CONTENTS

ADRENOCORTICAL RESPONSE IN COWS AFTER INJECTION OF ADRENOCORTICOTROPIC HORMONE

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Abstract

Adrenocorticotropic hormone (ACTH) challenge test is recognized as a method for evaluating some forms of stress. Six lacting cows, well trained to blood sampling were used for this study. Cows were randomly assigned to receive saline or an intramuscular single dose (0.5 µg/kg) of ACTH (Synacthen Depot). Blood samples (10 mL) were collected from the coccygeal blood vessels of all cows at 0 h (immediately before treatment) and every 30 min for 2 h to measure serum cortisol, glucose, creatinine and urea concentrations. Each blood collection included a separate puncture of the coccygeal blood vessels using a new needle. Respiratory frequency was measured for each cow at 0, 30, 60, 90 and 120 min. Serum cortisol concentrations of cows did not differ between treatments at the initiation of treatments; however, serum cortisol, glucose, creatinine, urea concentrations and respiratory frequency were affected by ACTH, time, and the interaction of ACTH x time. Administration of ACTH increased ($P < 0.05$) serum cortisol concentration in cows within 30 min of administration, and concentrations remained increased throught the blood sampling period. Cows that received ACTH had increased ($P < 0.05$) respiratory frequency within 30 min of administration. An increase in hypothalamic pituitary-adrenocortical activity, causes the rise of blood cortisol, indicates a physiological response to different stressors.

Key words: *adrenocorticotropic hormone, cortisol, cow.*

INTRODUCTION

Adrenocorticotropin (ACTH) is a polypeptide hormone composed of 39 amino acids that is secreted by corticotroph cells in the anterior pituitary gland. Since they share the same receptor in the adrenal gland the sequence $ACTH_{1-24}$ (Synacthen) has the same biological action as the whole molecule. Assessment of serum cortisol levels following the administration of adrenocorticotropic hormone is a recognised method for evaluating adrenal cortex function in human and veterinary clinical medicine (Verkerk et al., 1994; Pacak and Palkovits, 2001). Larger doses of ACTH are needed if the researcher wishes to maintain serum cortisol for a longer time period (Lay et al., 1996).

The aim of this study was to determine the adrenocortical response of Holstein-Friesian mix breed cows to a single dose of ACTH (Synacthen) as Tetracosactide Hexaacetate. Another objective was to examine the relationship between changes in cortisol concentrations in serum and some biochemical parameters, following activation of the hypothalamic-pituitary-adrenocortical axis.

MATERIALS AND METHODS

Six lacting cows, well trained to blood sampling were used for this study. Cows were randomly assigned to receive saline or an intramuscular single dose (0.5 µg/kg) of ACTH (Synacthen Depot 1mg/mL). Blood samples (10 mL) were collected from the coccygeal blood vessels of all cows at 0 h (immediately before treatment) and every 30 min for 2 h to measure serum cortisol, glucose, creatinine and urea concentrations with an automatic biochemistry device Cormay Accent 200. Each blood collection included a separate puncture of the coccygeal blood vessels using a new needle. Respiratory frequency was measured for each cow at 0, 30, 60, 90 and 120 min. For the statistical evaluation, SPSS 16.0 for Windows was used. The statistical analysis was made using t-test and Pearson correlations.

RESULTS AND DISCUSSIONS

Peak cortisol, the increment of peak above basal cortisol level, and the integrated cortisol response over time following ACTH treatment are used as measures of adrenocortical responsiveness (Bertoni et al., 2005).
In our study cortisol levels measured before and after ACTH injections in cows are shown in Figure 1. Compared to baseline (samples 0 min), serum cortisol increased significantly after ACTH administration to reach their maximal levels at 30 min and peak concentrations were on average 14 to 19 times greater than basal concentrations. In general, our results were similar to those observed by other authors in calves after ACTH administration (Veissier et al., 1999) and in cows during and after machine milking (Gorewit et al., 1992; Rushen et al., 2001; Negrao et al., 2004; Knights et Smith, 2007). Mean serum cortisol response was greater ($P<0.005$) in all ACTH-treated cows than in saline-treated cows at 30, 60, 90 and 120 min.

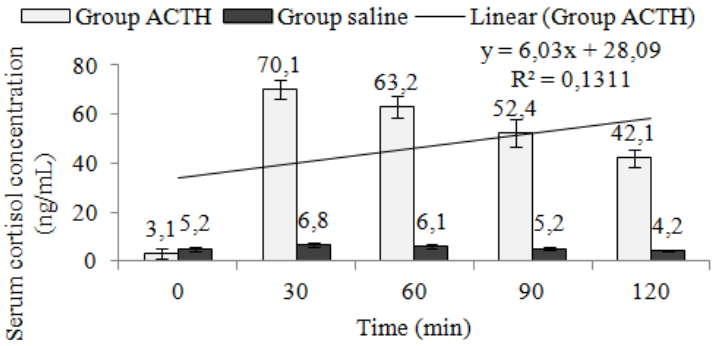

Figure 1. Mean serum cortisol concentrations for cows administered with saline (2 mL, 0.9% NaCl) or 0.5 µg of ACTH/kg BW

Typical metabolic consequence of cortisol is to increase blood sugar through gluconeogenesis (Desborough, 2000) and to increase respiratory rate (Schubert et al, 2009). Serum glucose (Figure 2) concentration of cows did not differ (P=0.48) between treatments at the initiation of treatment (time 0); however serum glucose was affected (P=0.05) by ACTH, time and the interaction of ACTH x time. Administration of ACTH increased serum glucose concentration.

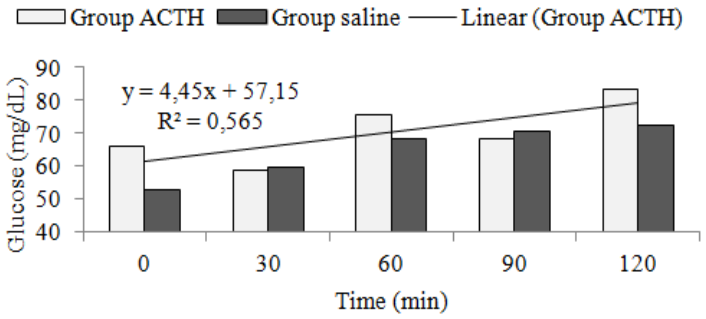

Figure 2. Mean serum glucose concentrations for cows administered with saline (2 mL, 0.9% NaCl) or 0.5 µg of ACTH/kg BW

Respiratory frequency value with (Group ACTH) and without (Group saline) ACTH treatment are summarized in Figure 3. Difference between Group ACTH and Group saline were not statisticaly significant (P>0.05). Respiratory frequency reached the maximal levels at 30 min after ACTH

administration followed by a decrease till the end of study. Study revealed a direct correlation (P=0.001, r=0.991) between serum cortisol and respiratory frequency.

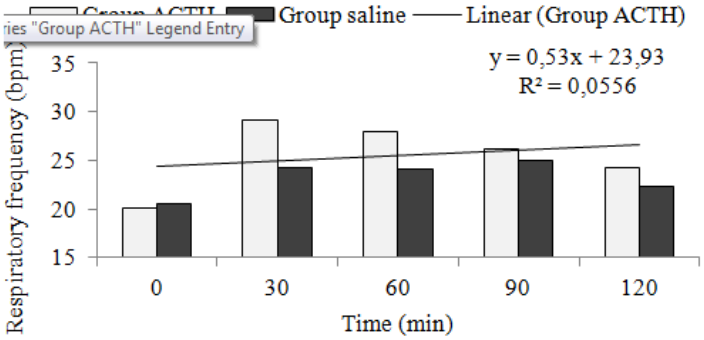

Figure 3. Mean respiratory frequency for cows administered with saline (2 mL, 0.9% NaCl) or 0.5 µg of ACTH/kg BW

Cortisol is known also to increase blood pressure, having a direct renal actions resulting in vasodilatation One possible mechanism is the reported increase in renal vascular resistance (Xe et al., 2006).

Urea and creatinine concentration are tests done to monitor kidney function. Serum urea and creatinine value with (Group ACTH) and without (Group saline) ACTH treatment are summarized in Figure 4 and 5. Difference between Group ACTH and Group saline values were not statisticaly significant (P>0.05). During the entire study, the urea and creatinine were placed in physiological limits for cows. Pearson statistical test showed no correlation between evolution of cortisol and urea.

Regarding creatinine concentration, the most elevated values were observed before the ACTH treatment (1.19 ± 0.02 mg/dL) and the lowest in those 30 min after the treatment (0.74 ± 0.09 mg/dL), but the differences were not statistically significant (P> 0.05).

Study revealed an indirect correlation (P=0.01, r=-0.933) between serum cortisol and creatinine.

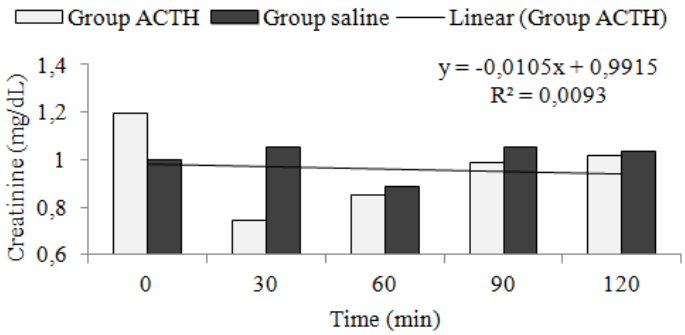

Figure 4. Mean serum creatinine concentrations for cows administered with saline (2 mL, 0.9% NaCl) or 0.5 µg of ACTH/kg BW

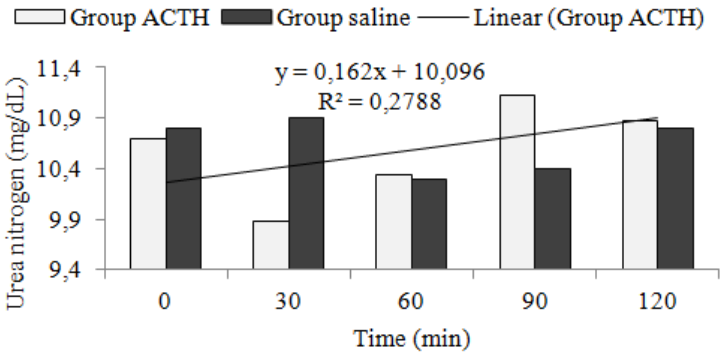

Figure 5. Mean serum urea nitrogen concentrations for cows administered with saline (2 mL, 0.9% NaCl) or 0.5 µg of ACTH/kg BW

CONCLUSIONS

Administration of ACTH increased (P<0.05) serum cortisol concentrations in cows within 30 min of administration, and concentrations remained increased throught the blood sampling period.

Cows that received ACTH had increased (P<0.05) respiratory frequency within 30 min of administration.

Study revealed a direct correlation (P=0.001, r=0.991) between serum cortisol and respiratory frequency, and an indirect correlation (P=0.01, r=-0.933) between serum cortisol and creatinine.

Administration of ACTH at the dose rate of 0.5 µg ACTH/kg of body weight will induce cortisol release and may be used to test the sensitivity of the adrenal gland during different stressors.

AKNOWLEDGEMENTS

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HEART RATE VARIABILITY FOR ASSESSING STRESS IN COWS

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Abstract

Measurement of heart rate variability (HRV) is a non-invasive technique that can be used to investigate the functioning of autonomic nervous system, especially the balance between sympathetic and vagal activity. HRV is measured by determining the constantly changing temporal distance between succeeding heartbeats (R-R intervals). Five lacting cows, well trained to blood sampling were challenged with an intramuscular single dose (0.5 µg/kg) of ACTH (Synacthen Depot). HRV was measured for each cow for 5 min, at 0 h (before treatment) and every 30 min for 2 h. HRV parameters were analysed in the time domain, frequency domain and nonlinear components. Blood samples (10 mL) were collected from the coccygeal vein of all cows at 0, 30, 60, 90 and 120 min, after the measurement of HRV, for serum cortisol. The heart rate of cows increased significantly ($P<0.05$) under the influence of Synacthen administration. All computed time domain parameters declined significantly after ACTH administration. The decline of root mean square of successive interbeat interval differences (RMSSD) was more pronounced than that for standard deviation of all interbeat interval (SDNN), after ACTH administration. The power of low-frequency component divided by power of the high-frequency band (LF/HF) increased also within 30 min of administration of ACTH. All nonlinear parameters (%DET and %REC) exhibited a significant rise 30 min after ACTH administration. Serum cortisol concentration also increased ($P < 0.05$) within 30 min of administration in cows. The nonlinear parameters were most important to indicate the level of stress in cows. HRV is a valuable physiological indicator for stress in cows.

Key words: *frequency domain, heart rate variability, nonlinearity, time domain, stress.*

INTRODUCTION

An increase in hypothalamic pituitary-adrenocortical activity, causing the rise of blood cortisol, indicates a physiological response to different stressors; consequently a measurement of serum cortisol is frequently used to study stress response (Sapolsky et al., 2000). Measures of cardiovascular parameters including heart rate (HR) have a long tradition as indicators of health and welfare in many species. The heart is under sympathetic and parasympathetic control, and HR is the effect of the non-additive regulatory functions of the interacting antagonistic branches of the autonomic nervous system (Berntson et al., 1991). As a consequence of the ongoing regulatory

mechanisms, HR is never constant but varies from beat to beat even in the absence of physical or psychological stress. This beat-to beat variability is referred to as HR variability (HRV) or heart period variability (Hagen et al., 2005).
The aim of the study was to evaluate the usefulness of heart rate variability (HRV) and its specific parameters as a new approach to assess stress load in cow.

MATERIALS AND METHODS

Five lacting cows, well trained to blood sampling were challenged with an intramuscular single dose (0.5 μg/kg) of ACTH (Synacthen Depot, 1mg/mL). The ECG with a Poly-Spectrum software was used for measurement of heartbeat activity in cows (R-R interval). The green electrode was placed on the sternum or better 2 or 3cm right from the sternum. The red electrode was placed on the left side of the cow thorax about 30 cm below the top of the thorax. The black electrode was placed approximately 10 cm above the red electrode. The yellow electrode was placed on the right side of the cow's thorax in a position similar to the red one on the left side. All electrodes need to be positioned in a way so that they can be tightly fixed. The jelly electrode liquid in the sponge of the adhesive electrode is able to penetrate the hair coat even if the cow is not clipped.
HRV was analyzed with Kubios HRV software, version 2.0 (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) from 5 min interval of each ECG recording: at 0 h (before treatment) and every 30 min for 2h. The HRV analyzed parameters were: standard deviation of all interbeat interval (SDNN); root mean square of successive interbeat interval differences (RMSSD); normalised power of the low-frequency band, boundaries 0.04– 0.25 Hz (LFnorm); normalised power of the high-frequency band, boundaries 0.25–0.58 Hz (HFnorm); LF/HF; percentage of recurrent points in the recurrence plot, i.e., vector-repetition in the multi-dimensional space (REC); percentage of recurrent points that appear in sequence, forming diagonal lines in the recurrence plot (DET).
Blood samples (10 mL) were collected from the coccygeal vein of all cows at 0 (T0), 30 (T1), 60 (T2), 90 (T3) and 120 min (T4), after the measurement of HRV, for serum cortisol.

Statistical analysis was performed with the SPSS statistics for Windows. All data were normally distributed (Kolmogorov-Smirnov test). ACTH effect was examined using ANOVA. Significance was set at $P<0.05$, and all values are given as the mean ± one standard error of the mean (SEM). The relationships between cortisol measured in serum and heart rate and HRV parameters samples were evaluated by Pearson correlation coefficients.

RESULTS AND DISCUSSIONS

Determination of hypothalamus-pituitary-adrenal (HPA) axis activity is the standard procedure to evaluate stress conditions in farm animals (Mormede et al., 2007). A well-known stimulus of HPA resulting in an increase of circulating cortisol is stress. In our study cortisol levels measured before and after ACTH injections in cows are shown in Figure 1. Administration of ACTH (0.5 μg/kg) increased ($P<0.05$) serum cortisol concentrations in cows within 30 min of administration, and concentrations remained increased ($P<0.05$) throughout the blood sampling period (120 min). Doses of ACTH have varied from 0.125, 0.25, 0.50, or 1 IU of ACTH per kilogram of BW (body weight) in pregnant Brahman heifers (Lay et al., 1996) to 1 mg/kg of B W for dairy cows (Bertoni et al., 2005). In our study, the peak plasma cortisol concentrations after 0.25 mg ACTH /500 kg BW (equivalent to 25 IU/500 kg BW) injection in the dairy cows were well comparable with the values in dairy cows reported by Bertoni et al. (2005). Also, Verkerk et al. (1994) that used very low (0.0125 mg) and relatively high doses (0.4 mg) of $ACTH_{1-24}$ determine almost the same peak level of cortisol.

Nevertheless the utilization of cortisol as an indicator of stress requires some caution for some type of stress, for the effect of circadian rhythms as well as blood sampling itself that can cause stress effects (Negrão et al., 2004). It has been suggested that the extent of corticosteroid raise may be more related to the capacity of the animal to learn about the situation than to the real aversion to it; in fact the stress input can decline very much during a prolonged stress situation due to habituation (Smith and Dobson, 2002).

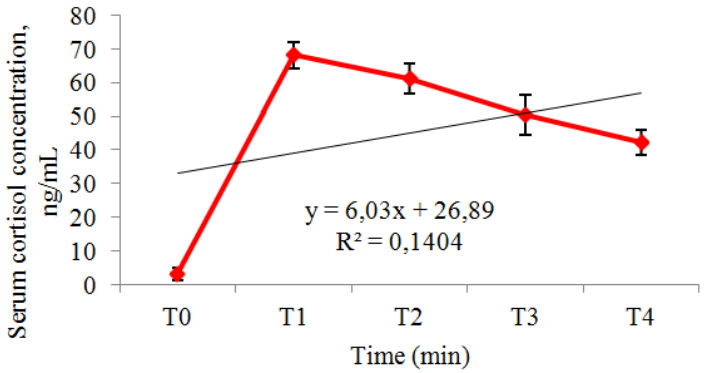

Figure 1. Mean serum cortisol concentrations for cows administered with 0.5 µg ACTH/kg BW

The heart rate (Figure 2) of cows increased significantly ($P < 0.05$) under the influence of Synacthen administration. Study revealed a direct correlation ($P=0.05$, $r=0.798$) between serum cortisol and heart frequency.
An increase in heart rate indicates increased sympathetic activity, decreased parasympathetic (vagal) activity or a combination of both (Borell et al., 2007). Rapid changes in heart rate are mostly caused by shifts in vagal regulation and occur within less than 5s while heart rate responses to sympathetic regulation occur more slowly (Hainsworth, 1995).
In our study heart rate reached the maximal levels at 30 min after ACTH administration (T1) followed by a decrease till the end of study.

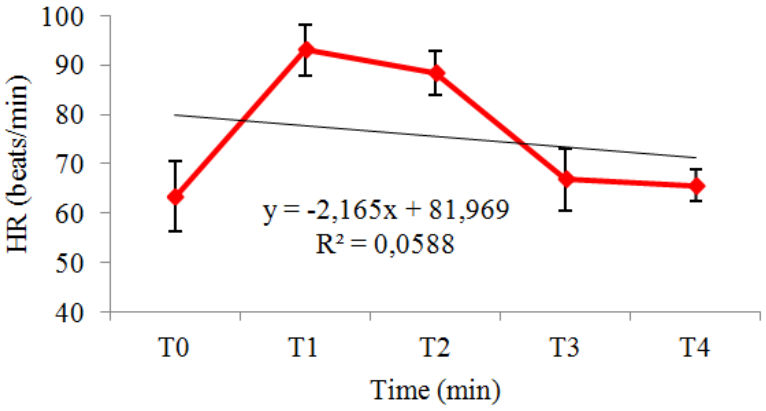

Figure 2. Heart rate at cows during the study

The R-R intervals (Figure 3) of cows increased significantly ($P < 0.05$) under the influence of Synacthen administration. Study revealed no correlation between serum cortisol and R-R intervals.
Normal R-R interval values of cows are 819±114.6 ms (Hagen et al., 2005). R-R intervals reached the maximal levels at 60 min after ACTH administration (T2) followed by a decrease till the end of study.

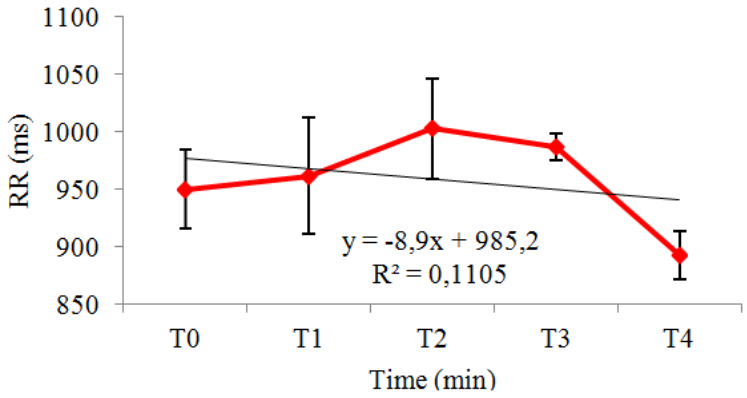

Figure 3. R-wave to R-wave interval at cows

All HRV parameters in time domain, frequency domain and nonlinear components are presented in Table 1. The time domain parameters declined significantly after ACTH administration.
RMSSD takes into account short-term, high-frequency components of HRV, strongly reflects vagal tone, and is thus highly correlated to other heart-rate variability measures such as HFnorm, LF/HF, recurrence and determinism (Hagen et al., 2005).

Table 1. Changes in HRV parameters at cows during the study

Time	Time domain		Frequency domain			Nonlinear parameters	
	SDNN (ms)	RMSSD (ms)	LFnorm	HFnorm	LF/HF	REC (%)	DET (%)
T0	39.15±8.1	14.55±3.4	83.3±4.3	16.7±5.2	5.17±4.2	45.20±4.7	93.4±0.3
T1	27.5±4.1	4.8±3.4	99.09±8.6	0.95±0.5	104.30±9.7	49.70±5.8	98.97±0.1
T2	16.2±2.1	17.4±3.1	66.9±12.1	33.1±2.5	2.02±0.7	43.23±4.4	95.2±0.6
T3	27.0±4.1	8.8±4.0	56.65±5.6	43.35±1.9	1.30±0.5	30±3.8	94.5±0.2
T4	37.1±2.2	5.4±3.2	81.85±7.3	18.15±6.1	4.50±2.1	35.52±4.1	96.1±0.3
Reference (Borell et al., 2007)	36±10.8	15±8.8	162±87	9.9±6.2	2.78±1.78	3.4±2.4	84±6.0

The decline of root mean square of successive interbeat interval differences (RMSSD) was more pronounced than that for standard deviation of all interbeat interval (SDNN), after ACTH administration. Pearson test revealed an indirect correlation (P=0.04, r=-0.842) between serum cortisol and RMSSD and no correlation with SDNN.
The higher HR after the treatment and lower RMSSD values suggest that cows were subjected to an increased level of stress. SDNN, the measure of total variability in the time domain, was not correlated with mean HR and sympatho-vagal balance LF/HF, and moderately correlated with RMSSD (P=0.048, r=0.735).
Like RMSSD in time domain, the HF band of HRV is regarded to be a good indicator for vagal activity (Friedman and Thayer, 1998). Simultaneously, we found higher values of LF, 30 min after ACTH administration. The LF oscillation of HRV has often been regarded as an accurate reflection of sympathetic activity (Malliani, 1995).
The power of low-frequency component divided by power of the high-frequency band (LF/HF) increased also within 30 min of administration of ACTH.
All nonlinear parameters (%DET and %REC) exhibited a significant rise 30 min after ACTH administration. Pearson test revealed a direct correlation (P=0.05, r=0.712) between serum cortisol concentration and %DET. Mohr et al. (2002) suggest that %DET indicates quantitative changes in the level of stress load.
The results of this study indicate that HRV is an indicator for the assessment of welfare at cows. Further studies are required and should consider intraindividual differences in the HRV exposed to various procedures, and large population of cows recordings to better determine the predictive value of HRV in the identification of individual vulnerability to stress.

