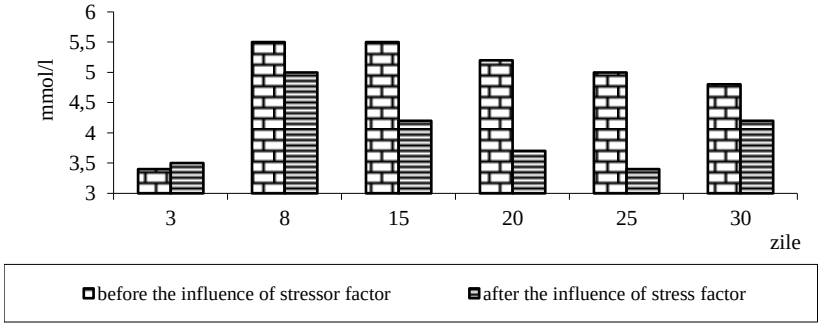

Fig. 1. The dynamics of concentration of glucose in blood plasma in calves during their early postnatal period while affected by thermal stressor

As could be observed from Fig.2, under the condition of thermal stressor the blood reaction in animals at their first days after birth turned to increased alkaline levels (at the 3-rd day by 6%, at the 8-th day by 17%) compared to the period before applying the stimuli. There was observed a decrease (by 22%) of alkaline reserve at the 8-th day of ontogenesis compared to the indices at the 3-rd day. At the 15-th, 20-th and 25-th days of thermal influencing stressor the alkaline reserve have been decreasing compared to its values registered before the application of stressor in average by 38%, then increasing again at 30-th day by 33%.

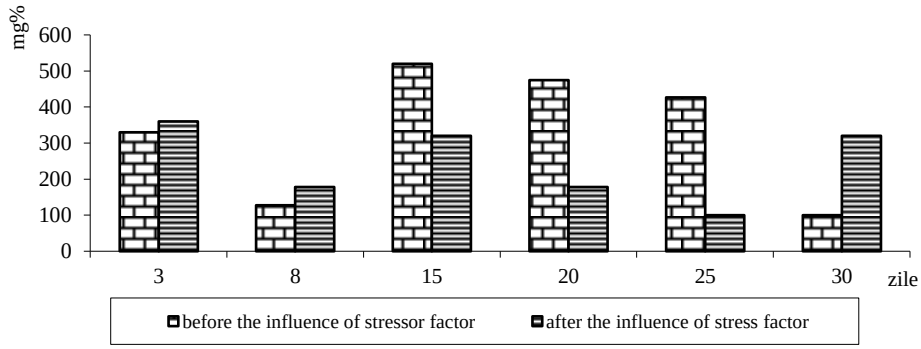

Fig. 2. The dynamics of the level of alkaline reserve in blood plasma of calves during early postnatal period while affected by thermal stressor

The obtained results show that changes in concentration of glucose and alkaline reserve in blood of the organism while affected by thermal stressor during the ontogenesis where observed being in a certain reciprocal relation, fact that have been revealed while studying the dynamics of these indices under similar conditions in swine [2]. The decrease of the levels of glucose and alkaline reserve in blood caused by the influence of the thermal stressor demonstrates the mobilization of energetic resources in organism as a stress-caused response to thermal stimuli as well as a level of their adaptation to new conditions.

The other blood indices (bactericide activity, cortisone concentration) were also researched that characterize the resistance of organism towards thermal stressor (Fig. 3& 4).

As could be observed from Fig.3, the influence of the thermal stressor provokes the increase of cortisone concentration in animal blood compared to indices before stimuli application that proves the fact that the development of the stress reaction implies intensification of suprarenal capsules and more abundant release of hormone including into the blood. The concentration of cortisone in blood have been increasing gradually up to 25-th day at birth being later on stabilized. The study of the control group animals revealed the stabilization of cortisone level starting with 15-th day at birth.

The data from literature resources [2, 8] indicate that the decrease of temperature from 21^0C up to 13^0C is accompanied by the reduction of protective resources of organism and diminishing of its resistance. In this way, the first days of postnatal ontogenesis are characterized by absent or weak humoral protective factors yet the cellular protective factors are pronounced. In this way the cellular protective factors appear earlier compared to the humoral ones; the phagocytosis mostly compensates the deficiency of humoral factors action.

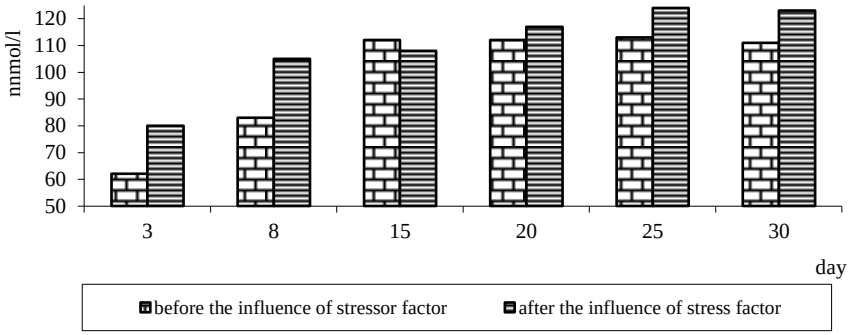

Fig. 3. The dynamics of cortisone concentration in blood of calves during their early postnatal period while affected by thermal stressor