CONCLUSIONS

The higher HR and the lower HRV after ACTH treatment, suggest a clear shift of the sympatho-vagal balance towards the sympathetic tone.
Pearson test revealed a direct correlation (P=0.05, r=0.798) between serum cortisol concentration and heart frequency.
The decline of root mean square of successive interbeat interval differences (RMSSD) was more pronounced than that for standard deviation of all interbeat interval (SDNN), after ACTH administration. The power of low-

frequency component divided by power of the high-frequency band (LF/HF) increased also within 30 min of administration of ACTH.
All nonlinear parameters (%DET and %REC) exhibited a significant rise 30 min after ACTH administration.
Pearson test revealed an indirect correlation (P=0.04, r=-0.842) between serum cortisol concentration and RMSSD, and direct correlation (P=0.05, r=0.712) with %DET.
HRV is a valuable physiological indicator for stress in cows.

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COMMENTS ON HISTOPATHOLOGICAL CHANGES IN RABBIT LIVER WITH EIMERIOSIS

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Abstract
The study was conducted on a total of 117 White New Zeeland breed rabbits, which were identified by feces examination with varying degrees of infestations with Eimeria sp., Eimeriosis hepatic lesions being identified in 20 of them.
Specific lesions were hepatic hypertrophy with presence of necrotic miliary nodular centres, vesicular looking angiocolitis, apostematous hepatitis and cirrhosis. Histopathological examination pursued in particular the consequences of sexual ongoing phase. Biliary ducts were dilated with hyperplastic epithelial reaction and the formation of papillary reactions with the presence of asexual and sexual stages of development.

Key words: *angiocolitis, eimeriosis, hepatitis, rabbits.*

INTRODUCTION

Considered of lesser extent than intestinal Eimeriosis, hepatic Eimeriosis caused by *Eimeria stiedae* is common worldwide and affects domestic rabbits and wild ones equally and possibly other leporidae. It is a serious form of coccidiosis which particularly affects rabbits older than 2-3 weeks.
Rabbits are responsive only from the 16 th day of life and at the age of 6 months are totally unresponsive due to an immunity installed after permanent contact with oocysts.
Immunity is installed in 21 days after infection. Full immunity under natural conditions is set after 3 months (Chowdhury and Fraser, 2008).
Main pathological lesions are angiocolitis, diffuse cirrhosis, nodular hepatitis, compression atrophy of hepatocytes around the bile ducts affected (Militaru et all., 1997).
Hyperplasia of bile duct epithelium is accelerated along sexual phase and epithelial damage and desquamation will prevail during the invasion of merozoites and schizonts (Chowdhury and Fraser, 2008). In histopathological analysis, severe congestion and dilation of central veins were observed and the endothelial lining were ruptured (Naimi et all., 2012).

Severe hyperplasia of the lining epithelium of the portal areas were detected forming fingerlike projections in the lumen of the bile duct (Ebtesam and Mathal, 2008).
Sinusoids were also congested in this connection and dilated with haemorrhagic spots.
Multiple areas of coagulative necrosis of hepatic cells surrounded with inflammatory cells also happened. In addition, cellular infiltration of lymphocytes in the infected liver was noticed (Zerrin and Yesari, 2007).

MATERIALS AND METHODS

The study was conducted on a total of 117 rabbits of New Zealand White breed (medium breeds) and half-breed from research stations, identified with parasitic infestations of *Eimeria* sp. in 97 (82.9%) of the total number of rabbits examined; the diagnosis was established by identifying oocysts of *Eimeria* sp. uninfested. Coproparasitological examination was performed through qualitative methods-Willis amended by Lungu and quantitative.
In heavy infestations or clinical, rabbits were slaughtered and morphopathological examination was performed upon the liver and biliar ducts, followed by histopathological examination after sampling the specific lesions, fixed in neutral formalin saline and further processed for inclusion in paraffin. Paraffin blocks were sectioned at 6 μm, stained preparations were obtained by the trichromatic method of Mallory, examined and microphotographied.

RESULTS AND DISCUSSION

In intestinal Eimeriosis, a distinction depending on the species couldn't be done, but *Eimeria stidae* species responsible for Eimeriosis liver could be differentiated due to the particularities of size (31-42 x 17-25 μm), shape (ellipsoidal, with one pole elongated), colour (pink orange) and layout of sporont (fig.1). Specific lesions were observed in the liver parenchyma and bile ducts caused by *Eimeria stidae* in Eimeriosis liver disease. In the center of the necrotic focus one could observe the presence of „old oocysts" with altered the absence of sporont, changed shape and altered wall structure.
Histopathological lesions in the liver showed the following symptoms: severe congestion and dilation of central veins, rupturing of the endothelial lining, hyperplasia of the epithelial lining of portal areas with finger-like

projections in lumen of the bile duct, congestion and dilation of sinusoids with haemorrhage areas (fig.2).

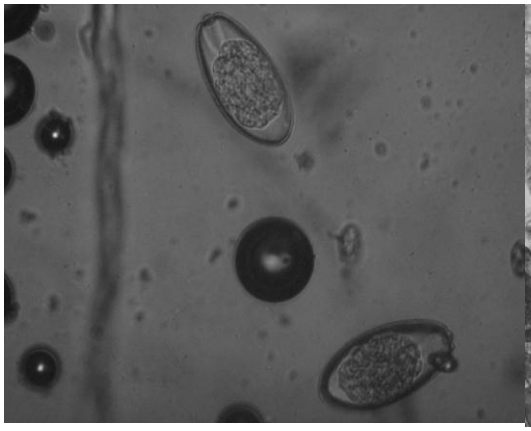

Fig. 1- Eimeria stiedae - unsporulated oocyst (20x)

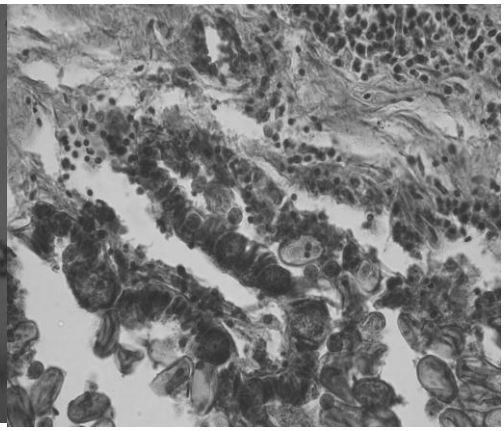

Fig. 2 – Hiperplastic bile ducts; Col. Mallory trichromic; Ob. 40x

Multiple areas of coagulative necrosis of hepatic cells surrounded with inflammatory cells were found.
On the surface of the parenchyma one could observe white-yellowish areas with irregular shape with sizes ranging from 0.3 cm to 1.5 to 2 cm (fig.3).

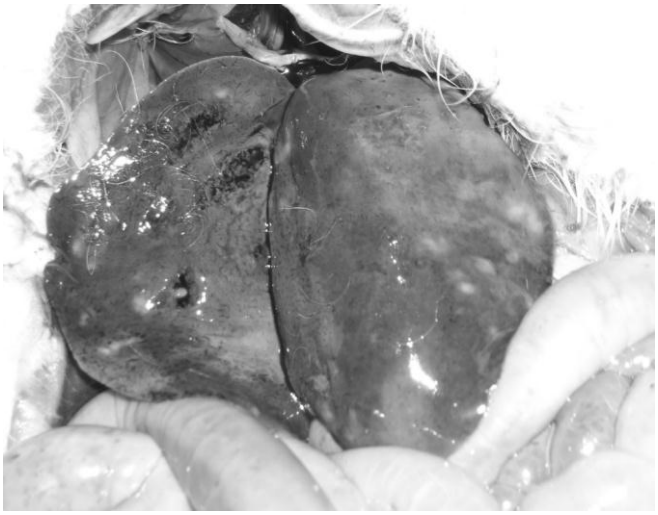

Fig. 3 – Nodular necrotic hepatitis

These are centres of necrosis bounded by a congestive-haemorrhagic edge produced by trophozoites and merozoites during asexual multiplication in the destruction of hepatocytes and sinusoidal capillaries rupture.
The bile ducts could be observed with necrotic lesions of diffuse or nodular type and colonies of *Eimeria*.
In the bile ducts located within bile-port space, hyperplastic reactions both intraepithelial and in the lumen occurred with presence of coccidia (fig.4).

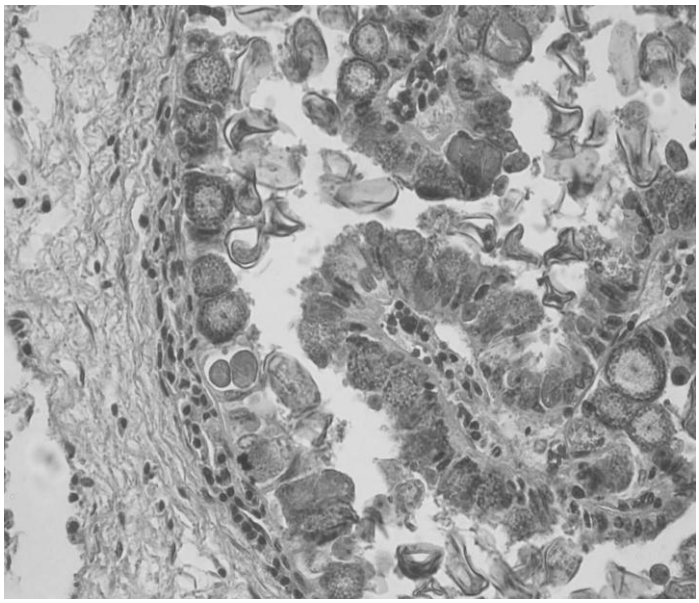

Fig. 4 – Angiocolitis; Col. Mallory trichromic; Ob. 20x

Hyperplastic bile ducts were surrounded by a conjunctival reaction type, accompanied by an inflammatory reaction of histiocytic lymph type.
The bile ducts were clearly dilated with hyperplastic reaction of simple prismatic epithelium, with formation of papillary reactions and the presence of asexual and sexual stages of development (micro- and macrogametocytes and oocysts in training).
In the lumen of the bile ducts there are numerous oocysts with thin shell of around 50 μm with a specific structure of *Eimeria stiedae* (fig.5).

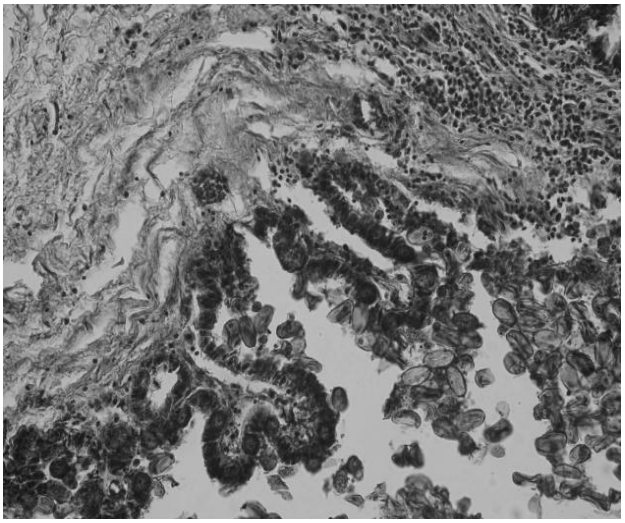

Fig. 5 – Angiocolitis with hyperplastic reaction of simple prismatic epithelium, with formation of papillary reactions; Col. Mallory trichromic; Ob. 20x

The liver lobules outlined necrotic lesions due to traumatic mechanical action of asexual forms (schizont and merozoite) and the destruction of liver parenchyma (Remack cords and sinusoidal capillaries). In the center of the necrotic area one can observe the presence of 'old' oocysts, with altered structure, characterized by the absence of sporont, changed shape and altered wall structure (fig.6).

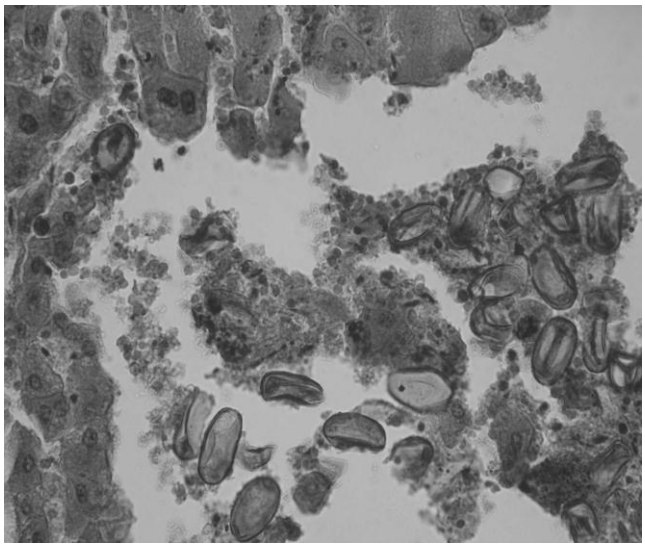

Fig. 6 – Necrotic hepatitis; Col. Mallory trichromic; Ob. 40x

CONCLUSIONS

The intensive growing conditions require the emergence and evolution of parasitary diseases where contributory factors (humidity 43-52%, temperature 17-20°C), feeding and manure disposal systems affect the development of infested forms (oocystes, eggs) in hepatic - Eimeriosis, intestinal Eimeriosis and some nematodosis – *Passalurus* sp.
Infestation with *Eimeria* sp. was identified in 97 (82.9%) of the total number of rabbits examined, 150, the highest incidence being in rabbits of 3-6 weeks which were identified in 82% of specimens examined.
Clinical expressions and lesions were evident in the liver Eimeriosis caused by Eimeria stiedae, characterized by necrotic hepatitis and angiocolitis in diffuse outbreaks.
Histological lesions are evident in hepatic Eimeriosis, in which bile duct hyperplasia and the occurrence of papillary reactions at the epithelial level with the presence of asexual stages of development could be noticed.

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CLINICAL, RADIOLOGICAL AND MORPHOLOGICAL ASPECTS IN CHYLOTHORAX IN CATS – 5 CASES REPORT

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Abstract

Chylothorax represents the acumulation of lymph (chyle) within the pleural space. In the radiology service of FMV Iasi, during the last year (2012), 13 cases of pleural effusion were radiologicaly diagnosed.
Thoracocenthesis was performed on the cats and macroscopical, cytological and microbiological exams followed.
Five cases were diagnosed with chylothorax. The etiological diagnostic was established: one case was trauma determined, two of them were caused by mediastinal lymphoma, one was congenital recurent chylothorax and one was caused by cardiac insufficiency.

Key words: *chylothorax, lymphosarcome, pleural effusion, thoracocenthesis.*

INTRODUCTION

Chylothorax represents the acumulation of lymph (chyle) within the pleural space. It results from the leakage of lymph from disrupted, obstructed or abnormal thoracic lymph channels (Fossum, 1993).
Common causes are: thoracic duct rupture (traumatic), lymphangiectasia of the anterior portion of the thoracic duct due to compresion from anterior mediastinal masses on anterior vena cava, cardiac insufficiency (dirofilariasis, cardiomyopathy), hyperthyroidism and anterior vena cava thrombosis (Fossum, 1993; Leib, Monroe, 1997). Other causes are mediastinal lymphome, torsion of a lung lobe, trauma, idiopathic or congenital
Clinical symptoms are: respiratory distress, cough, fainted heart sounds, fatigue and polydipsia. When the chylothorax is old, weight loss and anorexia are present (Leib, Monroe, 1997).

MATERIAL AND METHOD

In the radiology service of FMV Iasi, during the last year (2012), 13 cats were radiologically diagnosed with pleural effusion. The pacient's age varied from 6 months to 17 years. The symptoms were those of respiratory distress (tachipnea,

abdominal and open mouth breathing), weight loss, fatigue, none of them presented polidipsia.
Clinical examination revealed the loss of the thoracic elasticity, increased matitee on thoracic percussion, decreased lung sounds ventrally and increased broncho-alveolar sounds dorsally and bilaterally muffled heart sounds.
The thoracic x-ray examination was performed in left and right lateral incidence and dorso-ventral incidence, trying to keep the pacient as confortable as possible, knowing that respiratory distress may increase when the animal is stressed, especially in lateral recumbency.
The presence of various quantities of liquid was diagnosed by the large area of radioopacity that covered the lower part of the thorax in lateral incidences, covering the heart silhouette and different parts of the lung lobes, displacing the trachea dorsally and pressing the remaining normal lung lobes, modifying their possition and making them look like leaves, with round edges. In dorso-ventral position, the radioopacity – presence of liquid – surrounded the lungs, displacing them from the thoracic wall.
Thoracocentesis was performed on the cats, uni- or bilaterally, and macroscopic and cytological exams followed. Chylothorax was diagnosed in 5 cats.

RESULTS

In all the 5 cases of chylothorax, the radiographs were made before and after the fluid drainage, as seen in Fig.1 and 2. bellow. This helps evaluating the pulmonary tissue, respiratory capacity heart shape. The Fig. 3 presents the same case, after complete drainage and specific treatment.

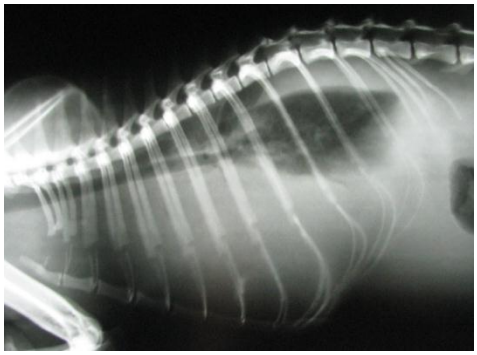

Fig. 1.—Rx lat, cat, plural and peritoneal effusion (before drainage)

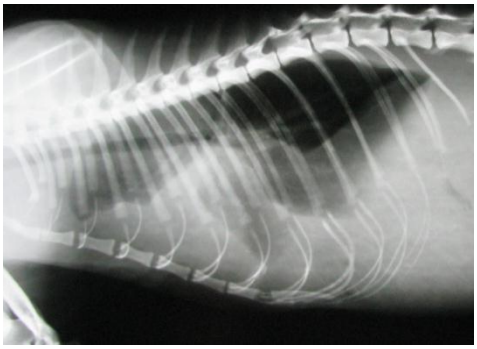

Fig. 2.—Rx lat, cat, plural and peritoneal effusion (after partial drainage)

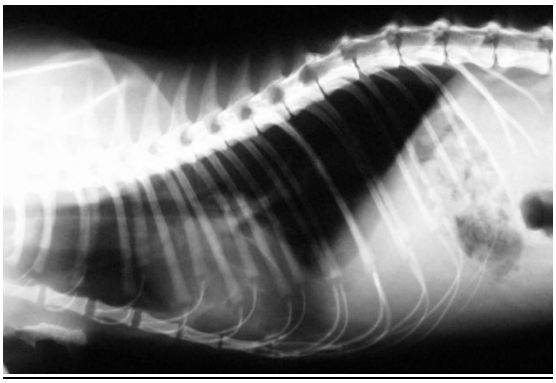

Fig. 3.—Rx lat, cat, plural and peritoneal effusion
(after complete drainage and treatment)

In the first image the small respiratory space is evident, the collapsed lungs, the elevated trachea, the radioopaque liquid that covers everything, even the shape of the heart. In the second image, the lungs are seen better, closer to the right position, the heart appears enlarged, still masked by the liquid and the trachea displaced dorsally.

The last image shows an almost normal appearence of the thorax, the trachea was less elevated, the heart was enlarged and the fissure lines between the lung lobes were present. At the moment of the first radiography, the nature of the collection wasn't suspected.

Pleural effusion was drain in every case, as much as possible. For this action, in the usual election place (7^{th} or 8^{th} intercostal space, on right, at the costo-condral junction, and the 6^{th} or 7^{th} intercostal space on left) the hair was shaved and the asepsie was made with alcohol or betadine.

Fig. 4.—Drainige of pleural fluid on the left side

The position of the pacients was on ventral recumbency, as confortable as possible, as seen on Fig. 4. At need, a light sedation was made.

The toracocentesis was performed using 20G or 22G intravenous catethers, depending on the density of the fluid. After the catether is placed, a sterile 2 ml seringe is drain out, then a 10 or 20 ml seringe can be used to drain the rest of the liquid.

Macroscopical examination of the fluid and refractometry vas performed.

The macroscopic appearance of the effusion and the quantity demonstrate a variety of colors (Meyer, Franks, 1987), as seen in Fig. 5-8. Chylothorax varied from milk white to pale rose and almost red. That depended on the etiology and the time of the accumulation. In traumas cases followed by the rupture of the thoracic channel, other vassels breake too. So blood was present in the effusion too. In lymphoma, cardiac insufficiency and in congenital chylothorax, the macroscopic aspect was dense white, pale white or pale rose.

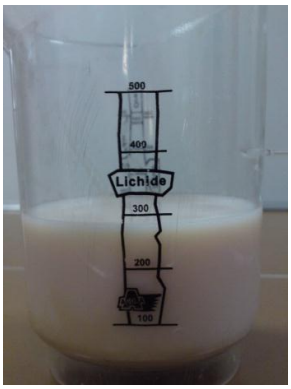

Fig. 5.— Important quantity (300 ml) of milky white liquid—chylothorax.

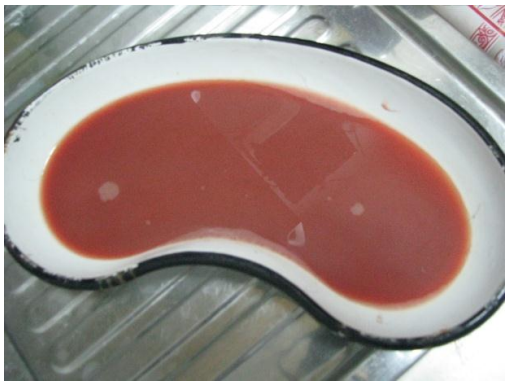

Fig. 6.— Large quantity (250 ml) of red, dense liquid—chylothorax with large quantity of blood cells.

Fig.7.— 200 ml of rose, dense fluid—chylothorax with small quantity of blood cells.

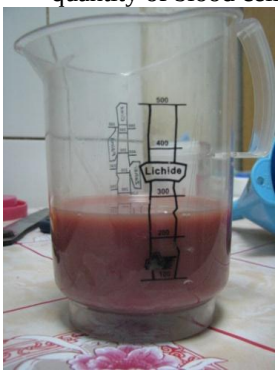

Fig. 8.— Important quantity (250 ml) of dense, almost red liquid—chylothorax and large quantity of old bood cells .

Cytological examination - during the punction of the pleural space, a sterile liquid sample is being prelevated on an EDTA tube (Fig. 9). The samples are cetrifugated, smeares are made and colored MGG and they are microsopicaly examined (Fig. 10 - 12)

Fig. 9.—Chylous effusion, lactescent aspect

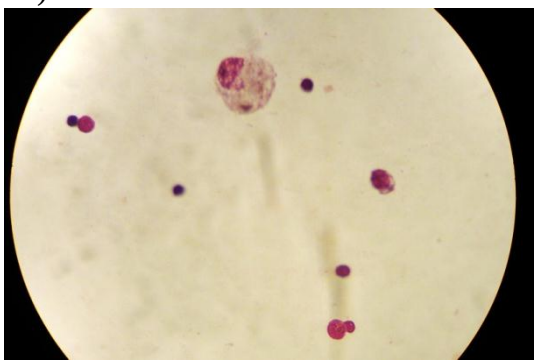

Fig. 10.— Cat. Chylous pleural effusion. Small lymfocites , G.N Macrophages. Col, MGG x 1000.

The EDTA samples are being sent to the cytology laboratory, were there are analysed. The samples were centrifuged, smeares were made from the sediment and coloured MGG, then examined under microscope, first using small lens (x10, x40) and later on immersion lens (x100).

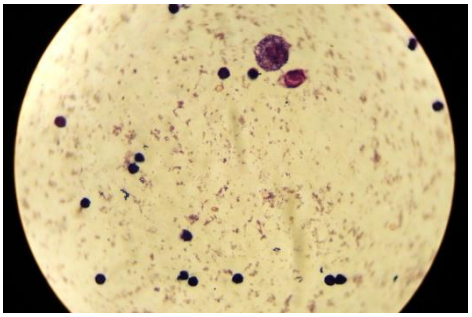

Fig. 11. - Cat. Chylous pleural effusion Small lymfocites, G.N.Macrophages, rare eritrocytes. Col, MGG x 1000.

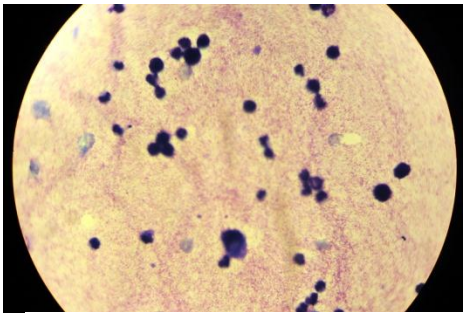

Fig. 12. - Cat. Chylous pleural effusion. Isolated and agglomerated lymfocites, G.N.Macrophages. Possible lymphoma. Col, MGG x 1000

Ultrasound examination of the mediastinum in search of mediastinal masses and the cardiac examination were performed. In one case a left heart dilatation was found.
Using all these methods, an ethiological diagnostic was established in each case. This is very important for the therapeuthic protocole that is to be followed.

CONCLUSIONS

Five cats were diagnosed with chylothorax, by the use of radiography, pleural punction, macroscopical evaluation and cytological examination.
The ethiology of these cases was established: one traumatic old chylothorax, two mediastinal lymphomas, one left heart cardiomiopathy and one congenital, recurent chylothorax, remittent under treatment.
The drainage of the pleural effusion is very important for the good quality radiography, the fluid analysis and for the releave of the pacient.

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HORNER`S SYNDROME- EYE OR NEUROLOGICAL DISEASE?

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Abstract

The interruption of sympathetic innervations at the head level is the main cause that produces the Horner`s syndrome. The damage of the nerve fibres may occur: central, preganglionic or postganglionic.
Most patients were sent for an ophthalmologic examination as a result of a sudden attack, often described by owners as "closed, paintful eye".
The purpose of this paper is to establish an etiologic differential diagnosis protocol in Horner`s syndrome.
The patients examined in the Surgery Clinics of the Faculty of Veterinary Medicine Bucharest presented enophthalmos, upper eyelid ptosis, palpebral slid reduction, third eyelid protrusion and miosis. The ophthalmologic examination was performed by direct methods and indirect methods, as Schirmer test, fluorescein test and the pupil size in the darkness. For the most patients, the disease started suddenly, with epiphora and very painful eye. Only for few of them, the onset was sudden and no ocular pain or epiphora were mentioned.
The results of the tests showed normal values for the Schirmer test, miosis, with negative response of the pupil in the darkness. The fluorescein test was negative and the internal face of the third eyelid presented no foreign bodies. In this cases, the etiology of the syndrome is idiopathic or secondary to media otitis, frequently subclinical.
It was achieved a diferential diagnosis between Horner`s syndrome and the superficial or deep corneal wounds, when the fluorescein test is positive and there were highlighter foreign bodies at the internal third eyelid.

Key words: *Horner`s syndrome, miosis, protrusion, otitis, foreign bodies.*

INTRODUCTIONS

Horner`s syndrome is characterized by unilateral protrusion of the nictitants, ptosis, anisocoria and miosis.
The most common causes of this syndrome include otitis media, preganglionary injuries as trauma or neoplasia, and idiopathic.

Horner`s syndrome results from impairment of ocular sympathetic innervations. The sympathetic innervations of the eye consists of a long, three neurone, pathway, extending from the diencephalon, through the timpanic bula, to the eye.

"Loss of sympathetic innervations causes a lack of tone in the orbital smooth muscle, with the result that the eye retracts slightly, producing enophtalmos. Loss of innervations to the muscle of the upper eyelid (Muller`s muscle) and sympathetically innervated tissue in the lower eyelid results in narrowing of the papebral fissure and incomplete elevation of the upper eyelid or ptosis. Lack of sympathetic tone and enophtamos result in protrusion of nictitants. Reduction of normal sympathetic tone to the iris dilator muscle result in the anisocoria and miosis in the affected eye."

Protrusion of the third eyelid and miosis are usually the most evident and bring the patient to medical attention.

Many patients present, besides these two symptoms, blepharospasm, epiphora, photopfobia and intense pain. The examination of the affected eye can be very difficult and the lesion is most often unilateral.

MATERIALS AND METHODES

Ophthalmologic examination of the patients who came in the Surgery Clinics of the Faculty of Veterinary Medicine Bucharest, during January 2010 - October 2012, presenting enophthalmos, third eyelid protrusion, miosis, palpebral slit reduction and signs of ocular pain performed by direct methods, as inspection, and indirect methods.

We examined 3 cats and 4 dogs.

In most patients, clinical signs appeared spontaneously, after a long walk outside. They presented pronounced miosis, third eyelid protrusion, with redness and edema, epiphora, photophobia and blepharospam (Figure 1). Because of the intense pain, the eye examination was done only after local anesthesia with benoxicaine drops.

The indirect methods performed are: Schirmer test, the examination of the internal face of the third eyelid and the fluorescein test.

In patients with no pain, to whom, the clinical signs occurred gradually, the eye examination was made easily. It was performed the test for a papillary near response (Figure 1). Schirmer test and the fluorescein test were also made. We examined the ear with the otoscope.

RESULTS AND DISCUSSIONS

Table 1. Indirect methods results

	Schirmer test	Fluorescein test	Observations	Diagnosis
Eye in pain	>20mm/min	positive	Internal face of the nictitant: foreign body	Superficial erosions
Eye without pain	<20mm/min	negative	Otoscope: otitis media	Horner syndrome

In patients with pain, fluorescein test is positive and revealed superficial lesions of the cornea at the inner canthus. The aspect is similar to the mark left by the windshield. (Figure 2)
The Schirmer test values were bigger than 20mm/min. On the internal face on the nictitant membrane we found foreign bodies. (table 1, figure 2)
In Horner`s syndrome, otoscopy showed the presence of otitis media with mucosa inflammation . The patients kept the head tilt on the same part as the affected ear (figure 3). Miosis persisted in dark (figure 4).

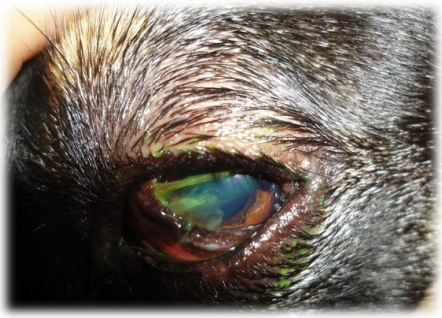

Figure 1.
Foreign body at the internal surface of the third eyelid (see the arrow)

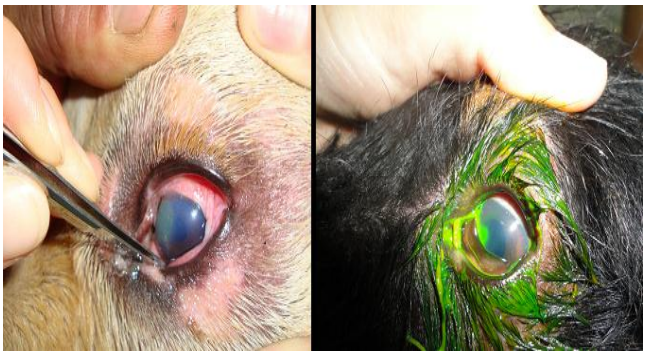

Figure 2.
Left eye: examination of the internal face of the nictitant.
Left eye: corneal superficial wound. Fluorescein test positive

Figure 3.
Left eye: Horner`s syndrome, secondary to chronic otitis. (note the enophtalmia, miosis, protrusion of the third eyelid and anisocoria)

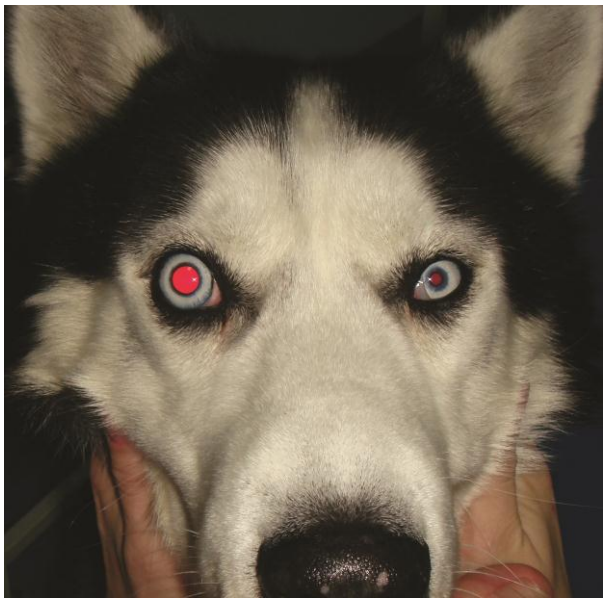

Figure 4.
Left eye: Horner`s syndrome, secondarytosubclinical otitis, in a Husky, 11 years old (note the anisocoria, enophtamia)

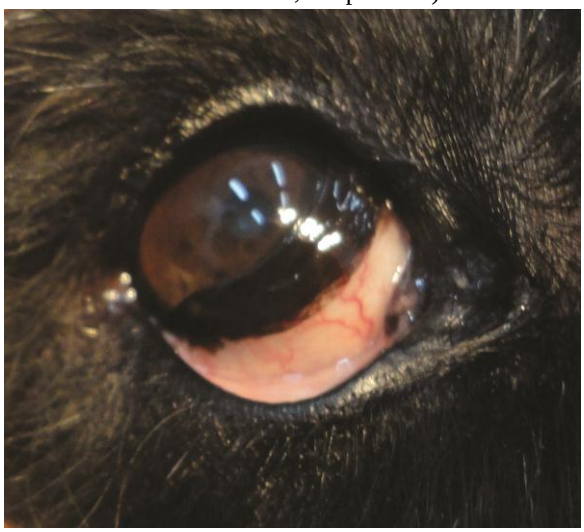

Figure 5.
Right eye: Idiopatic Horner`s syndrome in a Golden Retriever, 3 years old (note the miosis and the protrusion of the third eyelid)

CONCLUSIONS

Miosis and protrusion of the nictitant membrane can be a commune sign for many ocular diseases.

Horner`s syndrome often appear as a secondary manifestation of suclinical otitis media.

Incomplete examination of the eye may mask the presence of foreign bodies on the internal face of the third eyelid.

Treating the otitis is the most important things, Horner`s syndrome being a neurological disease appears like ophthalmological disease with full recovery in postganglionic lesions.

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STUDY OF SEASONAL DYNAMICS IN RESPIRATORY MICROBIAL FLORA IN EXTENSIVELY RAISED GOATS

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Abstract

An accurate evaluation of the seasonal dynamics of respiratory microbial flora in extensively raised goats represents the first step in early identification of potentially highly pathogenic bacteria in this species. The aim of the study was to monitor the seasonal influence on the bacterial flora of the animals, and therefore a comparative evaluation of changes during winter and spring seasons. The research was carried out on 20 healthy goats, raised under extensive conditions in Transylvania. Nasal discharge samples were cultured on simple media for isolation, and then identified by use of API 20 E and API 20 Staph biochemical tests. During the winter season, out of the total isolated bacterial strains, 26.8% to belonged to E.coli, 14.6% to each Enterobacter aerogenes and Erwinia spp, 12.1% to each Klebsiella pneumoniae and Enterobacter cloacae, 9.7 % to Staphylococcus xylosus and 4.8% to each Chryseomonas luteola and Staphylococcus lentus. During the spring season, changes in both percentages and isolated species soocred. The highest percentages were present in Enterobacter aerogenes (30.5%), followed by each Staphylococcus xylosus and the newly isolated Serratia fonticola (11.2%), sharply decreased E.coli (8.3%) and Erwinia spp. (5.5%), but increased Chryseomonas luteola (8.6%). Rahnella aquatilis (2.7%), Serratia ficaria (5.5%), Serratia liquefaciens (5.5%), Serratia marcescens (2.7%) and Serratia odorifera (8.3%) were present only in the spring season. The bacteria isolated from clinically healty goats could have a highly pathogenic character under critical/stressfull circumstances, which draws the attention to the importance of early identification of pathogens and the acurate sanitary management of the heard

Key words: *bacterial pathogenicity, goats, respiratory system*

INTRODUCTION

In present economical conditions Transylvanian farmers prefer goats raising to other livestock for their major benefits in meat and milk production (Peacock, 2005). Farmers are trying to increase quantitative and qualitative productions by applying god management practices that include rational feeding good housing conditions and disease prevention. Of all of goats maladies, those affecting the respiratory system can cause substantial loss through high morbidity and mortality. Most of the infectious agents that cause respiratory disease are usually common

inhabitants of the respiratory system (Emikpe, 2009). The pathogenic role of commensal organisms can be high considering that these animals sometimes are exposed to poor weather conditions. Furthermore, the carrier estate for various bacteria could have a major economic impact on productivity by decreasing milk production and also can affect the reproductive performance as well by obtaining undeveloped offspring. Although in Romania the goat population is increasing lately, the research directed towards the microbial flora carried by these animals is poor documented. Furthermore, the increasing imports of goats in order to improve the genetic potential of local breeds could also rise the variety of microbiological populations in local animals.
This study aimed to isolate and characterize bacteria from the nasal passageways of clinically healthy goats of local breeds (Carpatina and Alba de Banat) that are raised in extensive system in two antagonistic seasons like winter and late spring. The purpose of the research was to analyze and compare possible differences in bacterial strains isolated during this two seasons and there pathogenic role.

MATERIALS AND METHODS

The survey was carried out on total of 20 clinical healthy goats raised in extensive conditions in Tuşnad village, (Harghita County). From the studied animals were taken 20 samples from the upper respiratory tract in winter (January) and the same number of samples in late spring (May). Samples were collected using sterile swabs with transport medium. The samples were cultivated on simple broth for 24 h at 37^0C and inseminated on glucose agar plates for another 24h at 37^0C, to obtain isolated colonies. For a better identification the isolated colonies were inoculated also on MacConkey agar and Chapman agar. The isolates were identified by the use of API 20 E testing system for *Enterobacteriaceae* family and API 20 Staph. for bacterial colonies raised on Chapman medium (selective medium for *Staphylococcus spp*.).

RESULTS AND DISSCUTIONS

Even thought the samples were taken from clinical healthy goats we have isolated a large number of bacteria, most of them with increased pathogen potential The bacteria isolated in the winter and spring and there percentage are presented in the following table:

Table1. Number of bacterial strains and there percentage

Bacterial strains isolated from upper respiratory tract	Number of bacterial strains and there percentage			
	Winter	Bacterial organisms/total isolates (%)	Spring	Bacterial organisms/total isolates (%)
E coli	11	26.8	3	8.3
Erwinia spp.	6	14.6	2	5.5
Enterobacter cloacae	5	12.1	-	-
Enterobacter aerogenes	6	14.6	11	30.5
Chriseomonas luteola	2	4.8	3	8.6
Klebsiella pneumonie	5	12.1	-	-
Rahnella aquatilis	-	-	1	2.7
Serratia ficaria	-	-	2	5.5
Serratia fonticola	-	-	4	11.2
Serratia liquefaciens	-	-	2	5.5
Serratia marcescens	-	-	1	2.7
Serratia odorifera	-	-	3	8.3
Staphilococcus xylosus	4	9.7	4	11.2
Staphilococcus lentus	2	4.8	-	-
TOTAL	41	100	36	100

Escherichia coli it is a bacteria frequently involved in enteric disorders both in animals and humans, in most cases rebellious at treatment with ordinary antibiotics. The most severe cases are the ones of which the infection with pathogenic strains of *E.coli* afects the youg kids, because the survaving animals are phisicaly and economicaly dameged (Onderka and Wishart, 1988). The number of bacterial strains of *Escherichia coli* isolatede in winter is higher than in spring, one pssible cause can be the housing conditions, goats are restricted to limited space in barns during winter, but in spring are free on pasture and have more open space.

Klebsiella pneumoniae it is a world wide spred bacteria that can be resposable for artritis, and septicemic outbrecks in kids and newborns (Bernabe et all.1998) but is more frequent resonsable for pneomonya and necrotic damege of the lungs (Răpuntean et all., 2005). *Enterobacter aerogenes* and *Enterobacter*

cloacae are most frequntly responsable for enteric, respiratory and urinary infections (Lederberg et all., 2000).

Erwinia spp. are bacteria that are more common associated with plants rot but certain species are involved in pathogenic activity especially in patients with immunodeficiency. To explain the pathogenic role of *Erwinia spp* in animals, scientists made different genetic test involving genes sampling from *E.coli* and other bacteria from *Enterobacteriaceae* family (Chatterjee et all., 1972). The increased number of *Erwinia spp.* isolated in winter can be explain because in this season animals receive supplements of potatoes, carrots that can be contaminated with this bacterial strains.

Staphylococcus xylosus is a comensale bacteria on the surface of the skin common to hummans and animals (Schleifer et all 1975). An major fact that can not be ignored is that a few strins of *Staphylococcus xylosus* are responsable for opportunistic infections in hummans and animals (cows, shepp and lactating goats) (Pinna et all., 1999).

Staphylococcus lentus is a bacteria that can be associated with skin infectios in sheep, goats and swine (Quinn et all., 2003).The lactating goats are sensitive to the pathogenic mechanism of the *Staphylococcus lentus* strains that produce mastitis (Schleifer et al., 1975).

Chryseomonas luteola has an unclear habitat but it is frequently found in water, soil and other dump environments. Previous studies showed that *Chryseomonas luteola* may cause primarly septicemia, meningitis, osteomyelitis endocarditis, peritonoitis and it is capable to infect critically ill patients who have undergone surgical operations (Chihab et al., 2004).

During the spring season, the bacterial profail changes and is noticed a predominance of bacterial strains from *Serratia spp.* The notable changes in the bacterial population coincide with the normal changes of exploitation system in the spring season, the animals kept on the fields to pasture have full freedom of movement and are directly affected by weather conditions.. In the spring season we isolated a number of bacterial strains belonging to *Serratia spp.: Serratia fonticola, Serratia ficaria, Serratia liquefaciens, Serratia marcescens, Serratia odorifera*

Enterobacter aerogenes was the most isolated bacteria in the spring season, aspect notate in the table above. *Enterobacter aerogenes* may cause hospital related infections to the immunosuppressed patients, but in general it is an opportunist bacteria that aggravates symptoms in chronic diseases (Janda et al., 2006).The most frequent infections in which *Enterobacter aerogenes* has a major inpact are: respiratory related infections, gastroenteritis, urinary tract infections and nervous system infections (Lederberg et al., 2000).

The bacteria belonging to *Serratia spp* have an high pathogenicity in the respiratory tract. *Serratia odorifera* is a bacterial strain most frequently isolated from plants, and seldom is accused to produce respiratory infections in humans and animals. (Chmel et al., 1988). *Serratia fonticola* it is accused to be responsible for

respiratory infections in birds and has high pathogen potential for immune suppressed animals, it can also cause enteritis and diarrhea. (Choresca et al., 2008). *Serratia liquefaciens* it is an bacterial strain responsible for hospital related infections especially in the respiratory tract . There are surveys that show the presence of *Serratia liquefaciens* in goat hard paste cheese, as frequently contaminant, but the presence of this bacterial strein in the upper respiratory tract remains a mystery (Martin-Platero et al., 2009). *Serratia ficaria,* was for the first time isolated in 1979, and is a part of figs ecosystem and other akin trees like mulberry tree. Is pathologic action was revealed in few cases of biliary infections and septicemia (Stock, 2003). *Serratia marcescens* it is a worldwide bacterial strain that was thought to be harmless for more than two decades but more recent studies proved that this assumption was wrong. *Serratia marcescens* has a high level of pathogenicity and is involved in various infections affecting humans and animals. Most common infections with *Serratia marcescens* are urinary infections, endocarditis, miocarditis, and most frequent respiratory infections (Hejazi et al., 2004).

Rahnella aquatilis is a bacterial strain most often isolated from water, and wetlands. This bacteria is rarely involved in human and animals infections because of low virulence but may cause complications in immunossuppresed patients. Of the few case reports of *Rahnella aquatilis* in literature most describe infections are located in the urinary tract a patient with renal transplant, and sputum from a patient with chronic lymphocytic leukemia and emphysema (Carinder et al., 2001).

CONCLUSIONS

In our study we identified bacterial strains that normally are present in the respiratory tract of healthy goats that are raised in shelters but also bacterial strains with high pathogenicity that can cause harm in optimal conditions.

We observed notable differences in bacterial populations in the different seasons, winter and spring, and a major cause can be the housing conditions. In winter goats are raised in shelters, a compact and protective environment, and in this season we isolated from the upper respiratory tract *E.coli*, in 26.8% of the bacterial strains. *E.coli*, is a bacterial strain that normay populates the digestive field, but in shelter compact conditions can be found in the upper respiratory tract and is a major pathogen for both, human and animals.

The isolation of *Klebsiella pneumoniae* from goats represent a high risk for human contamination considering that this agent is implicated also in respiratory pathology. *Klebsiella pneumoniae* was isolated in winter, and can be a high pathogen also for the newborn kids.

The identification of *Staphylococcus xylosus* and *Staphylococcus lentus* in winter season suppose a risk for kids and workers in the shelter environment.

The detection in the winter season of bacterial strain like *Chryseomonas luteola* and *Erwinia spp* can be attribute to the food sources, since they populate succulent vegetables, food supplements for goats in this season.

The changes in the bacterial populations isolated in spring season, is directly related with housing conditions, the goats are raised on the pasture, in open field. Notable is the dominance of *Enterobacter aerogenes* 30% of total bacterial strains isolated an opportunist bacteria that produce respiratory related infections.

Isolation and identification of various bacterial strains of *Serratia species* like *Serratia fonticola, Serratia ficaria, Serratia liquefaciens, Serratia marcescens, Serratia odorifera* warns against posible infections since thease are potentialy pathogenic bacteria in immunosuppressed patients.

Identification of bacterial strains as *Rahnella aquatilis* most likly isolated from wetlands supose a riske because of the implication of this pathogen to imunocompromised patients.

All bacterial species isolated from goats in winter and spring season have a high risk of pathogenicity in proper conditions, therefore is necessary to take safety measures when handling goats, kids and respect proper hygiene protocols for public health in general.

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STUDY OF THE MORPHOLOGICAL BASIS IMPLICATED IN INHALATORY ANAESTHESIA AT DOGS: A PERSONAL RESEARCH

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Keywords: *anesthesia, pulmon, RX, Sevoflurane, surgical*

INTRODUCTION

The study is about narcosis, frequently used today in veterinary medicine, in order to make some surgical procedures, with therapeutical, economical or estethical purpose. The substance that we have studied is an anaesthetic with selective or general effects over different structures of the central nervous system, which produces similar results with others anaesthetic in the same class.

Materials and methods: Have been observed anesthesical effects of Sevofluranului administrated through mask induction and mentaining through endotraheal bore, at 5 dogs from different tales and rases) in a private clinique At every case have been made recordings of hematological and biochemical values and also the evaluations of vital constants: cardiac frecqency, electrocardiograma, respiratory frequency, temperature.

To apreciate the presence or absence of hurt sensibility it's reffered observation of member retraction reflex, interdigital reflexul and nociceptive sensibility reasearch of animal, through sting.

Results: after the inhalatory administration of Sevoflurane, we've noticed a slight increase of enzymatic activity for aspartataminotranspherase (GOT/AST), alaninaminotranspherase (GPT/ALT), gammaglutamiltranspherase (GGT), total amylase and also an increase of glycemia, comparing with the initial moment. There was no significant difference between before and after anaesthesia. The stages of anaesthesia were defined by a short time (5-10 minutes) of induction, with no significant respiratory complications (apnoea, larynx spasm, or cough) and an average time for getting out of anaesthesia of about 10-20 minutes.

Conclusions: using inhaled Sevoflurane as an anaesthetic agent, we didn't noticed any side effects, such as vomiting, convulsions or restlessness and the temperature, the heart rate, the respiratory rate and the oxygenation of

the peripheral tissues were in normal ranges. Aneshesia reprents an medical procedure to diminuate or suprimate, completed or partial of body sensibility, at pains realised through fizical and chemical agents. Anesthesia is using in surgical procedures to permit pacients to support surgical intervention with minimal hurting effects. Anesthesia is manifesting through: absence or dispppearing one of many kind of sensibility and reversible abolish of she, caused through utilisation of anesthesic agents. General anesthesia consists in reversal loss of conscience. General anesthesia is realised in three kind of actions: narcosis (represented by conscience loss or deep sleep), is owed administration of one anesthesic agent, or inhalation (Sevoflurane, Izoflurane, Halotane) or intervenous side; analgesia (means pain disappearing) which is obtained with analgezics; curing (inhibiting substances), who permits muscle relaxing for a good surgical intervetion need. Morphological base implicated in inhalatory anesthesia is represented by respiratory system with intra and extrapulmonary sides, integrity of those contributing in very much measure for good evolvingof all narcosis steps but also of wake up.