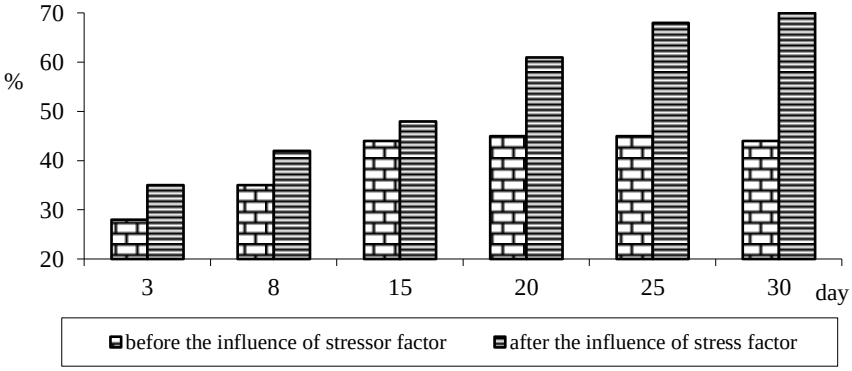

Fig. 4. The dynamics of level of bactericide activity in blood of calves during their early postnatal period while affected by thermal stressor

The bactericide activity serves also as an index of stress resistance. The data from Fig.4 indicate that the moderate thermal stressor facilitate the increase of this index compared to situation before its influence. In the dynamics the most expressed values of this index have been observed after the 25-th day at birth, and at the time before introducing stressor– after the 15-th day.

In this way, the influence of stress factor prolongs the period of adaptation of the organism compared to the control group of animals, fact proved by data on concentration of cortisone in blood and bactericide activity. At the same time it should be noted that these data confirm the increase of resistance and adaptive capacities of organism towards action of thermal stressor.

The experimental research conducted during early postnatal ontogenesis of the calves (n=10) infested with strongyles and eimeria for the purpose of determining the dynamics of the concentration of total proteins and protein particles in blood serum upon influencing these parasitic stressor has revealed the similar (to thermal stressor) effect. These modifications had the phasic character and were depending on the intensivity and extensivity of invasion. The

increased by 20% concentration of protein particles at the 7-8-th days and by 14% at the 25-30-th days and 22% decrease of β-globulins particles at 7-8-th days and 19% decrease of γ – globulins and correspondingly 20% and 12% decrease at 25-30-th days have been noticed during the early postnatal ontogenesis being compared to uninfected animals. In this way, the modifications of the protein metabolism have been studied in dynamics.

The concentration of glucose have been decreased up to 182 mmoli/l in the group of infested animals compared to 205 mmoli/l in the control group and was varying depending on the level of infestation, as well as concentration of cholesterol –from 565 mmoli/l to 466 mmoli/l. These data indicate the diminishing of resistance and adaptive capacities of the organism towards parasitic stressor.

The analysis rhythm of weight gain in calves while not affected or affected by thermal stressor during their early postnatal period has revealed that this was higher in the experimental group irrespectively of the timeframe comparatively to the animals from the control group (Fig. 5).

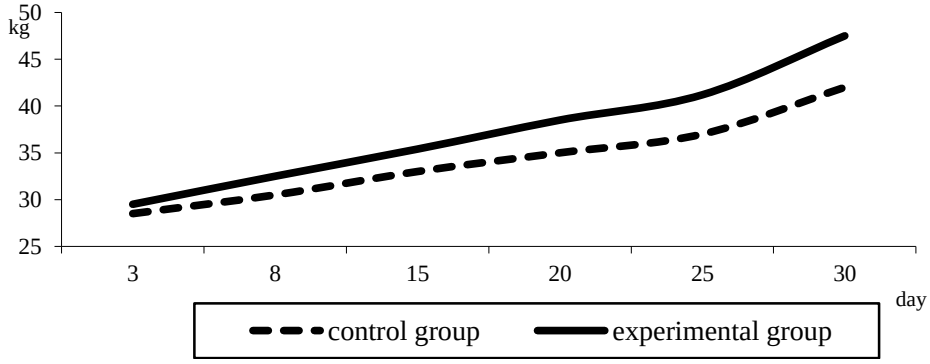

Fig. 5. The dynamics of the weight gain in calves during their early postnatal period while affected by thermal stressor

In this way, the obtained data on the influence of thermal and parasitic stressors on some indices of protein and saline metabolism, glucose, stress resistance and adaptive capacities in calves in their early postnatal period have revealed that the most labile were the following physiologic parameters: quantity of glucose, reserve of alkalines, macro elements, cortisone and bactericide activity. The most stable indicators were quantity of total proteins and protein particles.

The changes of the physiologic parameters tested during early postnatal ontogenesis in calves while affected by thermal stressor were characterized by phasic aspects, being probably determined by the periodicity of the development and growth processes, by maturing of the organs and organs systems and by the character of metabolism during various periods. The first peak of changes in the researched indices was registered at the age of 8 days at birth. This peak was manifested by decrease of glucose level, of alkaline reserve, albumins, γ-globulins, Ca, P, Na concentration in blood; by increase of the total proteins level, of α-and β- globulins, of cortisone and bactericide activity. The second peak of changes have been observed at the 25-th day at birth and was characterized by decrease of the level of glucose, of alkaline reserve, β-globulins, increase of total proteins, albumins, α-globulins, γ-

globulins, cortisone, bactericide activity, concentration of Ca and P. These changes took place in the critical periods of development characterized by imprinting, immunodeficiency status, depression of dominance and retardation.

The bactericide activity and concentration of cortisone in serum have been increased during the experimental timeframe of postnatal ontogenesis of animals affected by thermal stressor. These values serve as a test for the enhance of the stress resistance and adaptation capacities of animals.

The thermal stressor causes the changes in the metabolism that positively affects the functional status of systems and organs during the critical periods of the early postnatal ontogenesis and stimulates the rhythm of weight gain in calves.

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