MATERIALS AND METHODS

Have been observed anesthesical effects of Sevofluranului administrated through mask induction and mentaining through endotraheal bore, at 5 dogs from different tales and rases (which age between 3 and 6 years old, 3 females and 2 males, 2 rase european rases, 1 rotweiller, 1 caniche, 1 cocker) in a private clinique. Surgical interventions was not very hard to be realised and don't have been more than 60 minutes, and have received the same pre medication.At every case have been made recordings of hematological and biochemical values and also the evaluations of vital constants: cardiac frecqency, electrocardiograma, respiratory frequency, temperature, oxygen saturation of periferical tissues, induction time, wakeing up time, metabolisation and secundary products removment. Have been monitorised the effects above cardiovascular, respiratory, neuromuscular and renal systems and also on liver.Anesthesic circuit used is cclosed kind types which includes a tubes system which assure oxygen need and removing of carbon dyoxide through his absorbtion by sodata calce,in this mode can be realised artificial ventilation. In closed circuit it's producing total reinhalation of gase mix assuring adecquate oxygen need.

The method used present the advantaje of a small consum of volatile anesthesic, for making a deep narcosis with dirijable time and posibillity of controlling pulmonar ventilation.
I've been made endotraheal intubation in the follow mode:
-opening mouth cavity
-viewing through larinx opening and tongue exteriosation, laringoscope apllying at her base;
- head fixing ina an ortopneic position to reduce much the orotraheal angle, and then the well have been introduced on the trachea till the third anterior level. This well also can be viewed the the help of RX imagistics.
-air is introducing in well ballon with help of syring and then the termination is closing - exterior head of tube
-for well verify if it's correctly intratracheal positioned i've been executed pulmonary hearing with stetoscope after insuflation with ballon. Vezicular murmur and the pulmon distension once with air insuflation have confirmed the correct localisation of well.
-the swell is ataching at anesthesic circuit and continue the narcosis
- the well is fixing on animal maxila for not permitting any moves intratracheal and don't produces injuries.

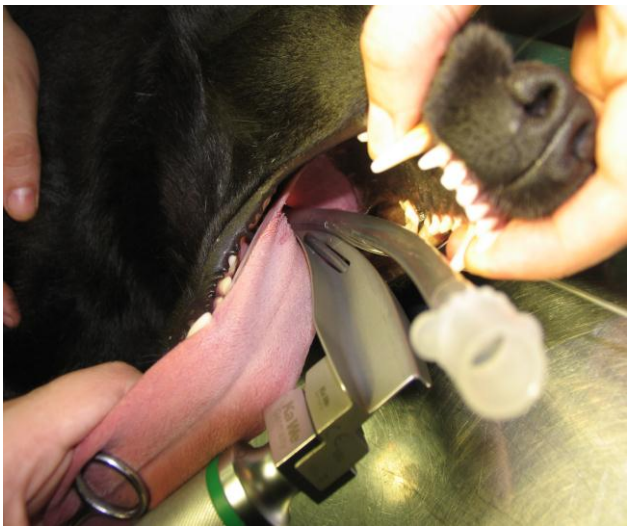

Fig.1 Installing endotracheal well

I've used facial masl only for induction, she can be used also for mentaining but it can be risk as a gas part to enter in stomach and the gastric regurgitate to be aspired, causing dangerous pulmonar complications. Also it have been

determined at anesthesia mentaining only from mask appears many anestehesic quantities lost.

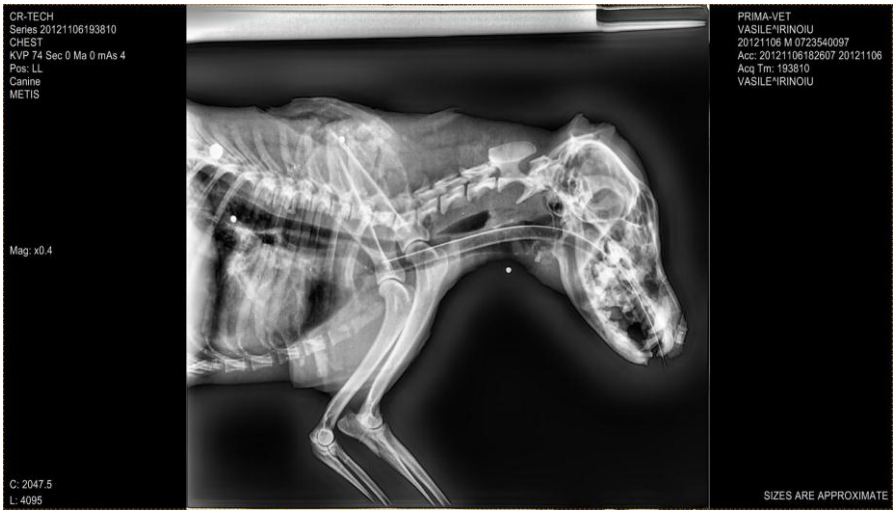

Fig.2 RX view of endotracheal well.

Fig.3 Pacient monitoring

RESULTS AND DISCUSSIONS

After inhalatory administration of Sevoflurane have observed: easly grow of enzimatic activities for aspartat aminotranferase (GOT/AST), alaninaminotranferase (GPT/ALT), gama glutamil transferase (GGT), of total

amilase, glicemia, creatininemia and ureea near by initial moment, seeing that aren't major differences between initial moment and after anesthesia. Steps of anesthesia have described through anesthesia induction for a short time (5-10 minutes), without any notable respiratory complications (apnea, larynx spasm and cough) ending of anesthesia have produced in 10-20 minutes. Minimal alveolar concentration (MAC) of Sevoflurane is aproximative 2,5% during waist and the age of animal, she beeing the most deducted at old pacients at a high concentration of anesthesia at a long time, producing respiratory depresia quick then an young body. Deep anesthesia permits endotraheal intubation without miorelaxing using. The odor, iniritating has permitted induction through mask with a small frecquency a respiratory tricky (apnea, larynx spasm, stop breathing and cough). The odor dont't affects negatively the induction. Rapidly growing of inspirated alveolar concentration with Sevoflurane is translating into an quickly anesthesia induction. Salivation frecqency, apnea, larynx spasm, stop breathing, are more reduced than Halotan and aren't controlable. Anesthesia induction can be realised throurgh growing inspirated concentration of Sevoflurane in progresive steps.Using mask induction of general anesthesia with Sevoflurane, induction time was shorted without growing of tricky frecquency (cardio-respiratory), using by a high concentration, circuit tehnique prepared (amorsed) comparing with conventional method of progressive induction.

Through practical tests have been demonstrated that the substance easily enter in the system, when she is administrated through inhalation, and after the ending of administration in blood the concentration of anestheic quickly decreased.

Also I've demostrated the nefrotoxicity and hepatotoxocity of Sevoflurane. Concording phisico and chemical dates in organism biotransforming of Sevoflutane is limitated (5%) producing hexafluoroizopropanol (HFIP), with realising by anorganic fluorure (F) and carbon dioxide. Once formed HFIP is rapidly conjugate with glucuronic acid, and after that is rapidly removed as an urinary methabolit. At dogs, metabolised quantity represents less than 2,5 % from anesthesic absorbtion and methabolits excretion is finished in 48 hors.

As point of view of nefrotoxicity and hepatotoxocity biochemical tests pre and postsurgical have demonstrated non alterating of renal and hepatic functions at dogs.

As another inhalatories anesthesies, Sevoflurane in high blood concentrations cause respiratory depresia and growing of arterial partial presure of CO_2.

CONCLUSIONS

No adversal reactions have been seeing at administration with Sevoflurane (vomit, convulsions or agitation) and the temperature, cardiac and respiratory frecvqency, peripherical tissues in oxygen saturation was in normal parameters.

Inhalatory anesthesia don't modified major bood constants post-surgical.

Removing inhalating anesthesic on respiratory side (over 95% in case of Sevoflurane) permits anesthesia control and quickly restore in case of an complication.
The odor permit induction trhourgh mask and intubation without using miorelaxing.
I've noticed that negative efects above organs and systems are minimal.

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RESEARCH REGARDING EVALUATION OF RAM'S SEMEN, COLLECTED BY ELECTROEJACULATION, OUT OF THE BREEDING SEASON

Borzan Mihai Marian, Groza Ioan Stefan, Morar Iancu Adrian, Morar Glad

Abstract

Our study was conducted out of the breeding season (March, 2012). An experimental study was conducted to assess the external genitalia in rams, along with artificial collection of semen, using the electroejaculation method by electrostimulation with an electroejaculator standardized for small ruminants, produced by MINITUBE-Germany. The semen obtained was macroscopically (number of stimulus necessary for obtaining the semen, volume, color, smell) and microscopically (sperm waves, mobility, viability, concentration) evaluated.

Key words*: electroejaculation, microscopical and macroscopical evaluation, reproduction, ram, semen.*

INTRODUCTION

Adult males of many ruminant species present importand changes of involution and growth of testicular volume in season and out of the breeding season, including a tranzition between the intense activity and the arrest of spermatogenesis. (Asher et all., 1999).

In season, the morphologic changes of the entire reproductive sistem, production of spermatozoa and testosteron ensure the success of breeding in the short period of the breeding season. (Goeritz et all., 2003).

Collection of semen is made by ejaculation, without sexual contact between male and female (Noakes D.E. et all, 2009) Being an very important operation, many scientists tried to improve the collection methods for obtaining the semen, but without affecting the health state of the male (Ptaszynska M., 2009). The methods for semen collection have to assure the collection of semen without losses, without affecting the spermatozoa, and also a high quality sanitation for the collected semen is required (Grazyna P.et all., 2002). If electroejaculation is practiced for too long time, the reproduction potential of the animal can be compromised **(**Lasley B.L. 1994, Groza I.Ș. 2006).

The existing methods for collectin the semen from reproductive males are divided into: urethral methods, which allow obtaining the semen directly from the male urethra, and vaginal methods, in which semen is collected in the female vagina, after the matting act.

MATERIAL AND METHOD

An experimental study was conducted to evaluate the reproductive potential of rams, outside of the breeding season. For this purpose, testicol size and semen were evaluated. The semen was obtained by electrical stimulation and then a microscopical and macroscopical evaluation was conducted.
The study was carry out in March 2012, out of the breeding season, on a batch of 11rams.
The including criteria of the rams in the study were: males, sexual mature, clinically healthy.
The excluding criteria from the study were: sexual imature rams or at andropause.
Clinical examination. The general examination was made on the main systems and then the male genitalia was examined. The examination was conducted on natural light and the general evolution, attitudes and conformation were evaluated.
The skin from different areas, the joints, the hooves were palpated, being very important in the examination of the reproduction rams.
Local and general changes that could affect the reproductive abillity and behaviour of the rams were evaluated.
The examination was done standing and in movement by inspection, palpation and ascultation.
Examination of the male genitalia. The examination was made by inspection and palpation of the genitaila, or the scrotum examination, testes, epididyms, the testicular cord, prepuce and penis.
In testes it was noted: mobility, size, consistency, tenderness to palpation, the position of testes and testicular measurements were made to lenght and circumference of the testes. Note that all this findings were made out of the breeding season.
The examination of head, body and tail of epididym was conducted.
The deferend ducts were easy to feel on palpation, the thickness and sensibility was assessed in the testicular and funnicular segment.
Also , the testicular cord was palpated and thickness, consistency and mobility of the cord was evaluated, by comparison with the other.
The penis was examined, then it was exteriorised and volume and integrity was assesed. Also an examination of the prepuce was made. (Table 1)

Table 1. Clinical examination of genitals and testicular measurement in the studied rams

Ram	Breed	Testicular length		Testicular circumfe r-ence (cm)	Testis	Penis	Prepuce
		Left testis (cm)	Right testis (cm)				
RO112932623	Schwarzkopf	9	9	36	Normal	Normal	Normal
RO110293577	Schwarzkopf	10	10	38	Normal	Normal	Normal
RO1552592912	Schwarzkopf	9,5	9,5	37	Normal	Normal	Normal
RO1102932644	Schwarzkopf	9	10	39,5	High consistence on the right testis	Normal	Normal
RO1551948912	Schwarzkopf	11	10	39	High consistence on the left testis	Normal	Normal
RO1102932601	Schwarzkopf	10,5	10	38	Normal	Normal	Normal
RO1075345130	IDF (Ille de France)	9,5	9,5	40	Normal	Normal	Normal
RO1075345140	IDF (Ille de France)	10,5	10,5	39,5	Normal	Normal	Normal
RO1075345069	IDF (Ille de France)	10	10	39	Normal	Normal	Normal
RO1075345070	IDF (Ille de France)	9	10,5	41	High consistence on the right testis; hard epididimis	Balano-posthitis	Balano-posthitis
Ram	**Breed**	**Testicular length**		**Testicular circumfe r-ence (cm)**	**Testis**	**Penis**	**Prepuce**
		Left testis (cm)	**Right testis (cm)**				
RO11210002323	Schwarzkopf	10	10	40	Normal	Normal	Normal
Average		9.71	9.86	38.67			
STDEV		0.76	0.38	1.58			

Collection of semen

The first step for evaluating of the semen is the collection. The collection of semen was made using the electrostimulation method.

Semen was collected with an manual electroejaculator standardized for small ruminants. In this type of electroejaculator, the electrical stimulation is made by the user and not by a program, like in electronic electroejaculators which have an diferent program that changes automaticlly the stimuli voltage depending on the species.

For collection, after restraining, the ram is laid on the left side. A local toiled was performed by shaving and washing the prepuce with sodium bicarbonate 3% followed by the drying of the area. An enema was performed to eliminate the feces and for a better conductibility. The penis was exteriorised from the prepuce, acting on the sigmoid flexure, and the vermiform appendix was introduced in the collection recipient.

The electrode of the electroejaculator (30cm long and 2 cm in diameter) was inserted into rectum after the lubrication, for about 15 cm.

The collector recipient was placed in front of the urethra to collect the semen.

Collection was made in special sterile glass (sperm friendly), dry and heated at body temperature, which were changed at every semen ejaculate, to avoid the risk of urine contamination in the whole ejaculate.

The electrical stimulus was applied for 5 seconds, and breaks of 10 seconds between stimuli. The device is connected to a source of continuous electric charge (accumulator) with tension variations of 2.5-9V.

The semen was obtained after 3-5 stimuli, and the process occurs without erection.

The semen was obtained with the same protocol at all the collections and to all studied individuals. Then a macroscopical and microscopical examination was performed.

The macroscopical examination included the measurement of the ejaculate volume with an graduated cylinder, also the color and smell of semen were assessed.(Table 2)

Table 2. Macroscopic examination of semen

No crt	**Ram**	**Breed**	**Number of stimulations**	**Semen volume (ml)**	**Color**	**Consistency**	**Density**	**Smell**
1	RO112932623	Schwarzkopf	6	2,5	White	creamy	dense	normal
2	RO110293577	Schwarzkopf	5	2	White	creamy	dense	normal
3	RO1552592912	Schwarzkopf	8	2	White	creamy	dense	normal
4	RO11029326 44	Schwarzkopf	7	2	White	creamy	dense	normal
5	RO1551948912	Schwarzkopf	8	2	White	creamy	dense	normal
6	RO11029326 01	Schwarzkopf	9	0,75	White	creamy	dense	normal
7	RO1075345130	IDF (Ille de France)	8	2	White	creamy	dense	normal
8	RO1075345140	IDF (Ille de France)	8	2	White	creamy	dense	normal
9	RO1075345069	IDF (Ille de France)	8	1,5	White	creamy	dense	normal
10	RO1075345070	IDF (Ille de France)	8	-	-	-	-	-
11	RO11210002 323	Schwarzkopf	9	5	White	creamy	dense	normal

The microscopical examiantion of semen evaluated the presence of sperm waves, the mobility, viability and concentration of spermatozoa.
The mobility was evaluated by the number of spermatozoa with forward movements and their movement energy, on a slide at 37^0C.
Viability of spermatozoa was assessed by Hancook-Dott method- the intravital staining with eosin and nigrozin.
Over 500 spermatozoa were evaluated and then expressed as a percentage their share (Table 3).

Table 3. Microscopic examination of semen

No. crt	**Ram**	**Breed**	**Sperm waves**	**Mobility (%)**	**Viability (%)**	**Concentration**	**Observation**
1	RO112932623	Schwarzkopf	+ + +	90	92	over 1 x 10^9/ml	-
2	RO110293577	Schwarzkopf	+ + +	90	93	over 1 x 10^9/ml	-
3	RO1552592912	Schwarzkopf	+ + +	95	95	over 1 x 10^9/ml	Exfoliate cells
4	RO1102932644	Schwarzkopf	+ + +	50	60	over 1 x 10^9/ml	Exfoliate cells and cellular detritus
5	RO1551948912	Schwarzkopf	+ + +	85	80	over 1 x 10^9/ml	-
6	RO1102932601	Schwarzkopf	+ + +	95	90	over 1 x 10^9/ml	-
7	RO1075345130	IDF (Ille de France)	+ + +	95	93	over 1 x 10^9/ml	-

No. crt	Ram	Breed	Sperm waves	Mobility (%)	Viability (%)	Concentration	Observation
8	RO1075345140	IDF (Ille de France)	+ + +	90	90	over 1 x 10^9/ml	-
9	RO1075345069	IDF (Ille de France)	+ + +	100	100	over 1 x 10^9/ml	-
10	RO1075345070	IDF (Ille de France)	-			-	-
11	RO11210002323	Schwarzkopf	+ + +	80	80	over 1 x 10^9/ml	-

CONCLUSIONS

At clinical evaluation 3 rams presented testicular modifications, and one ram was diagnosticated with balanoposthitis.
Collection of semen failed on one ram (aspermia) and 5-9 electrical stimulations were needed to obtain semen.
Volume of semen ranged between 0,75 and 5 ml and had white-creamy color.
At microscopical examination sperm waves were present, mobility ranged between 50 and 100%, viability ranged between 60 to 100% and concentration was over 109sperm/ml semen.
Total number varied between 1 and 7 x 109 sperm.
After andrological evaluation, 2 out of 11 rams were excluded from reproduction process.

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CLINICAL AND IMAGISTIC CORRELATION IN PACIENTS WITH RENAL FAILURE

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Abstract

Kidneys are organs that receive a large amount of blood, approximately 20% of the heart blood flow. Many renal diseases have an important vascular component, some systemic diseases, such as hypertension, are vascularly mediated through the juxtaglomerular component. The study has been carried out on a number of 25 cats and 18 dogs, inside the Faculty of Veterinary Medicine Bucharest, sedated and awake as well as with different renal, hepatic and heart diseases. The resistance index and pulsatility index have been appreciated in renal level from the renal arteries, interlobar arteries and arcuate arteries, with superior specificity in pathologic conditions (diagnosis and prognosis)
.
Key words: *Doppler ultrasonography, resistivity index, pulsatility index.*

INTRODUCTION

Kidneys are organs that receive a large amount of blood, approximately 20% of the cardiac blood flow. Many renal diseases have an important vascular component, some systemic diseases, such as hypertension, are vascular mediated through the juxtaglomerular component.
All of these characteristics make the kidney suitable for Doppler examination, because it is expected, in renal and renal vascular diseases, for vascular arterial and venous and micro vascular circulation to be modified.
Hemodynamic alterations that decrease renal perfusion are characterized by systemic vasodilatation. This systemic vasodilatation determines an increase in blood flow in the splahnic region that will determine compensatory increase of heart flow. In this moment, kidneys can't adapt due to mechanism of self regulation regarding renal circulation that becomes functional only in arterial pressures over 50-70 mmHg. Renin-angiotensin-aldosteron system is activated in order to compensate hypovolemic status.
In dogs and cats, chronic renal failure represents the most common cause in increased blood pressure. When present, hypertension can be inconstant in dogs to cats. Studies revealed that two thirds of a cat population and 50-93%

of dogs present hypertension associated with chronic renal failure (Ettinger and Feldman, 2010)

MATERIALS AND METHOD

This study has been carried out inside the Faculty of Veterinary Medicine, on a number of 35 cats and 27 dogs, sedated or awake, healthy or with renal, hepatic and cardiac affections. Sedation protocols have been selected regarding the affection, following the significant alterations of resistivity and pulsatility indexes.

Examination modalities used were colour Doppler, pulsatory emission Doppler. Fundamental information obtained by Doppler examination are: presence, way, speed and blood flow character and estimation of red blood cells that contribute in generating Doppler signal.

Preparation technique for ultrasonography examination were similar with standard abdominal examination. In some cases sedation was required and was correlated and adapted with the patients status based on blood analysis(hematological, biochemical, electrolytes), ECG and arterial pressure.

Systolic pressure has been determined 5 to 10 minutes prior to examination, in order to let the pacient relax. Systolic pressure has been determined with a high definition tensiometer, by applying a sleeve on the proximal tars in order to tighten the tibial cranial artery. For each patient, a number of three determinations were carried out, using the average value.

Resistivity and pulsatility indexes were obtained by average values of at least five waves, in three different levels/places. Examination was carried out by positioning the patient dorsal and lateral. Before Doppler examination, kidneys size was determined in transversal, sagittal and coronary sections.(Fig.1). Ecogenity of cortical reported with the spleen and liver was also determined, and the ratio of cortical and medullar.

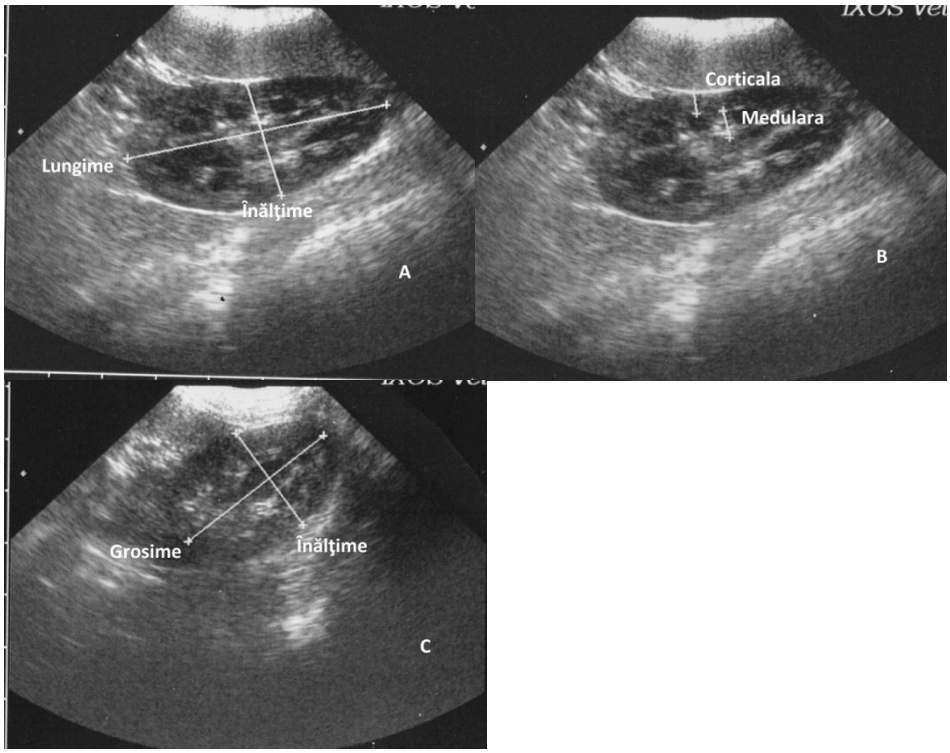

Fig.1 Appreciation of renal size (A. Transverse, B. Transverse, C. Sagittal)

Blood flow along the renal arteries has a pulsatile character due to permanent forwarding in the form of successive accelerations and decelarations. This pulsatile character is influenced by two main factors: rhythmic contraction of the heart and arterial walls elasticity. At venous level, blood forwarding has a continuous character depending on cardiac and respiratory function.

Vascular ultrasonography investigation begins with vascular anatomy appreciation and vascular hemodynamic appreciation. Anatomical evaluation is carried out with B mode and appreciates the anatomical position of the examined vessel, its pattern (linear or sinuous), wall and vascular diameter assessment. Doppler is used for vascular hemodynamic, following, in the first place, vascular flow presence, way of motion, determining blood flow speed(maximum, minimum and medium), evaluation of peripheral resistance and blood flow throughout the vessel.

Renal artery is identified by following the aorta medial related to the kidney and posterior to the cranial mesenteric artery. Both arteries are recognized fairly easy at this level due to the curve aspect and pulsatile blood flow

synchronized with the heart. Recordings are made throughout the artery length, following any variation of obtained parameters.
Evaluating resistivity and pulsatility indexes are very important in assessment of renal affections prognosis and for development and treatment management. In acute renal failure, internal medium homeostasis is disturbed and its capacity of eliminating metabolic residues. Chronic renal failure consists in permanent alteration of renal function, which contributes to the installment of disturbances regarding hydro-electrolytic homeostasis, metabolic residue discharge, hormone secretion(renin or erythropoietin), with irreversible evolution and character.
Resistivity and pulsatility indexes were calculated automatically by the ultrasound machine software after maximum systolic and diastolic value measures:

$$Pulsatility\ index = \frac{Maximum\ systolic\ speed - Maximum\ dyastolic\ speed}{Maximum\ speed}$$

$$Resistivity\ index = \frac{Maximum\ systolic\ speed - Maximum\ dyastolic\ speed}{Maximum\ systolic\ speed}$$

RESULTS AND DISCUSSIONS

Ultrasonographic values obtained regarding renal size in cats were:

Length (cm)	**Heigth (cm)**	**Thickness**
2,7 – 4,7 Average 3,5 cm	1,7 – 2,8 Average 2,1 cm	2,65

Ultrasonographic values obtained regarding renal size in dogs were:

Body/Weight	**Length(cm)**	**Height (cm)**	**Thickness**
Small 2 – 10 kg	5,1	3,2	2,9 – 3,2
Medium 10 – 30 kg	6,6	5,1	3,0 – 3,6
Large ≥ 30 kg	8,6	6,2	3,3 – 3,8

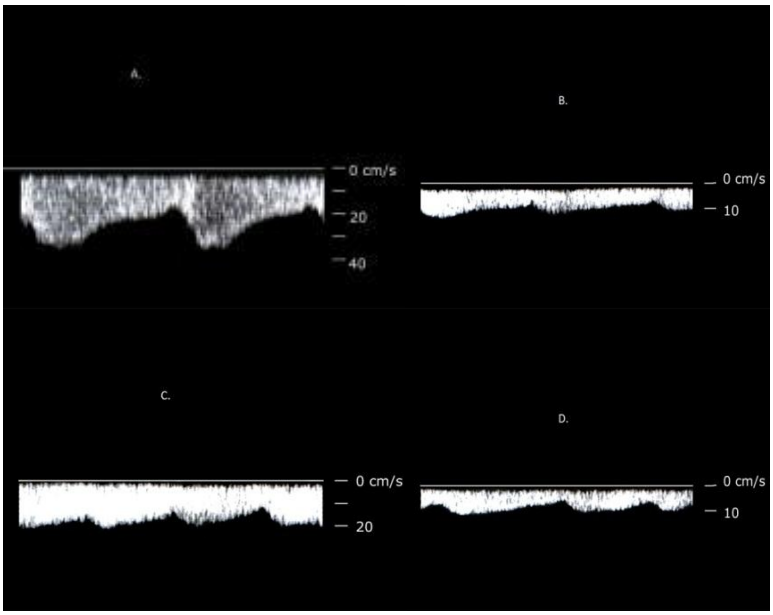

Fig.2 Doppler morphology of renal veins(A), arcuate veins(B), interlobar veins(C) and interlobular veins(D)

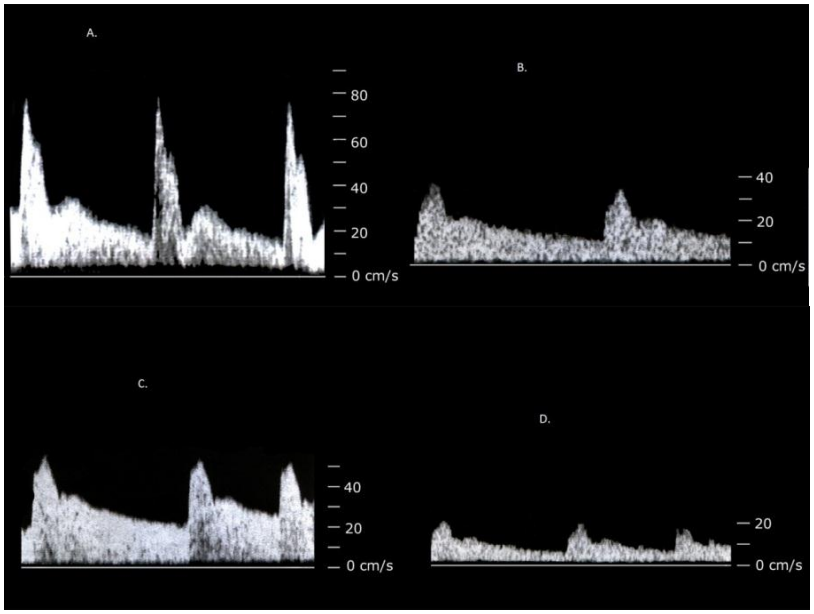

Fig.3 Doppler morphology of renal artery(A), arcuate artery (B), interlobar artery (C) and interlobular artery (D)

Resistivity and pulsatility indexes were calculated at renal, interlobar and arcuate artery.Diagnosis and prognosis relevance was determining those indexes at arcuate and renal arteries.Differences regarding resistivity and pulsatility indexes between the left and the right kidney were 2-3%.Pulsatility index at intrarenal arteries was approximately similar with renal arteries, but for peripheral arteries, recorded values were smaller, in some cases impossible to determine.
Resistivity index represents the ratio between systolic and dyastolic speed.Therefore, an increase in vascular resistance decreases dyastolic speed, any modification regarding resistivity index increase, by obstruction or vasoconstriction, reduces renal blood flow.If renal flow decreases, glomerular filtration rate also decreases.
Obtaining intrarenal resistivity index is useful in confirming renal diseases when, in B mode examination, kidneys look normal.Normal renal function is correlated with the degree of perfusion, resistivity and pulsatility index are used in evaluating vascular renal response to different degrees of hypovolemia.
In dogs, average resistivity index value was ≤1.55, and pulsatility index was≤0.66.In cats, average resistivity index value was≤1.09 and for pulsatility index was≤0.53.Obtained values were similar to those quoted in speciality literature.

CONCLUSIONS

In renal level, with high relevance in blood flow evaluation, resistivity and pulsatility indexes were appreciated in renal, interlobar and arcuate arteries, with high specificity in pathologic states for diagnosis and prognosis
Increase in resistivity index decreases systolic speed and is a precise indicator of renal blood flow reduction(indicating also the decrease of glomerular filtration rate)
Determining and appreciating resistivty index is useful in confirming renal diseases in cases where ultrasonographic modifications in B mode are reduced, inexistent or irrelevant.
Ultrasonographic evaluation has revealed, in chronic renal failure, superior resistivity and pulsatility values, associated with reduction of rena size, whereas in acute renal failure, resistivity and pulsatility values were normal in most patients.
In hypovolemic states, resistivity and pulsatility indexes are used in evaluating renal vascular response(directly correlated with the degree of renal tissue perfusion)

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TRANSPLANTATION OF BIOPSIED, SEXED AND CRYOPRESERVED BOVINE EMBRYOS

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Abstract

Embryo biopsy performed in order to obtain a small amount of blastomeres needed for embryo sexing is an invasive method that damages the zona pellucida and therefore decreases the survival capacity of the embryo that is subsequently submitted to cryopreservation. The aim of the present study was to evaluate three biopsy techniques applied in bovine embryos according to their capacity to maintain embryo viability after cryopreservation. Three embryo biopsy techniques (needle, blade and aspiration) were applied on 120 bovine embryos divided into 3 batches (n=40) in order to harvest the blastomeres needed for polymerase chain reaction (PCR) embryo sexing. After the biopsy, the embryos were frozen/thawed using the one step method with ethylene glycol and then transferred into synchronized recipients. DNA was extracted from blastomeres and amplified using bovine Y-chromosome specific primers, in order to determine the sex of the embryo. The pregnancy diagnosis and the assessment of pregnancy rate were performed 30 days after transfer using an ultrasound scanner. There was a significant difference in pregnancy rates according to the biopsy method used: 55% for the needle biopsy, 45% for the aspiration method and 30% for the microblade technique. The accuracy of the PCR sexing method was comparable in all batches, and therefore was not influenced by the biopsy method. The needle method of embryo biopsy proved to be the most suitable as it yielded the highest pregnancy rates and can be successfully applied when harvesting blastomeres for embryo sexing.

Key words: *bovine, embryo sexing, cryopreservation, biopsy*.

INTRODUCTION

Embryo gender determination is one of the biotechnologies that created great emulation among the specialists studying in vitro fertilization and embryo transfer. Embryo biopsy performed in order to obtain a small amount of blastomeres needed for embryo sexing is an invasive method that damages the zona pellucida and therefore decreases the survival capacity of the embryo that is subsequently submitted to cryopreservation. The aim of the present study was to evaluate three biopsy techniques applied in bovine embryos according to their capacity to maintain embryo viability after cryopreservation.

MATERIALS AND METHODS

Embryo production

Donor selection was made according to the general criteria accepted by the International Society for Embryo Transfer (IETS). Only healthy individuals, with no history of reproductive disorders and with regular sexual cycle were taken into consideration. Superovulation was induced with a total dose of 1000 IU porcine FSH-LH (Pluset, Carlier), administered according to the manufacturer's indications. Artificial insemination was performed 48h after the last administration of Pluset, followed by a second and third insemination, 12h and 24h later respectively. Embryo recovery was made using the non-surgical flushing method 6.5-7.5 days later. Morphologic evaluation of embryos was done taking into consideration the IETS criteria and only grade 1 morulae and blastocysts were used for biopsy and further study.

Embryo biopsy

Embryo biopsy was performed using a Nikon Eclipse TS100 inverted microscope, equipped with an Olympus Narishige ONO-131 three axis hydraulic micromanipulator. The embryos were divided into 3 batches (n=40) according to the biopsy method used. The first batch was biopsied using the needle technique (Tominaga and Hamada, 2004), the second batch by blastomere aspiration (Lopatarova et al. 2010), while the third batch was biopsied using a fine microblade (Akiyama et al., 2010).

Cryopreservation, transplantation, sexing and pregnancy diagnosis

Embryo cryopreservation was achieved using the one step method with 1.5 M ethylene glycol described by Voelkel and Hu, 1992. Thawing of embryos was made in 37ºC water bath for 10 seconds, followed by direct transfer into synchronized recipients. Embryo sexing was made using the PCR method described by Peura et al., 1991 using the BRY4a primers. Pregnancy diagnosis was carried out 30 days after embryo transfer using an ultrasound scanner and the pregnancy rate was assessed on this occasion. The results were statistically analyzed using the In Stat Graph Pad software and the unpaired t-test.

RESULTS AND DISCUSSIONS

The pregnancy rate obtained after the transfer of biopsied, sexed and cryopreserved embryos was 57% for batch 1 in which the needle biopsy technique was applied, 43% for batch 2 in which the aspiration biopsy

method was used and 31% for batch 3 in which the microblade biopsy technique was applied.
All pregnancies progressed to term and resulted in healthy calves. The accuracy of the sexing method was of 100% in all batches, all calves presenting the same morphological sex as predicted earlier by PCR.
The statistical analysis showed the following significant differences between batches:
Batch 1 (needle biopsy) vs. Batch 2 (aspiration biopsy): $p=0.0010$, extremely significant; Batch 1 (needle biopsy) vs. Batch 3 (microblade biopsy): $p<0.0001$, extremely significant; Batch 2 (aspiration biopsy) vs. Batch 3 (microblade biopsy): $p=0.0030$, very significant.
Various authors, performing embryo biopsy for different reasons, obtained comparable results. The pregnancy rate after the transfer of fresh, biopsied embryos varied from 53 to 62%, the microblade biopsy method being usually preferred (Bredbacka et al., 1996, Herr and Reed, 1991, Roschlau et al., 1997, Thibier and Nibart, 1995) and is comparable to the one obtained for fresh, intact embryos. Sex determination can also be performed in bisected embryos if transferred freshly, without cryopreservation, with good conception rates, up to 56.5% (Lopatarova *et al.*, 2008).
The results obtained for frozen/thawed biopsied embryos varied quite a lot among various researchers and ranged from 33 to 66% when needle or aspiration was used, but was of only 23% to 28% when a microblade was used (Nibart *et al.*, 1997, Shea 1999).
Other data shows that the efficiency of producing bovine embryos, transferable after vitrification on day 7 was higher when the biopsy was performed on day 4 rather than day 7.5 (Vajta et al., 1997).

CONCLUSIONS

Biopsied frozen/thawed bovine embryos have a better chance to survive the cryopreservation process if damage of the zona pellucida is minimized as much as possible.
This can be achieved by carefully choosing the embryo biopsy method, the needle technique showing obvious advantages, as presented above.
As biopsied embryos are rarely transferred directly into recipients, and are usually cryopreserved for later use, the choice of an adequate biopsy method has a direct influence on embryo viability after thawing.

ACKNOWLEDGEMENTS

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PREVALENCE OF MOBILE SEROVARS OF *SALMONELLA SPP.* ISOLATED FROM BREEDING HENS, LAYING HENS AND BROILER CHICKENS IN 2010

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Abstract

For the control of mobile Salmonella presenting a high zoonotic risk and also involving property damage through mortality, a National Program was implemented in Romania to control mobile Salmonella infections in breeding hens, which includes relevant serovars represented by S. enteritidis, S. infantis, S. hadar, S. typhimurium and S. virchow and relevant serovars represented by S. enteritidis and S. typhimurium for laying hens and broiler chickens.
The faeces samples were collected with a frequency established by the Community legislation while the program aimed at reaching a prevalence of 1% or less of the flocks with a confidence interval (confidence limit) of 95% for breeding hens and broiler chickens and up to 2% for flocks of laying hens.
Following the study conducted, 655 strains of Salmonella enterica subsp. Enterica were isolated.
For the breeding hens, serovar S. enteritidis had the highest incidence - 15 strains were identified, for serovar S. typhimurium only one strain was isolated, while the other relevant serovars represented by S. infantis, S. hadar and S. Virchow were not identified.
For the laying hens, S. enteritidis had the highest incidence - 19 strains were identified, while for serovar S. typhimurium 5 strains were identified.
In farms of broiler chickens S. enteritidis had the highest incidence as well - 38 strains were identified, while serovar S. Typhimurium was not identified.

***Key words*:** *breeding hens, broiler chickens, laying hens, Salmonella.*

INTRODUCTION

In intensive poultry farming there was an increase in the frequency of mobile Salmonella infections, namely paratyphoid infections, associated with the occurrence and circulation of new serovars involved in the etiology of these diseases. Occurrence and evolution of paratyphoid infections cause losses through mortality, implementation of prevention and control measures and restrictions on the trade in poultry (Clep, 2011; Gast, 2008).
Besides the economic issues, mobile Salmonella infections are also important in terms of health, due to the high zoonotic risk of the serovars

involved. Poultry products (eggs, meat and their derivatives) are the major source of human infection with mobile Salmonella (Clep, 2011; Gast, 2008). In breeding hens, laying hens and broiler farms, the folowing measures are taken in order to prevent the mobile Salmomonella spreading: biosecurity, vertical transmission avoidance and usage of barrier flora, the latter one being a new concept based on probiotic products usage and competitive exclusion of flora. The usage of the barrier flora method, is replacing the preventive therapy based on antibiotics and sulphonamides, which is currently forbiden in the EU countries.
Based on the Community legislation in this field, a National Programme for control of mobile Salmonella infections with zoonotic risk for species *Gallus gallus* in breeding, laying and broiler farms was developed in our country (Clep, 2011).
Research that is subject of this paper was conducted in order to analyze epidemiologically the effectiveness of measures within the program performed in 2010.

MATERIALS AND METHODS

In order to prepare this paper, a longitudinal epidemiologic study was conducted throughout 2010. That year was the second year of the National Programme for control of mobile Salmonella infections with zoonotic risk for species *Gallus gallus*. Such longitudinal study was based on primary data collected from holdings with breeding hens, laying hens and broiler chickens nationwide.
This study analysed the prevalence of serovars isolated from farms with breeding hens, laying hens and broiler chickens.
The primary data collected nationwide were presented in tables, processed and presented graphically to be interpreted.
In **holdings with breeding hens** legislation provides for two types of controls:
- self control (at the initiative of farmers);
- official control (performed by the County Sanitary Veterinary and Food Safety Directorate)

Within the self control, samples are taken every two weeks from the hatchery or the holding. Currently, in Romania, the sampling is performed in the farm.
As regards the official control, samples are taken in the farm, three times during the production cycle:

- four weeks following the beginning of laying;
- eight weeks before the end of the laying period;
- in the middle of the laying period.

Samples taken are represented by faeces and disposable footwear (boot swab) made of absorbent material.

The programme aims at reaching prevalence up to 1% of the flocks with a confidence interval (confidence limit) of 95%. Samples are represented by at least 1 g of fresh faeces taken from several points of the housing, directly proportional to the number of poultry of the flock, as it is shown in Table 1.

Table 1

Number of locations from which the samples are prelevated

Number of birds kept in the breeding flock	Number of points that are collected faeces samples to be taken from the breeding hens flock
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

In **holdings with laying hens** legislation provides for two types of controls in terms of samples taken:

- self control performed at the initiative of farmers;
- official control performed by the state veterinary services.

Production stages to be covered by sampling through the self-control programme of the farmer are as follows:

- one day old chickens;
- pullets two weeks before the beginning of laying or transfer to the layer house;
- every 15 weeks during the laying period (Daneș, 2010);

The state veterinary services take samples at least:

- annually in a poultry flock but in holdings with at least 1 000 poultry;
- when poultry reach the age of 24 weeks (with a margin of plus or minus 2 weeks), in flocks of laying hens kept in houses where the previous poultry flock was infected with *Salmonella.*

The state veterinary services should obligatorily take samples when infections with *S. enteritidis* or *S. typhimurium* are suspected following an epidemiological survey on the foodborne illness outbreaks. It is also necessary to take official samples in all other flocks of laying hens of the holding, where the presence of *S. enteritidis* or *S. typhimurium* is detected in one of the flocks of laying hens of the holding.
The program aims at achieving prevalence up to 2% of the flocks with a confidence interval (confidence limit) of 95%.
In **broiler chicken farms,** legislation provides for two types of controls:
- self control performed at the initiative of farmers;
- official control performed by the state veterinary services.

Sampling within the self-control should take place 3 weeks before slaughtering the broiler chickens.
As regards the official control carried out by the state veterinary services, it must include every year at least one flock with broilers of 10% of the broiler farms with more than 5000 heads.
Faeces samples are taken to reach prevalence up to 1% of the flock with a confidence interval (confidence limit) of 95%.
Designated persons who use disposable footwear (socks, slippers) walk inside the house on a well-established route corresponding to the surface (permanent litter, grids). Footwear used is priory moistened with diluting solutions recommended by the National Reference Laboratory (0.8% sodium chloride, 0.1% peptone, distilled or double-distilled water, pH = 7). The routes used by the designated persons must represent 50% of the housing.
Samples taken were sent under refrigeration conditions to the accredited county laboratories within 24 hours, where they were processed within 48-96 hours from sampling.
Bacteriological examinations are conducted in accordance with ISO 6579-2002/ Amendment 1:2007 - Horizontal method for detection of *Salmonella spp.* developed by the Community Reference Laboratory for *Salmonella spp.* isolated from poultry in Bilthoven, the Netherlands. This methodology is used by the authorized county sanitary veterinary laboratories.

RESULTS AND DISCUSSIONS

In 2010, following the analysis of samples taken from flocks with breeding hens, laying hens and broiler chickens, 655 strains belonging to the species *Salmonella enterica subsp. Enterica* were identified.

In case of detecting mobile Salmonella in holdings with breeding hens in our country, in 2010, 55 strains of mobile Salmonella belonging to 10 serovars were isolated; the results are presented in Table 2.

Table 2

Serovars of mobile Salmonella isolated from flocks of breeding hens in Romania in 2010

No.	Breeding hens: 55 strains		%
1	S. agora	1	1.8
2	S. amsterdam	2	3.6
3	S. enteritidis	15	27.3
4	S. ifantis	1	1.8
5	S. livingstone	5	9.1
7	S. mbandaka	3	5.5
8	S. montevideo	13	23.6
9	S. thompson	10	18.2
10	S. typhimurium	1	1.8
11	S. uganda	4	7.3

Frequency of serovars and strains isolated from the breeding flocks is variable. Thus, serovar *S. enteritidis,* with 15 strains isolated and identified, had the highest frequency, while serovars *S. typhimurium, S. ifantis* and *S. agora* had the lowest frequency, with one strain each.

Following the analysis of the frequency of the mobile serovars we notice that serovars *S. enteritidis* and *S. typhimurium* were identified out of the five serovars relevant for the breeding hens, namely *S. enteritidis, S. infantis, S. hadar, S. typhimurium* and *S. virchow.*

As regards the mobile Salmonella in the holdings with laying hens in our country, in 2010, 179 strains were isolated, belonging to 15 serovars; the results are presented in Table 3.

Following the analysis of the frequency of the mobile serovars in laying hens we notice that both serovars relevant for flocks of laying hens were

identified, namely serovar *S. enteritidis* within which 19 strains were isolated and identified and *S. typhimurium* within which 5 strains were isolated and identified. Serovar *S. livingstone* – a serovar that is not relevant - had the highest frequency, with 45 strains isolated and identified, while serovars *S. gallinarum*, *S. senftenberg* and *S. agora*, with one strain each, had the lowest frequency.

Table 3

Serovars of mobile Salmonella isolated from flocks of laying hens in Romania in 2010

No.	Laying hens: 179 strains		%
1	S. agora	1	0.6
2	S. bredeney	2	1.1
3	S. enteritidis	19	10.6
4	S. gallinarum	1	0.6
5	S. hadar	3	1.7
7	S. ifantis	7	3.9
8	S. livingstone	45	25.1
9	S. mbandaka	6	3.4
10	S. montevideo	33	18.4
11	S. newport	4	2.2
12	S. senftenberg	1	0.6
13	S. tennessee	5	2.8
14	S. thompson	42	23.4
15	S. typhimurium	5	2.8
16	S. uganda	5	2.8

In case of detecting mobile Salmonella in holdings with broiler chickens in 2010, 421 strains of mobile Salmonella belonging to 18 serovars were isolated; the results are presented in Table 4.

Serovar *S. ifantis*, with 267 strains identified, had the highest incidence in farms with broiler chickens. Out of the two serovars relevant for broiler chickens, namely *S. enteritidis* and *S. typhimurium*, only *S. enteritidis* was identified, with 38 strains identified and isolated.
Serovar *S. virchow* was not identified in breeding hens, laying hens and broiler chickens.
Some serovars considered exotic for Romania, such as *S. senftenberg* and *S. thompson*, occurred due to imports of replacement chickens and day-old chickens from third countries, where frequency of such serovars is increased. Exotic serovars entered free countries, including Romania, due to the epidemiology track of Salmonella, worldwide, determined firstly by the trade in poultry from third countries where legislation is more permissible on the control of mobile *Salmonella* infections.

Table 4

Serovars of mobile Salmonella isolated from flocks of broiler chickens in Romania in 2010

No.	Broiler chickens: 421 strains		%
1.	S. amsterdam	1	0.2
2.	S. bredeney	2	0.5
3.	S. enteritidis	38	9.02
4.	S. glostrup	1	0.2
5.	S. hadar	26	6.2
6.	S. ifantis	267	63.4
7.	S. insangi	3	0.7
8.	S. kentucky	11	2.6
9.	S. kottbus	2	0.47
10.	S. liverpool	2	0.47
11.	S. livingstone	4	0.95
12.	S. mbandaka	1	0.2
13.	S. montevideo	3	0.7
14.	S. orion	2	0.47
15.	S. senftenberg	14	3.3
16.	S. taksony	33	7.8
17.	S. tennessee	7	1.7
18.	S. thompson	4	0.95

Much less serovars were isolated from holdings with breeding hens compared to the farms with broiler chickens because the imported flocks are smaller, biosecurity rules are very strict and control performed through sampling is rigurous.

Frequency of mobile *Salmonella* serovars isolated in our country was variable in recent years, whereas number of serovars and strains isolated increased. The results provided by other authors were influenced largely by the developments of intensive poultry farming and trade in poultry.

Volintir quoted by (Verdeş, 2001) showed in 1975, following a study, that a percentage of 63-93% *S. typhimurium* was isolated from broiler chickens and hens, while a much lower percentage of other serovars was isolated and Sicoe quoted by (Daneş, 2010) showed in 1988 that serovars *S. typhimurium* and *S. enteritidis* had the highest frequency.

Following the liberalization of trade in poultry in our country (Draghia *et al*., 1993) showed that 5% of the breeding hens were carriers of mobile *Salmonella*, while the dominant serotypes were *S. enteritidis* (47,4%) and *S. typhimurium* (18,6%).

During 2001-2005, (Tatu-Chitoiu *et al.*, 2006) studied 2807 mobile *Salmonella* strains, out of which 2402 were isolated from poultry, while *S. enteritidis* was the dominated serovar, with a frequency of 43.3%, and serovar *S. djugu* had the lowest frequency (1.25%). In this study, 57 serovars were identified, out of which 7 were considered new serovars for our country.

Worldwide, frequency of the mobile serovars isolated from breeding hens, laying hens and broiler chickens is variable and changes periodically, depending on many factors. In USA, serovars *S. heidelberg*, *S. kentucky*, *S. enteritidis*, *S. seftenberg* are frequently isolated in *Gallus gallus* species, while 21 mobile serovars were isolated in the EU, out of which 5 were considered relevant serovars for breeding hens due to their high frequency and zoonotic risk (Gast, 2008; Popa *et al*., 2006), and 2 serovars, namely *S. enteritidis* and *S. typhimurium* are considered relevant for broiler chickens and laying hens.

Serovars isolated in 2010 from breeding hens under the National Program are represented by high frequency serovars isolated both in USA and the European Union, but their number and the number of strains isolated is much lower compared to the laying hens and broiler chickens.

Due to the intervention of favorable factors, the mobile *Salmonella* track is complex and promotes the circulation of certain serovars. Thus, in 2010, in

our country, out of the relevant serovars, serovar *S. enteritidis* was dominant in breeding hens, laying hens and broiler chickens.

CONCLUSIONS

Pathological material samples were collected in accordance with the legislative provisions for holdings with breeding hens, laying hens and broiler chickens.
Bacteriological examinations are conducted in accordance with the legislative provisions in authorized county laboratories and National Salmonella Reference Laboratory within IDAH Bucharest where serotyping is performed as well.
In 2010, 655 strains of mobile Salmonella were isolated from breding hens, laying hens and broiler chickens. For the breeding hens, relevant serovar *S. enteritidis* had the highest incidence - 15 strains were identified, for relevant serovar *S. typhimurium* only one strain was isolated, while the other relevant serovars represented by *S. infantis*, *S. hadar* and *S. Virchow* were not identified. For the laying hens, out of the two relevant serovars, *S. enteritidis* had the highest incidence - 19 strains were identified, and 5 strains were identified for serovar *S. typhimurium*. In farms of broiler chickens *S. enteritidis* had the highest incidence as well - 38 strains were identified, while serovar *S. Typhimurium* was not identified.
Following the analysis of data, we notice that serovar *S. enteritidis* was identified in breeding hens, laying hens and broiler chickens. As regards serovar *S. virchow,* it was not identified in any category of poultry. Also, serovars considered exotic for Romania, such as *S. senftenberg* and *S. thompson* were isolated.
Most serovars isolated in 2010 from breeding hens, laying hens and broiler chickens are often isolated in EU or non-EU countries.

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IRIS MELANOMA IN CATS

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Abstract

Iris melanoma is a primary intraocular tumor with a high potential risc for metastasis, characterized by the presence of a single or a multiple hyperpigmentation focal areas, or diffuse hyperpigmentation of the anterior epithelium of the iris. This hyperpigmentation is due to an abnormal growth and proliferation of melanocytes. However, not any hyperpigmentation should be handled as a melanom, is required differential diagnosis with melanosis, iris nevi, iris cysts, iridal discoloration due to inflammation, or melanosis secondary to chronic inflammation process. Depending of the expanding and the size of the tumor, it may cause complications as glaucoma and uveitis. The enucleation, despite the metastasis risk, represents the only treatment option that can be considered.

Key words: *enucleation, feline, iris, melanoma, Russian Blue.*

INTRODUCTION

The most common primary intraocular neoplasm in cats is malignant melanoma of the anterior uvea, usually is unilateral, is found in cats of all ages, with no breed predisposition (Boydell and Enache, 2012). A melanoma is clinically characterized by malignant growth of melanocytes, cells that are dark in appearance due to the inclusion of the melanin pigment, or, on the contrary, unpigmented in amelanotic iris melanoma.

The iris involvement is characterized by the presence of one, or more, golden to dark, brown pigmented foci, that slowly (over months to years) coalesce to form larger pigmented areas and eventually involve most of the iris as it becomes diffusely hyperpigmented, thicker, and less mobile.

Iris melanomas in cats usually arise from the front of the iris surface, with extension to the ciliary body and choroid (Gelatt, 2007).

In the cat, ocular melanomas are more common than oral and dermal ones, and ocular and oral ones are more malignant that dermal ones, with higher rates of mortality and metastasis (Pigatto et al., 2010).

Not all iris hyperpigmentation lesions are melanomas or malignant lesions. Brown hyperpigmentation of the anterior surface of the iris may start out as single or multi-focal pinpoint, flat regions, termed iris freckle or nevus. The

hyperpigmentation may progress to diffuse iris hyperpigmentation or coalescing freckles but still without changing the contour of the eye, is termed iris melanosis. This is considered a benign process, but increasing darkness and size of pigmented areas can be observed over months to several years, and the cells may eventually undergo malignant transformation into iris melanoma. Melanoma must be differentiated from non-neoplastic lesions, including pigmented cysts, freckles, nevi, discoloration consequent to granulomatous or nongranulomatous inflammation; and other intraocular tumors: adenoma or adenocarcinoma, lymphosarcoma, and metastatic tumors (Peiffer et al., 2002).

MATERIALS AND METHODS

In the Clinics Department of Surgery from the Faculty of Veterinary Medicine Bucharest, two Russian Blue males had been examined and diagnosticated with iris melanosis. Periodical ophthalmic examinations highlighted the transformation of the hyperpigmentation area into tumoral, nodular masses which spreads and blocks the iridocorneal filtration angle. Evolution of the cases was different, two years, respectively five months.

Case A, Russian Blue, male, 6 years, in may 2010 the left eye presented a light-brown hyperpigmentation of the iris root, without changing the contour of the eye (Figure 1). This is considered a benign process, but increasing darkness and size of pigmented may eventually undergo malignant transformation into iris melanoma. Because of that the cat was closely monitoring through regular medical checks.

After 1 year the brown hyperpigmented area expanded with changing the contour of the pupil and distortion of the iris root, ring-shaped melanoma. After performing drug mydriasis, dyscoria was observed (Figure 2). Ultrasonography was performed, which revealed irregular iris thickening, a iris mass of 0.48 / 0.52 cm. Enucleation was recommended, but the owner did not accepted the treatment, thus that surgery was not performed. At the end of the year the hyperpigmented area is darker, increased in size, and anisocoria is obvious (Figure 3). The enucleation was performed after 2 years from the first visit.

Case B, a 6 year old cat, male, Russian Blue was examined in may 2012, presenting a brown hyperpigmentation of the iris root, localized at ”3 o'clock”, left eye, diagnosticated with iris melanosis. After 5 months the hyperpigmented area increased in size, deforming the sclero-corneal limbus. The complications, glaucoma and buphtalmia occurred, so that the eye is

painful. Physical examination was normal. As complementary exams had been realized complete blood cell count and serum chemical profiles, that were in physiological range. The abdominal ultrasonography did not shown any evidence of metastasis.

Treatment, the same for the both cases, was the enucleation of the eye, followed by cytological and histological examinations (Gelatt, 2001).

Surgical treatment, in terms of evolution, was applied differently, the enucleation was realized after 2 years, for case A, and after 5 months for case B.

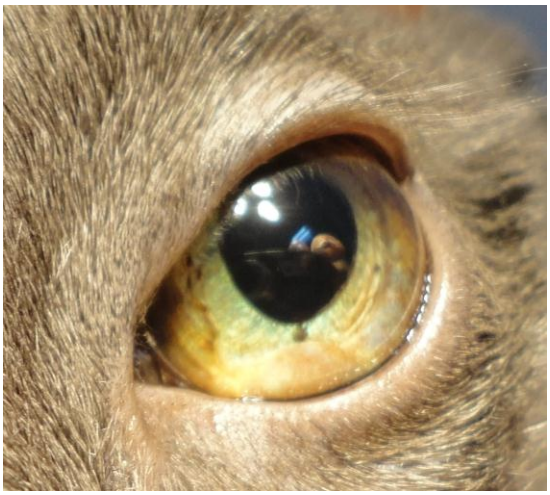

Figure 1. Case A, first medical examination, iris melanosis.

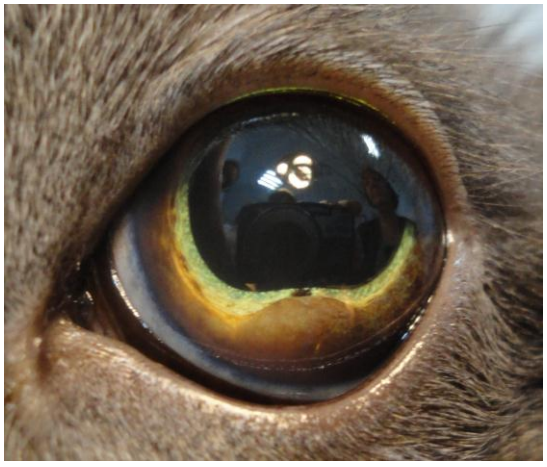

Figure 2. Case A, 1 year after the first medical examination.Dyscoria

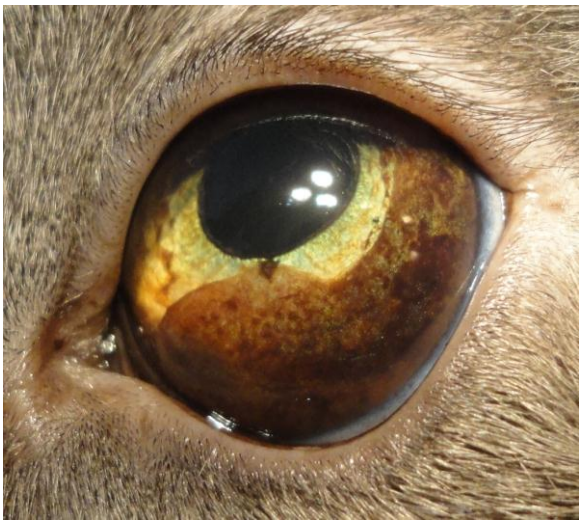

Figure 3. Case A, after 1and a half year from the first medical examination.

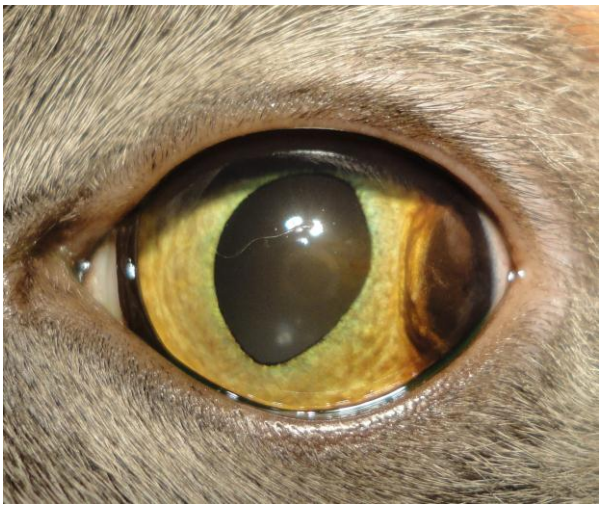

Figure 4. Case B, dark-brown hyperpigmentation of the iris root.

RESULTS AND DISCUSSIONS

Case A – after 2 years from the first visit the enucleation was performed, followed by histological and cytological examinations. Histologically, malignant transformation is characterized by a change in the histological

features of the cell. Tumor cells are exfoliated into the anterior chamber, implanted in the iridocorneal angle, and invaded the iridal stroma. Transformed cells tend to be round, with a large round nucleus and a proeminent nucleoli. Cytological appearance reveales tumoral melanocytes, epithelioid cells, with anisocytosis and anisokaryosis, brown intracytoplasmic granules (Figure 5). Histologically, irido ciliary melanoma with pleomorphic melanic cells, epithelioid and spindle, with moderate pigmentation and clear criteria of malignancy (Figure 6).

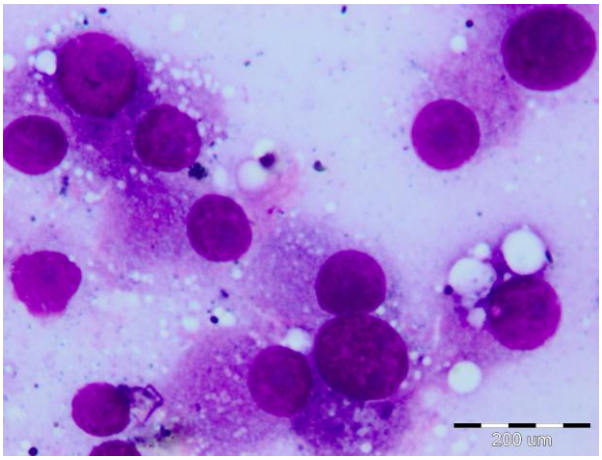

Figure 5. Case A, tumoral melanocytes, with anisocytosis and anisokaryosis. M-G G stain, ob. x 100

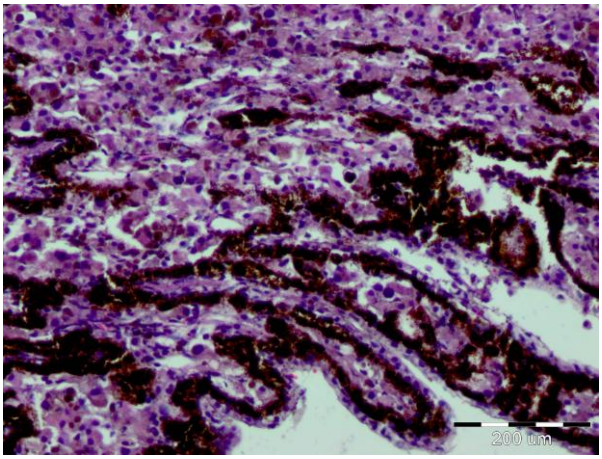

Figure 6. Case A, irido ciliary melanoma. HE stain, ob. x 20

Case B presented a rapid, a shorter evolution, for only 5 months, contrary to the case A, which had a 2 year evolution. The diagnostic in iris melanoma is estabilished after a complete ophthalmic examination: ophthalmoscopy, tonometry and eye ultrasound to evaluate thickening of the iris root and ciliay body, to define the tumor shape and the extent of local invasion. Depending on the lesion's size and invasiveness, complications such as secondary glaucoma, corneal edema, hyphema and anterior uveitis may occur. When complications like glaucoma and buphtalmia occures, such eyes fall into the category of the "blind painful eye" (Peiffer et al., 2002).
Cytologically are observed tumoral melanocytes, with anisocytosis, anisokaryosis, frequent karyomegaly and binucleation, along with numerous melanophages (Figure 7). The histopathological evaluation confirmed the tumor as a malignant melanoma with involvement of the iris stroma and the ciliary body. Predominantly spindle melanocytes, pleomorphic, with evident melanic pigmentation. Spindle cell tumors are arranged in streams and interweaving bundles (Figure 8).

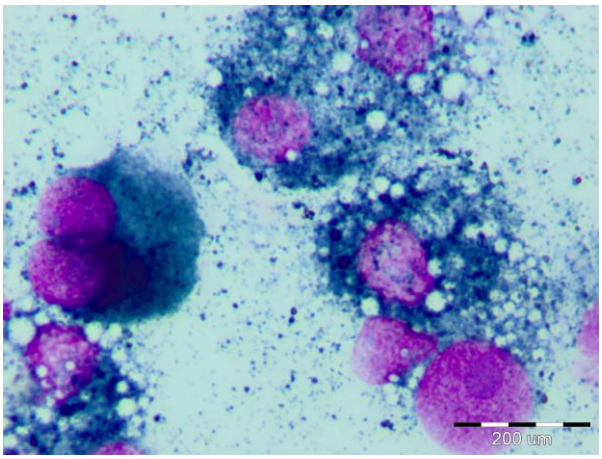

Figure 7. Case B, melanophages and binucleated cell. M-G G stain, ob. x 100

Differential diagnosis should include iris freckles or nevi, melanosis, pigmented uveal cysts, iridal discoloration due to inflammation and other uveal neoplasia. The diagnosis through of the fine needle aspiration

cytology of anterior segment is not recommended due to potential intraocular complications. (Boydell and Enache, 2012)

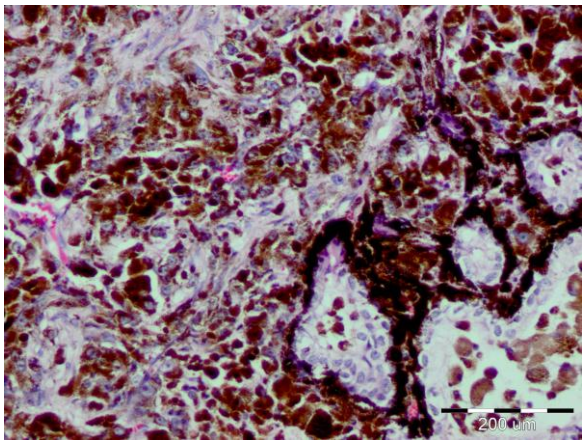

Figure 8. Case B, spindle melanocytes, pleomorphic. Spindle cell tumors are arranged in streams and interweaving bundles. HE stain, ob. x 20

Diffuse iris melanomas should be regarded as potentially aggressive malignant neoplasms with a potential for metastatic disease that can have long latency periods. They may extend through the sclera into the orbit or extend to the cranial cavity via the optic nerve and may spread to distant organs. The tendency of feline uveal melanomas is to metastasize first to regional lymph nodes and later to all visceral organs and to the skeletal system. Metastasis to the lungs, pleura, heart, pericardium, mediastinum, hilar lymph nodes, diaphragm, omentum, liver, spleen, bone, and brain has been documented (Peiffer et al., 2002).

A systemic examination should also be performed to evaluate and check for metastatic disease. This may include a complete blood profile, including a blood count and serum chemistry panel, thoracic and abdominal radiography or abdominal ultrasonography. This is necessary due to the risk of metastasis in organs such as regional lymph nodes, lungs and liver, being the main sites for metastasis. Feline intraocular melanoma is considered to have a greater metastatic potential than in dogs (Peiffer et al., 2002).

In both cases the treatment was surgically, the enucleation of the affected eye, and cytology and histopathology exams were performed from the

excised eye. After enucleation, the definitive diagnosis is confirmed by histophatology.

CONCLUSIONS

Ocular globe enucleation, despite the metastasis risk, represents the only treatment option that can be considered for iris melanoma.
The earlier the enucleation is performed, the better is the prognosis.
Iris melanoma represent the most common primary intraocular neoplasm in cats.
For iris melanosis and small iris freckles the treatment recommendation is monitoring closely through regular medical checks.
Not any melanosis should be treated as a melanoma, neither as melanoma should not be treated as melanosis.
Thoracic radiographs and complete blood work should be performed prior to enucleation.
The globe should always be submitted for histopathology examination in order to confirm the neoplastic disease.
The cats are alive, returns periodically to the clinic for regular checks.

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A CASE OF BLISTER DISEASE TO *BOA CONSTRICTOR*

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Abstract

Blister disease is a common condition in reptiles due to poor environmental management – that is, housing the reptile in overly-moist or dirty surroundings. It is also known as vesicular dermatitis. Later, these scales become swollen and infected by opportunistic bacteria (Pseudomonas spp.). Small reptiles or those with weakened immune system (either from previous illness, malnutrition or stress) can go downhill rapidly and die very fast from blister disease. This report describes a diagnostic and treatment strategy for an infectious dermatitis in a boa (Boa constrictor).

Key words: *Blister disease, Boa, Pseudomonas, reptiles*

INTRODUCTION

Naturally, boas can get a number of different health problems. We now wish to address the health problems that are most commonly encountered in captivity (Elliott, 2007, Harkewicz, 2001, White et al., 2011).
One of the most common reasons captive reptiles are presented to a veterinarian is for dermatologic disease (Harkewicz, 2001, White et al, 2011).
The most important cause for dermatopathies is inadequate management, especially species inappropriate temperature and humidity (Hatt 2010).
Blister disease (it is also known as vesicular dermatitis, scale rot, or necrotizing dermatitis) is a common condition in reptiles due to poor environmental management – that is, housing the reptile in overly-moist or dirty surroundings (Elliott, 2007, Mark and Thomas, 2009). As the animal is forced to lie on damp substrate saturated with rotting food or feces and urates, the skin becomes infected. Watery blisters are the first sign. Later, these scales become swollen and infected by opportunistic bacteria (*Pseudomonas spp.*). The infection may pass into the body causing septicemia and passing to internal organs.

Small reptiles or those with weakened immune system (either from previous illness, malnutrition, environmental or psychological stresses and other infections) can go downhill rapidly and die very fast from blister disease (Elliott, 2007, Paterson, 2006, White et al., 2011).
The skin may rot away from the initial blister, leaving the body more susceptible to bacterial and fungal invasion and thermal burns (Hatt, 2010, White et al, 2011).
This report describes a diagnostic and treatment strategy for an infectious dermatitis in a boa (*Boa constrictor*).

MATERIALS AND METHODS

One young snake (a 4-month-old male *Boa constrictor*) was submitted for clinical examination. About 4 days present clinical signs that included restless movements, anorexia, changes of skin colors into dark nuances and vesicular formation disposed in the third quarter of the body. The first clinical sing observed and described the owner was a pink to red appearance of the bottom most scales. The snake were housed individually in primary enclosures standard 40 Litre aquaria with screen lids. Water was provided in plastic water bowls. Light was provided by fluorescent light located in room ceiling with a 12 hour light/dark cycle. The ambient room temperature range was 28-32^{0}C, with the relative humidity of 60-80%. The snake was feed two juvenile mice weekly. Bedding consisted whole paper towels, this is changed every two weeks and were wet.

For the diagnosis, using a sterile syringe has been extract a reddish liquid from vesicle, and collected skin samples from infected area on sterile cotton swab previously soaked in a sterile saline solution were submitted for bacteriological (including test for susceptibility to antibiotics) and cytological investigations. Also were collected samples was inoculated on to sheep blood agar, MacConkey agar and Columbia agar. The plates were incubated at 37^{0}C, aerobically, for 72 hour, immediately after inoculation. Aerobic bacteria were characterized using standard phenotypic and biochemical properties.
Diagnosis is based from cultural and sensitivity taken aseptically from blister fluid is essential to isolate the pathogen involved (Paterson 2006).

RESULTS

Cultural examination of the fluid was positive for *Pseudomonas aeruginosa*. Cytological examination did reveal the presence of increased numbers of Gram negative bacillary bacterial cells.
After susceptibility test established a treatment for seven days, use enrofloxacin 5 mg/kg IM q 24 houer (Baytril®5%, Bayern Animal Health GmBH, Germany).
The blisters or inflammation should be treated as above and the enclosure thoroughly cleaned out of all residues. If the condition is the result of toxic substrates, the material not only needs to be removed and discarded, but the inside surfaces of the enclosure must be washed out with hot soapy water to remove all residues from the oils in the substrate. After thorough rinsing and disinfection, the enclosure may be outfitted with a proper substrate for the duration of the healing period.
Clinical condition of snake after therapy returned to normal.

CONCLUSIONS

Most of the lesions are on the ventral or underside of the animal which is why it is easy to miss. These fluid filled blisters may become infected with aggressive opportunistic bacteria, and if not treated promptly may lead to severe tissue (skin) damage, septicemia (blood poisoning caused by bacteria or their toxins) and death. You must examine your pet snake regularly in order to catch problems.
Poor condition may have created a suitable microenvironment for opportunistic *Pseudomonas* infection.
Early treatment is essential and the animal must be seen by a specialist vet. Other conditions can present in ways which may be mistaken for blister disease and so it is very important that the diagnosis is made correctly. When the treatment has been completed and the snake is well, it is very important that the environment is kept clean and dry at all times.
At the first suspicion of this disease, you must seek veterinary help.
Naturally, a proper examination of a sick boa requires a vet, who then prescribes the necessary medications and conducts those treatment methods that are beyond competence of the snake keepers.

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NECROTIZING FASCIITIS IN DOG – CASE STUDY

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Abstract

Streptococcus spp are common opportunistic pathogens of mammals and are associated with a variety of diseases affecting multiple organ systems. Necrotizing fasciitis is a severe, debilitating disease in adult dogs that can result in systemic illness and death. Toxic shock–like syndrome, a typically fatal sequel of necrotizing fasciitis in dogs. In dogs, S. canis is the most common streptococcal species isolated in cases of toxic shock–like syndrome associated with necrotizing fasciitis. This report describes a diagnostic management if necrotizing fasciitis in dog.

Key words: *necrotizing fasciitis, dog, Streptococcus*

INTRODUCTION

Necrotizing fasciitis is a severe, debilitating disease in adult dogs that can result in systemic illness and death (Barkha et al., 2012, Cătană, 2001, Jenkins et al., 2001). Toxic shock–like syndrome, a typically fatal sequel of necrotizing fasciitis in dogs (Barkha et al., 2012). In dogs, *Streptococcus canis* is the most common streptococcal species isolated in cases of toxic shock–like syndrome associated with necrotizing fasciitis (Lam et al., 2010, Lyskova et al., 2007, Miller et al., 1996).
Streptococci are a family of gram-positive bacteria some of which can cause either localized or systemic infections in both humans and animals. Some strains rarely cause disease and are often considered to be commensal (normal) inhabitants of the skin and mucosal surfaces (oral, nasal, intestinal), while other strains are capable of causing serious or even life-threatening infections (Cătană, 2001, Dewinter et al., 1999, Lyskova et al., 2007).

In dogs, Streptococci are known for their ability to occasionally cause septicemia (blood born infections) in puppies and a range of localized diseases in adults (Lyskova et al. 2007).

MATERIALS AND METHODS

One male German Shepard dog (a 12-year-old) was submitted for clinical examination, present bad clinical status, depression, in lateral recumbence, with high fever (40.5º C), 38 bpm respiratory rate, 172 bpm heart rate, short capillary refill time (CRT <1 second), bounding pulses, peripheral vasodilation, intensely painful subcutaneous lesions, and lameness in right side of the neck area. This clinical signs were associated with coughing up blood, bleeding from the nose, severe bruising of the skin, and bloody diarrhea. Aggressive supportive care included intravascular fluid therapy (Isotonic crystalloids 10-15 mL/kg - Lactated Ringer's solution; Colloid 40 mL/kg/day – Hetastrach 6% in 0.9 % NaCl HE span; Dexamethasone sodium phosphate, 4 mg/kg, IV; Vitamin K_1 – 2.5 mg/kg, SC), intravenous antibiotics (Clindamycin, 20 mg/kg/12 hour, IV) and nutritional support (Duphalyte, 30 mL/5kg). After four hours of treatment, rapidly develop severe hypotensive shock and disseminated intravascular coagulation and died. Post-mortem was revealed lesions of septicemia and gangrene. Samples were taken for bacterial culture and hematology (blood, skin and tissue). Hematology focused on the following parameters: complete blood count, prothrombin time, partial thromboplastin time, fibrinogen, d-dimer, clinical chemistry panel and blood gas evaluation. Cultivation and identification of bacterial species was performed by standard methodology. Samples collected were sown to achieve environment cultural examination on calf blood agar 5% and BHI agar. The plates were incubated for 24 hours in normal atmosphere at 37°C. For the rapid identification of the specific antigens of Lancefield group of streptococci was used Pastorex Strep® (Bio-Rad Laboratories).
Biochemical characteristics were assessed using API 20 STREP® multi test system (bioMérieux).

RESULTS

Clinical and laboratory findings suggested sepsis (25% band neutrophils and 23500 White blood cell count). Rapid progression of the infection, as well

as anatomopathological findings were characteristic for necrotizing fasciitis of a neck area caused by β-haemolytic *Streptococcus* infection. Cultural examination confirmed the presence of group G β-haemolytic streptococci associated with *Streptococcus canis*.

Typically, infected dogs are found in lateral recumbence, either being too weak to move or experiencing rigidity with mild convulsions. Rapid, uncontrolled fine muscle fasciculation's are often noted. A consistent and important clinical finding is a very high temperature (40.5º C).

Dogs that develop this disease appear to be normal and healthy prior to being recognized as very sick only a short time later. The course of the disease, from initial recognition of illness to death, can be as short as 6 hours. It is not uncommon for the dog to appear normal at bedtime and to be found dead the next morning.

As the disease progresses, a deep, non-productive cough, typical of pulmonary edema, develops. Rapid, spontaneous hemorrhaging, typical of disseminated intravascular coagulation, develops. This can be associated with coughing up blood, bleeding from the nose, severe bruising of the skin, and in some cases bloody diarrhea. Profound hypotension and toxic cardiomyopathy may develop.

CONCLUSIONS

Streptococcal septicemia in older dogs is often a sequel to localized infections, such as with necrotizing fasciitis. Streptococci are important opportunistic pathogens in the neonatal and adult dog. Streptococcal infection can result in septicemia as well as life-threatening localized infections in the skin and lung. Thus, isolation of *Streptococcus* does not necessarily correlate with disease and must be interpreted with consideration of clinical and pathologic findings.

Clindamycin seems to be particularly useful in acute cases because it halts the metabolism of the streptococci, stopping the cascade of toxins responsible for the high fever, shock and Disseminated Intravascular Coagulation (DIC).

The reason for the emergence/re-emergence of canine necrotizing fasciitis (toxic shock–like syndrome) is unclear and very little is known about transmission, prevention, or immunity following possible exposure.

Unfortunately, to date, advances in detection and prevention have been few. No vaccine has been developed, no medication has been found to be

effective in preventing the infection, and no test has been beneficial at identifying those animals at risk.

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RETROSPECTIVE STUDY OF THE EPIDEMIOLOGICAL AND MORPHOLOGICAL ASPECTS OF CUTANEOUS MALIGNANT MESENCHYMAL TUMORS IN DOG

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ABSTRACT

This study evaluated the epidemiological and morphological features, along with the efficiency of the cytological and histopathological diagnosis of cutaneous malignant mesenchymal tumors (MMT) in dogs. The study included 325 dogs with cutaneous MMT presented during five years (2007 – 2011) at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest. It was intended to establish: the predisposition of breed and sex, location of lesions, the incidence by years, seasons and months, and what sampling and type of investigations were used for the diagnosis. During these five years, a total of 3643 dogs with various lesions were subjected to pathological diagnosis and 1262 (34.5%) of them presented cutaneous/subcutaneous masses, of which 325 (25.7%) dogs were diagnosed with MMT. Breed predilection was not detected, but the affected dogs were mainly of medium and large breeds. Sex predilection was not apparent, both sexes being almost equally represented.

The incidence of the cutaneous MMT ranged from 14% in 2009, to 26% in 2011. The median age of the affected dogs increased from 8 years in the first 3 years, to 9 years in the last year of study. About half of the neoplasms were located on the limbs (49%). The attempt to correlate the incidence of the tumors with the season concluded that the majority of the diagnoses were established during spring (20.5%) with the fewest, during summer (12.5%). The samples were obtained by fine needle aspiration (54%) and surgical excision (46%). The most frequent MMT were mast cell tumor, (39%), hemangiopericytoma (24%) and histiocytic tumors (12%).

***Key words**: cutaneous, malignant, mesenchymal, tumors, dog*

MATERIAL AND METHODS

The study included 325 dogs with cutaneous MMT presented during five years (January 2007 – December 2011) at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest.

The main objectives of the study was to determine the incidence of cutaneous MMT, related to age, sex, breed, location and the morphological characteristics of MMT. Other objective of the study was to evaluate the importance of cytology and histopathology in diagnosing of cutaneous lesions.

The cytological samples were collected by fine needle aspiration (FNA) or surgical excision (SE). Impression smears and/or scraping were made from the surgical samples, followed by May–Grünwald Giesma (MGG) and Diff-Quick staining. The surgical excisions were fixed in 10% formaldehyde solution and Bouin's fixative and routinely processed. The sections were cut at 4-6 microns and stained with trichrome Masson or hematoxylin eosine (HE).

RESULTS AND DISSCUTIONS

A total number of 3643 dogs were presented at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine Bucharest, between January 1st, 2007 and December 31, 2011. A number of 1262 (34,6%) were diagnosed with cutaneous lesions, which brings cutaneous pathology of dog on the first position in our department.
Out of the 1262 dogs with cutaneous lesions, 325 (25.7%) were diagnosed with MMT. These 325 dogs were evaluated for the incidence of MMT related to breed, sex and location and the morphology of MMT. A special consideration was give also to the method of sample collection and examination. The possible correlation between epidemiology and morphology was evaluated by relating the epidemiological data to every year, month and season of the study.
Out of the 500 dogs examined in 2007, 172 (34.4%) had cutaneous lesions, and 55 (32%) of these 172 dogs, had MMT.

Table 1. Cases of cutaneous MMT diagnosed in 2007

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCATION**				**SAMPLING METHOD**		**EXAMINTION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	7	5F/2M	11 (9-13)	1	0	2	4	4	3	4	0	3
FEB	6	4F/2M	10 (9-12)	0	0	2	4	5	1	5	1	0
MAR	2	2F/0M	9 (5-13)	0	0	1	1	1	1	1	0	1
APR	8	6F/2M	9 (6-12)	1	1	3	5	6	2	6	0	2
MAY	6	3M/3F	10 (6-12)	0	1	2	3	3	3	4	1	1
JUN	3	3M/0F	8 (7-10)	1	1	3	1	2	1	2	0	1
JUL	4	3F/1M	5½ (4-7)	1	0	2	2	3	1	3	0	1
AUG	3	2F/1M	12 (11-13)	0	0	1	2	1	2	2	0	1
SEPT	5	3F/2M	5 (3mon-11)	1	0	2	2	1	4	2	0	3
OCT	7	4F/3M	8 (4,5-12)	2	0	1	4	4	3	4	0	3
NOV	2	2F/0M	10 (8-12)	0	0	2	1	1	1	1	1	0
DEC	2	2F/0M	9 (8-10)	0	0	0	2	0	2	1	0	1
TOTAL	**55**	**39F/16M**	**8 (3mon-13)**	**7**	**3**	**21**	**31**	**31**	**24**	**35**	**3**	**17**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

A number of 39 (71%) were females and 16 (29%) were males. This data contradicts data reported in previous studies, where males were more affected, with a raport of 2:1. The mean age of the dogs in this study was 8 years (range, 3 months – 13 years), is in agreement with other studies.

The cutaneous MMT were located mainly on the trunk and limbs, followed by head and neck. Some dogs had multiple lesions, located in two or three body regions. FNA was used in 30 cases (56.4%) and 25 of these were SEs. Of these 25 cases, 5 (20%) were submitted only to cytological examination, 3 (12%) were submitted only to histopathological examination and 17 (68%) cases were submitted both to cytological and histopathological examination.

Out of the 735 dogs examined in 2008, 277 (37.7%) had cutaneous lesions, and 66 (23.8%) of these 277 dogs, had MMT. Out of the 66 dogs with MMT, 31 (47%) were females and 35 (53%) were males. The mean age was 8 years (range, 3 months – 16 years), similar to the previous year.

Table 2. Cases of cutaneous MMT diagnosed in 2008

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCATION**				**SAMPLING METHOD**		**EXAMINATION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	5	4F/1M	10 (6-16)	1	0	3	2	2	3	3	0	2
FEB	7	3F/4M	9 (5-15)	1	1	3	2	4	3	5	0	2
MAR	7	0F/7M	9,5 (4,5-13)	2	1	2	2	5	2	6	1	0
APR	8	3F/5M	8 (1-13)	5	0	3	2	5	3	5	1	2
MAY	8	5F/3M	8 (9 mon-13)	1	0	2	6	5	3	6	0	2
JUN	9	6F/3M	8 (1-14)	0	0	5	4	5	4	5	3	1
JUL	4	3F/1M	4 (5 mon-9)	0	2	1	3	1	3	3	1	0
AUG	0	0F/0M	-	0	0	0	0	0	0	0	0	0
SEPT	1	1F/0M	8	0	0	0	1	0	1	0	1	0
OCT	6	2F/4M	8 (3mon-13)	0	1	1	6	5	1	6	0	0
NOV	7	2F/5M	10 (7-12)	0	0	3	4	3	4	3	2	2
DEC	4	2F/2M	9 (9-11)	1	0	0	3	3	1	3	1	0
TOTAL	**66**	**31F/ 35M**	**8 (3mon-16)**	**11**	**5**	**20**	**35**	**38**	**28**	**45**	**10**	**11**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

The limbs were the most affected, followed by trunk and head. The neck was the least affected, less than 10% of the lesions had this location. FNA was used in 57.5% (n=38) cases and 42.5% (n=28) of these were SEs. Of these 28 cases, 7 (25%) were submitted only to cytological examination, 10 (35.7%) were submitted only to histopathological examination and 11 (62.7%) cases were submitted both to cytological and histopathological examination. Out of the 700 dogs examined in 2009, 218 (31%) had cutaneous lesions, and 48 (22%) of these 218 dogs, had TMM.

Table 3. Cases of cutaneous TMM diagnosed in 2009

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCTION**				**SAMPLING METHOD**		**EXAMINTION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	3	0F/3M	7½ (2-13)	0	0	3	0	1	2	1	0	2
FEB	5	0F/5M	9 (1-12)	0	0	4	2	2	3	3	0	2
MAR	3	1F/2M	7½ (1-11)	1	0	3	1	2	1	3	0	0
APR	4	3F/1M	7 (5mon-10)	0	0	3	2	4	1	3	0	1
MAI	9	2F/7M	6 (5mon-15)	5	0	3	4	8	1	9	0	0
JUN	4	2F/2M	12 (9-15,5)	0	0	0	4	3	1	3	0	1
JUL	3	2F/1M	10 (9-11)	0	0	2	1	1	2	1	0	2
AUG	1	1F/0M	13	0	0	0	1	1	0	1	0	0
SEPT	2	1F/1M	10 (6-14)	0	0	3	1	2	0	2	0	0
OCT	4	2F/2M	9 (5-12)	0	0	0	4	2	2	3	0	1
NOV	9	4F/5M	9 (1-14)	3	1	6	4	7	2	8	0	1
DEC	1	0F/1M	7	0	0	0	1	0	1	0	0	1
TOTAL	**48**	**18F/30M**	**8 (5mon-15)**	**9**	**1**	**27**	**25**	**32**	**16**	**37**	**0**	**11**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

In 2009, cutaneous MMT affected significantly the males (62.5%, n=30), in comparison with the females (37.5%; n=18). The cutaneous MMT were located mainly on the trunk and limbs. FNA was used in 66.6% (n=32)

cases and 31.0% (n=16) of these were SEs. Of these 16 cases, 5 (31%) were submitted only to cytological examination and 11 (69%) cases were submitted both to cytological and histopathological examination. No sample was submitted only to histopathological examination. Out of the 807 dogs examined in 2010, 236 (29.5%) had cutaneous lesions, and 70 (29.6%) of these 236 dogs, had MMT.

Table 4. Cases of cutaneous MMT diagnosed in 2010

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCTION**				**SAMPLING METHOD**		**EXAMINATION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	2	0F/2M	7 (3-9)	0	2	2	1	2	0	2	0	0
FEB	5	3F/2M	11 (7-13,5)	1	0	1	3	4	1	4	0	1
MAR	11	7F/4M	8 (3mon-13)	3	0	4	6	5	6	6	0	5
APR	8	6F/2M	10,5 (7-14)	0	0	2	6	4	4	5	0	3
MAY	9	4F/5M	8 (1-14)	1	0	3	6	6	4	6	1	2
JUN	2	2F/0M	8 (4-12)	0	0	1	2	1	1	1	0	1
JUL	1	0F/1M	9	0	0	0	1	1	0	1	0	0
AUG	5	3F/2M	11 (9-12)	1	0	2	2	1	4	4	1	0
SEPT	4	0F/4M	8 (6mon-10)	2	0	1	1	2	2	2	0	2
OCT	8	2F/6M	7,5 (1-13)	0	1	4	5	3	5	4	1	3
NOV	9	4F/5M	8 (2mon-14)	3	1	3	3	3	6	5	2	2
DEC	6	2F/4M	5 (1mon-10)	2	1	1	3	2	4	5	0	1
TOTAL	**70**	**33F/ 37M**	**8½ (1mon-14)**	**13**	**5**	**24**	**39**	**33**	**37**	**45**	**5**	**20**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

In 2010, the number of males (n=37) was almost equal to the number of females (n=33), and the mean age increased with approximately 6 months. There were more cases of MMT located on the head, but the trunk and limbs remained the most affected regions.
In the previous year, FNA was the dominant method of sampling, but this year, FNA and SE were used almost in the same proportion.

Cytological examination remained the main method of examination, but the number of cases submitted both to cytological and histopathological examination increased. The main reason for this could be the interest of clinicians to receive both a fast preliminary diagnosis (cytology), but also a final diagnosis (histopathology). Another reason could be the increase in the level of responsibility of the dog owners, who acknowledge the major role of a final diagnosis in the prognosis and management of the therapy.
Out of the 901 dogs examined in 2011, 359 (39.8%) had cutaneous lesions, and 86 (23.9%) of these 359 dogs, had MMT.

Table 5: Cases of cutaneous TMM diagnosed in 2011

MONTH	**NO. CASE**	**SEX F/M**	**MEAN AGE (years)**	**LOCATION**				**SAMPLING METHOD**		**EXAMINATION METHOD**		
				H	**N**	**T**	**L**	**FNA**	**SE**	**C**	**H**	**C+H**
JAN	4	3F/1M	9 (1-14)	1	0	1	2	1	3	2	0	2
FEB	5	3F/2M	9 (2-12)	3	0	2	1	2	3	2	1	2
MAR	7	4F/3M	9 (2,5-13)	2	0	2	2	3	4	4	1	2
APR	6	2F/4M	3½ (4mon-7)	2	1	2	3	3	3	4	0	2
MAI	8	1F/7M	10(6mon-15)	0	0	3	5	6	2	7	0	1
JUN	11	5F/6M	11 (6-15)	3	0	3	5	1	10	5	1	5
JUL	7	3F/4M	10 (7-11)	0	1	2	4	4	3	5	1	1
AUG	6	4F/2M	9 (5-11)	2	0	3	2	4	2	5	1	0
SEPT	9	3F/6M	8½ (4-12)	1	2	2	5	5	4	5	1	3
OCT	10	6F/4M	10 (7-14)	2	2	4	2	4	6	5	4	1
NOV	5	2F/3M	10 (4-14)	1	2	2	1	4	1	4	1	0
DEC	8	3F/5M	8½ (6-14)	1	0	3	4	5	3	6	2	0
TOTAL	**86**	**39F/ 47M**	**9 (4 mon-15)**	**18**	**8**	**28**	**36**	**42**	**44**	**54**	**13**	**19**

F=female, M=male, H=head, N=neck, T=trunk, L=limbs, FNA= fine needle aspiration, SE= surgical excision, C=cytology, H=histopathology

In 2011, the males (54.6%) were more affected than the females (45.4%). The mean age increased to 9 years, but the age range remained the same. The incidence of the lesions located on the head increased; 21% of the MMTs developed in this location. 50% of the samples were collected by FNA, and 50% were collected by SE. Of these 44 SEs, 12 (27%) were submitted only to cytological examination, 13 (29%) were submitted only to histopathological examination and 19 (44%) cases were submitted both to cytological and histopathological examination. There was a constant and significant increase in the number of cases presented for diagnostic pathology. Thus, in 2011 there was a 80% increase in the number of cases, compared to 2007.

The number of cases with cutaneous lesions in 2011 increased with 90%, compared to 2007. The number of cases of cutaneous MMT increased in 2008, compared to 2007, but decreased in 2009. In 2010 and 2011, there was a constant and significant increase in the number of the cases with MMT. The reason of the 80% increase of the number of cases with cutaneous lesions in 2008, compared with 2007, could be the heat wave in the summer, when the temperature rised above 35°C for long periods of time. The dogs with long hair were more represented, knowing their sensitivity to the heat. The highest and the lowest number of cases with cutaneous MMT were diagnosed during spring and summer, respectively. Possible explanations could be the higher incidence of parasitic infestations (fleas, ticks, and mosquitoes) and allergies, the damaging effects of UV radiations and high temperatures during spring (Cranganu, 2009). The small number of cases in the summer could be correlated with the vacation time of the dog owners and the shorter working hours in our department.

The mean age of the dogs with MMT remained constant in the first 3 years of the study (8 years, range 3 months – 16 years), and increased in 2010 (8.5 years) and 2011 (9 years). The age ranges remained the same, during the 5 years study, but there were an increase in the 9-10 years category.

No breed was overrepresented and the breeds of the dogs in the study were German Shepherd, Boxer, Rottweiler, Labrador, cross-breed and mixed-breed. The breed distribution could be explained by preference of Romanian dog owners for these breeds. The over representation of the large breeds in this study is in agreement with the data reported in previous studies.

FNA was predominantly used between 2007 and 2009 and in the following two years, but the number of SEs increased. Even though histopathological examination is more expensive, it is more reliable in the final diagnosis of the lesion. Cytological examination was the main examination method.

The most frequent MMT were mast cell tumor (MCT) and hemangiopericytoma, followed by histiocytic proliferative disorders and fibrosarcoma. There were infrequent cases of liposarcoma, hemangiosarcoma and myxoma. The type and incidence of cutaneous MMT, according to every year of the study can be found in Table 6.

Tabel 6. The incidence major categories of MMT

Year	***Fibro***	***MFH***	***Lipo***	***Hem***	***HPD***	***MCT***	***Hemperi***	***Other***	***Total***
2007	9	3	0	2	2	26	10	3	**55**
2008	6	3	2	1	5	29	18	3	**66**
2009	8	0	3	0	3	14	17	3	**48**
2010	2	4	3	1	13	29	15	3	**70**
2011	8	1	0	1	17	30	19	9	**86**
Total	**33**	**11**	**8**	**5**	**40**	**128**	**79**	**21**	**325**

Fibro – fibrosarcoma; MFH – malignant fibrous histiocytoma; Lipo – liposarcoma; Hem – hemangiosarcoma; HPD – histiocytic proliferative disorders MCT – mast cell tumors Hemperi – hemangiopericytomas Other – other MMT

In 2009 and 2007, MCT had the highest incidence (29% and 48%, respectively). The incidence of hemangiopericytoma was approximately constant, but it increased in 2009 (35%). Between 2007 and 2009, there were 2-4 (4-8%) HPDs, but their incidence increased to 13 (19%) and 17 (20%) in 2010 and 2011, respectively. The incidence of HPDs increased significantly. The incidence of fibrosarcoma fluctuated from 16% in 2007, to 9% in 2008 and 17% in 2009. Few cases of MFH and hemangiosarcoma were diagnosed, their incidence ranging from 1 to 6%.

The cytological and histopathological aspects of the main MMTs are presented, as follows.

The diagnosis of MCT by cytology is in most of the cases straight forward. The smear is often highly cellular; the cells have round shape and metachromatic cytoplasmic granules. The nuclei are predominantly round and 1 or 2 nucleoli are evident. The cells could show heavy granulation of the cytoplasm, which obscures the nucleus or extended degranulation. The

background contains numerous free granules. The MCTs are rich in fibrous tissue, thus the cytological smear contains fibrocytes and fibroblasts (Fig. 1) The MCTs have various histopathological aspects (Weiss, 1994, Baba, 2002). The neoplastic cells show various grades of anisocytosis and anisokaryosis and are arranged in cords and sheets (Meuten, 2002, Manolescu, 2009). The arrangement of the cells between collagen bundles results in the multilocular appearance of the tumor (Fig. 2).

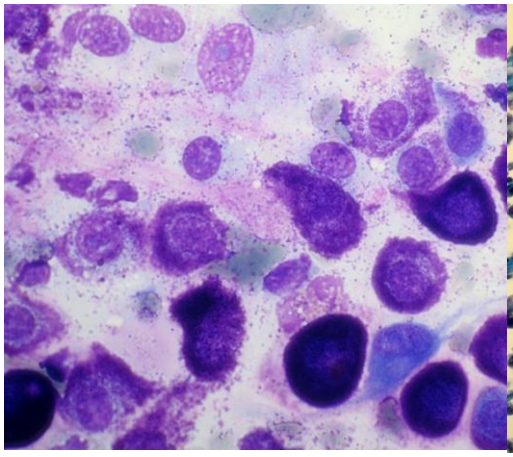

Figure 1. MCT. Highly cellular smear. Round cells with metachromatic cytoplamic granules. M-G.G. stain. x100

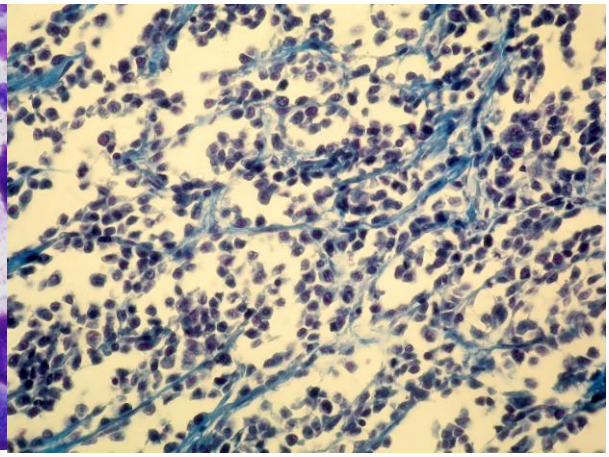

Figure 2. MCT. The cells are arranged in cords, between fibrous bundles. HEA stain. x 40

Hemangiopericytoma is included in the category of spindle cell tumors (Hendrick, 1998). The smears contains a monomorphous population of isolated or aggregated cells with elongated nuclei, 1-2 nucleoli and cytoplasm with short projection or star shaped, which is slightly basophilic (Fig. 3). The majority of the cells are dysplastic or slightly anaplasic.

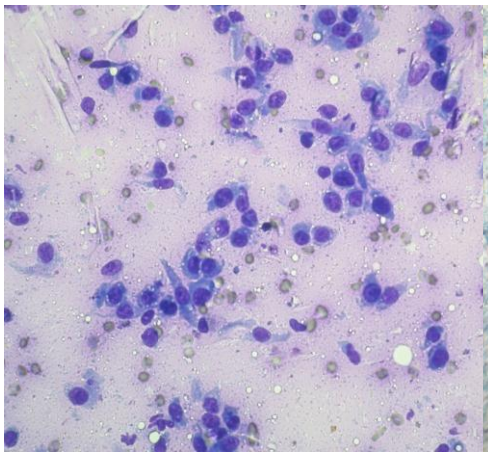

Figure 3. Hemangiopericytoma. Highly cellular smear. Spindle cells, isolated or aggregated, with cytoplasm with short projections or star shaped. M-G.G. stain. x40

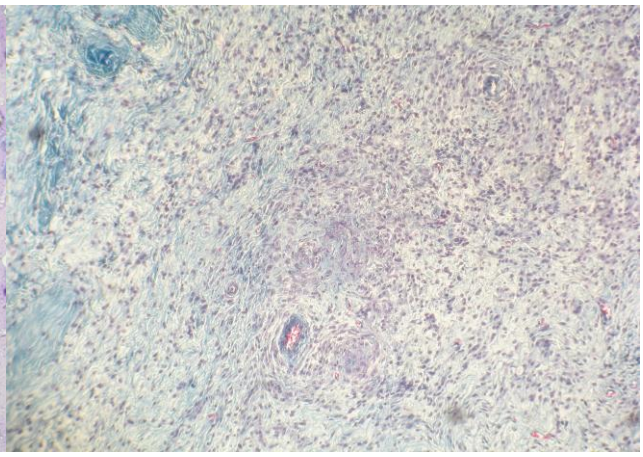

Figure 4. Hemangiopericytoma. Whirls of spindle cells arranged around blood vessels, which results in the "fingerprint" pattern of the tumor. HE stain, x10

The histopathological aspects are characteristic (Goldschmidt, 1992, Meuten, 2002). The spindle cells arearranged in whirls around blood vessels. The "fingerprint" pattern of the tumor can be seen in Fig. 4. The cells proliferated around collapsed capillaries (Baker, 2001, Raskin, 2010).
The cytology of ***fibrosarcoma*** depends on the grade of malignancy of the tumor. In low grades of malignancy, the spindle cells are dysplastic and/or with low anisocytosis and anisokaryosis. In high grade of malignancy, the pleomorphism is more obvious. The isolated cells have oval euchromatic nuclei, evident nucleoli, anizocaryosis and anisocytosis (Fig. 5).

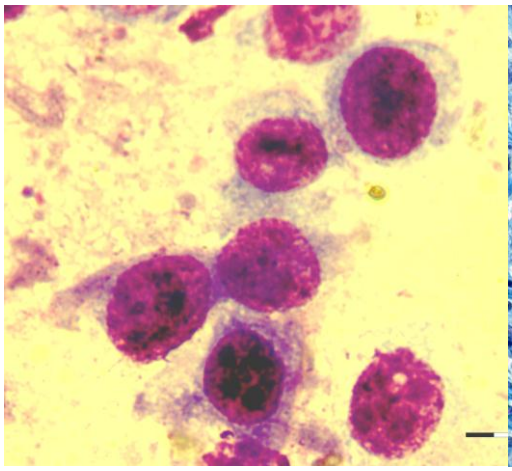

Figure 5. Fibrosarcoma. Monomorphous highly pleomorphic and malignant spindle cells. M-G.G. stain. x100

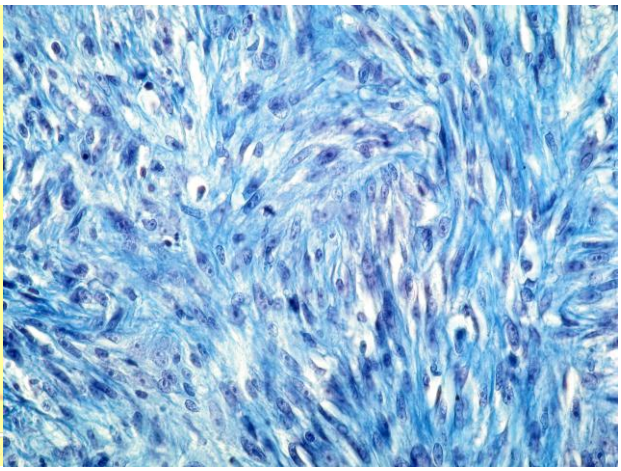

Figure 6. Fibrosarcoma. The neoplastic cells are arranged in intersecting cords. HEA stain. x40

The neoplastic cells are arranged in intersecting cords. The nuclei with marked anisokaryosis are located in a richly collagenic stroma (Fig. 6).

Malignant fibrous histiocytoma is one of the most aggressive MMT in dogs. Cellular pleomorphism, numerous multinucleated cells are usually seen in histopathological sections. The neoplastic cells are included in a richly collagenic mass, arranged in intersecting bundles and wirls (Fig. 7)

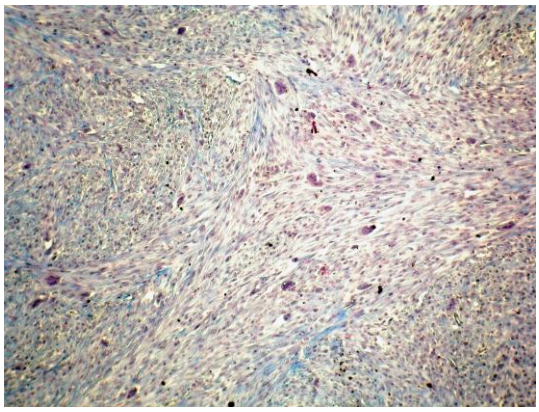

Figure 7. Malignant fibrous histiocytoma. Intersecting bundles of neoplastic cells and numerous multinucleated cells. HEA stain, x10

CONCLUSIONS

MMT represented 25.7% of the cases of cutaneous lesions presented for diagnostic pathology between 2007 and 2011.
The highest and the lowest incidence was in 2011 (26%) and in 2009 (14.7%), respectively.
There was no sex predilection. Out of the 325 dogs with MMT, 165 were males and 160 were females.
The mixed-breed and the cross-breed were overrepresented. MMT was diagnosed in Boxer, German Shepherd and Rottweiler, as well.
The mean age increased constantly, from 8 years in 2007, 9 years in 2011. The age range (1 month – 16 years) remained constant.
MMT developed mainly on the trunk (40% - 50%) and limbs (28% - 43%). The fewest lesions appeared on the neck (2% - 9%).
MMT is mainly a solitary lesion, but cases of multicentric location were noted.
Cytology by FNA or SEs was the main examination method.
MCT (29% - 48%), and hemangiopericytoma (18% - 35%) had the highest incidence of all the diagnosed MMTs.

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OVERVIEW OF THE EPIDEMIOLOGICAL AND MORPHOLOGICAL ASPECTS OF THE CUTANEOUS MALIGNANT EPITHELIAL TUMORS IN DOG

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Abstract

The malignant epithelial tumors (MET) are frequent and very important in the pathology of the dog's skin. The aim of the present study was to evaluate these tumors, both epidemiologically and morphologically. During 2007-2011, a total of 3643 dogs have been specifically examined; 224 of them had MET. The specimens were obtained by fine needle aspiration (60%) and surgical biopsy (40%). During these five years, a total of 3643 dogs have been specifically examined and 1262 (34.5%) of them had cutaneous lesions, and 224 (17.7%) dogs were diagnosed with MET. The incidence of the cutaneous MET increased with a constant rate, from 12% in 2007, to 23% in 2011. No predilection of breed was observed, but the majority of the dogs were medium and large breeds. 57% were males and 43% were females. The median age of the affected dogs was 9 years. The neoplasms were located on the trunk (34%), head (32.5%), limbs (30.5), neck (3%). The attempt to correlate the incidence of the tumors with the season concluded that the majority of the diagnoses were established during spring (30.5%) with the fewest, during summer (12%). Cytological examination was the single method for investigating 47% of the surgical samples. Sole histological examination was used for 17% of the surgical samples and 36% of the cases were diagnosed by both methods of investigation. The most frequent MET were squamous cells carcinoma, (31%), tumors with adnexal differentiation - malignant trichoepithelioma, malignant pilomatricoma (24%) and basal cell carcinoma (21.5%), but rarely were diagnosed: sebaceous carcinoma, apocrine carcinoma, and eccrine carcinoma. A constant increase of the incidence of cutaneous MET was observed in dog and the importance of cytological and histological examination was also demonstrated.

Key words: *cutaneous, dog, epithelial, malignat, tumor*

MATERIALS AND METHODS

The study was conducted between January 2007-December 2011 in the Department of Pathological Anatomy, Faculty of Veterinary Medicine Bucharest. From a total of 3643 dogs specifically examined, 1262 were diagnosed with cutaneous lesions. Of those 1262 dogs with skin lesions, 224 were diagnosed with MET, this representing the cases included in the study. Was followed MET incidence, epidemiological aspects (breed, age, gender, location) and morphology and the importance of anatomopathological,

cytological and histological exams in the diagnosis of cutaneous lesions in dogs. For the cytological exam were performed fine needle aspiration (FNA) or imprinting of the operatory piece (OP). The smears performed for cytological exam were stained with May-Grunwald Giemsa (M-G G) or quick Giemsa. The histological exam assumed the harvesting of tissue fragments, they were processed through the classical histological method, with the inclusion in paraffin, sectioned at 4-6 microns and stained with Masson trichrome method and HE.

RESULTS AND DISCUSSION

Between January 2007-December 2011 in the Department of Pathological Anatomy, Faculty of Veterinary Medicine Bucharest 3643dogs were specifically examined. Of whom, 1262 (34.5%) were diagnosed with cutaneous lesions, and among them 224 have been diagnosed with MET, which represents 17.7% of all diagnosed skin lesions.

In the year 2007, 500 dogs were examined, of which 172 (34.4%) had presented skin lesions. Of these 39 (22.7%) were diagnosed with MET.

Table 1.

Presentation of diagnosed cases in 2007 with MET

MONTH	**CASES**	**SEX F/M**	**AGE years**	**LOCALIZATION**				**RECOLT-ATION TYPE**		**DIAGNOSTIC TYPE OP**		
				H	**N**	**T**	**L**	**FNA**	**OP**	**C**	**H**	**C+H**
Dec.	1	1M	5	0	0	1	0	1	0	0	0	0
Jan.	9	2F/7M	10	4	0	3	2	7	2	1	1	
Feb.	4	3F/1M	9	1	1	0	2	1	3	2	1	0
	14/36%											
March	6	2F/4M	11	1	0	1	5	1	5	0	3	2
April	5	2F/3M	8	2	0	2	2	2	3	0	0	3
May	3	1F/2M	11	1	0	0	2	2	1	0	0	1
	14/36%											
June	2	1F/1M	7	1	1	0	0	2	0	0	0	0
July	3	2F/1M	9	0	0	2	1	3	0	0	0	0
Aug.	1	1F	7	1	0	0	0	1	0	0	0	0
	6/15%											
Sept.	2	2F	11	0	0	1	1	2	0		0	0
Oct.	2	1F/1M	12	1	0	1	0	2	0	0	0	0
Nov.	1	1F	6	0	0	0	1	1	0	1	0	0
	5/13%											
Total	**39/100%**	**18F/21M**	**9**	**12**	**2**	**11**	**16**	**25**	**14**	**3**	**5**	**6**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP = operatory pieces, C+H=cytologic and histologic.

In the year 2008 has been recorded a total of 735 cases, 47% more than in 2007. Among these 277 (37.7%) presented skin lesions and 54 (19.5%) were diagnosed with MET.

Table 2.

Presentation of diagnosed cases in 2008with MET

MONTH	**CASES**	**SEX F/M**	**AGE years**	**LOCALIZATION**				**RECOLTATION TYPE**		**DIAGNOSTIC TYPE OP**		
				H	**N**	**T**	**L**	**FNA**	**OP**	**C**	**H**	**C+H**
Dec.	2	1F/1M	10	1	0	1	0	2	0	0	0	0
Jan.	4	1F/3M	9	0	2	1	1	1	3	1	0	2
Feb.	5	1F/4M	11	1	1	2	1	3	2	1	0	1
	11/20%											
March	5	2F/3M	10	0	1	3	1	5	0	0	0	0
April	5	5M	9	1	0	3	1	2	3	1	2	0
May	4	4M	7	1	1	0	2	3	1	0	1	0
	14/26%											
June	9	4F/5M	9	3	0	2	5	5	4	2	1	1
July	3	1F/1M	9	0	0	1	2	2	1	0	1	0
August	3	2F/1M	8	1	1	1	0	2	1	0	0	1
	15/28%											
Sept.	5	1F/4M	8	3	1	1	0	2	3	0	3	0
Oct.	4	4F	12	2	0	2	0	2	2	0	0	2
Nov.	5	3F/2M	9	2	1	1	1	3	2	0	1	1
	14/26%											
Total	**54/100%**	**20F/34M**	**9**	**15**	**8**	**18**	**14**	**32**	**22**	**5**	**9**	**8**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C+H=cytologic and histologic.

In the year 2009, were examined 700 canine, 218 (31%) of them had skin lesions, of these 26, respectively 12% were diagnosed with MET.

Table 3

Presentation of diagnosed cases in 2009 with MET

MONTH	CASES	SEX F/M	AGE years	LOCALIZATION				RECOLTATION TYPE		DIAGNOSTIC TYPE OP		
				H	N	T	L	FNA	OP	C	H	C+H
Dec.	2	1F/1M	11	0	0	2	0	2	0	1	0	1
Jan.	4	1F/3M	9	2	0	0	2	3	1	2	1	1
Feb.	1	1F	11	1	1	0	0	1	0	0	0	1
	7/27%											
March	4	1F/3M	5	1	1	1	1	3	1	2	1	1
April	3	2F/1M	8	0	0	0	3	1	2	3	0	0
MaY	4	3F/1M	7	0	0	3	1	4	0	3	0	1
	11/42%											
June	1	1M	10	0	0	1	0	1	0	1	0	0
July	1	1 M	12	0	0	1	0	1	0	1	0	0
August	1	1 M	12	1	0	0	0	1	0	0	0	1
	3/12%											
Sept.	1	1 M	9	0	0	1	0	1	0	1	0	0
Oct.	2	2 M	8	1	0	1	0	2	0	1	0	1
Nov.	2	1F/1M	1	0	0	2	0	2	0	1	0	1
	5/19%											
Total	**26/100%**	**10F/16M**	**9**	**6**	**2**	**12**	**7**	**22**	**4**	**16**	**2**	**8**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C+H=cytologic and histologic.

In 2010 has been registered an increase in the number of cases, compared to the previous years, respectively 807. Of those 807 dogs examined, 236 (29.5%) were diagnosed with cutaneous lesions, of whom 35, respectively 14.8% with MET.

Table 4

Presentation of diagnosed cases in 2010 with MET

MONTH	CASES	SEX F/M	AGE years	LOCALIZATION				RECOLTATION TYPE		DIAGNOSTIC TYPE OP		
				H	N	T	L	FNA	OP	C	H	C+H
Dec.	2	1F/1M	11	1	0	1	1	1	1	0	0	1
Jan.	1	1M	3	0	0	0	1	0	1	0	0	1
Feb.	4	2F/2M	11	1	1	1	1	1	3	2	0	1
	7/20%											

March	6	4F/2M	10	3	1	3	0	5	2	0	0	2
April	5	3F/2M	10	1	0	0	4	1	4	2	0	2
May	1	1F	11	0	0	1	0	1	0	0	0	0
	12/34%											
June	4	1F/3M	9	3	0	0	1	2	2	1	1	0
July	2	2F	9	1	0	1	0	2	0	0	0	0
August	1	1M	8	0	0	1	0	0	1	0	0	1
	7/20%											
Sept.	2	1F/1M	7	1	0	1	0	0	2	0	2	0
Oct.	3	1F/2M	11	2	0	1	0	2	1	0	0	1
Nov.	4	4M	8	2	1	0	1	0	3	1	0	2
	9/26%											
Total.	**35/100%**	**16F/19M**	**9**	**15**	**3**	**10**	**9**	**15**	**20**	**6**	**3**	**11**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C + H = cytologic and histologic.

In 2011 the number of examined cases was 901 dogs. Among those, 359 (39.8%) were diagnosed with cutaneous lesions, of which 70 or 19.5% presented TEM.

Table 5

Presentation of diagnosed cases in 2011 with MET

MONTH	**CASES**	**SEX F/M**	**AGE** years	**LOCALIZATION**				**RECOLTATION TYPE**		**DIAGNOSTIC TYPE OP**		
				H	**N**	**T**	**L**	**FNA**	**OP**	**C**	**H**	**C+H**
Dec.	9	6F/3M	9	5	0	2	2	3	6	2	1	3
Jan.	3	3F	8	0	1	1	1	3	0	0	0	0
Feb.	3	2F/1M	11	2	0	1	0	2	1	0	0	1
	15/21%											
March	7	3F/4M	11	5	0	0	2	5	2	0	0	2
April	7	3F/4M	10	4	0	2	1	7	0	0	0	0
May	3	1F/2M	11	0	0	1	2	2	1	0	0	1
	17/25%											
June	4	4M	9	1	0	1	2	2	2	0	1	1
July	7	5F/2M	11	1	0	3	3	5	2	0	0	2
August	4	1F/3M	12	0	0	3	1	2	2	0	0	2
	15/21%											
Sept.	9	3F/6M	10	3	0	4	4	6	3	1	1	1
Oct.	7	3F/4M	8	1	0	4	3	2	5	0	3	2
Nov.	7	3F/4M	9	3	0	3	2	4	3	0	1	2
	23/33%											
Total	**70/100%**	**33F/37M**	**9**	**25**	**1**	**25**	**23**	**43**	**27**	**3**	**7**	**17**

F=female, M=male, H=head, N=neck, T=torso, L=limbs, FNA=fine needle aspiration, OP=operatory pieces, C+H=cytologic and histologic.

According to the data presented, it can be observed that in 2011 was registered the highest number of diagnosed cases with skin lesions (n = 359),of which 70 presented MET and considering that the thermal comfort in the summer of 2011 was exceeded by high temperatures, over 35°C for long periods of time, can be suspected that the thermal factor, solar radiation in some cases, have been implicated as predisposing factors in triggering or activation MET. Although there is no one certain breed predisposition, breeds like Rottweiller, Cocker, German Shepherd, Caniche, but also their crossbreeds and common breeds presented a higher incidence of malignant epithelial tumors.
In the gender distribution was noted that in all five years studied, from 2007-2011, the most affected were males, with a rate of 54% in 2007, 63% in 2008, 61.5% in 2009, 54% in 2010, 53% in 2011, compared with females. In 2008 and 2009 we can notice a higher distance of males compared with females, on the incidence of malignant skin tumors, at the same time remarking that in the years 2007, 2010 and 2011, the gender differences are very low. Although the specialized literature indicates that the males are more likely to develop skin lesions, in the our studied cases it is shown that the gender differences are small or insignificant. Regarding to the location of malignant skin tumors, it can be seen that there are constant elements, but also fluctuates from year to year. Thus, the localization of the head and torso was maintained at a high level in 3 out of the 5 years studied, respectively in 2008, 2010 and 2011, except in 2009 when the first places were occupied by the locations on the torso and limbs, and also, except in 2007, when the first place was occupied by the limb localization, the only year in which the limb localization was paramount. Regarding to the location of malignant skin tumors, it can be seen that there are constant elements, but also fluctuates from year to year. Thus, the localization of the head and torso was maintained at a high level in 3 out of the 5 years studied, respectively in 2008, 2010 and 2011, except in 2009 when the first places were occupied by the locations on the torso and limbs, and also, except in 2007, when the first place was occupiedby the limb localization, the only year in which the limb localization was paramount.

In 2011, the head and torso localizations are maintained at an equal level, recording a maximum of incidence and closely followed by limb localization.
In 2007 limb localizations were the most numerous, while in 2008 and 2009 the torso localizations to sum up most of the cases, as well as head localization in 2010.Neck localization showed the lowest incidence, and in 2011 from a total of 70 cases, only in one case the lesions were located on the neck.
Fine needle aspiration and excisional biopsy represents the main methods of sampling intended for cytological and histological diagnosis.In general, in diagnosed cases with malignant epithelial skin tumors fine needle aspiration was the most frequently used, reaching a peak in 2011, just in 2010 the main sampling method was the excisional biopsy, and in 2008 have been equally used both sampling methods.Fine needle aspiration is an easily performed method, fast and cheap, allowing a rapid diagnosis (several hours) which makes it to be recommended by clinicians and preferred by owners.The high percentage recorded in 2011 it may be correlated with the economic crisis situation faced by our country, so that the owners preferred this method from economically reasons.It is true that the fine needle aspiration and cytological examination have their limits and some error margin, compared to operatory pieces and histological examination, whose relevance is higher.

Table 6

Collecting method of the used samples

Year	Cytological	Histological	Cytological& Histological
2007	11	5	23
2008	23	16	15
2009	16	2	8
2010	18	4	13
2011	38	10	22
	106	**37**	**79**

Collected samples through the fine needle aspiration puncture were examined and diagnosed only cytologically, while the excisional biopsy samples were examined and diagnosed or cytologically, or histologically, or also cytologically and histologically.As shown in chart 9 and the following table, the cytological examination has experienced significant increase in 2011, when 38 cases, respectively 54% were diagnosed through this method, compared to 2007 when many of the clinicians, but also the owners

preferred to perform both cytological and histological exams. The histological examination was preferred in 2008, following in frequency the cytological examination.

The lesional range encountered in the casuistry under study is presented in the following table:

Table 7

Anatomopathological examination results

Year	Cases	SCC	BCC	BSC	MTE	MP	AGC	EGC	SGC	MGC	CGC	Other MET
2007	39	10	13	0	8	1	2	2	0	0	0	3
2008	54	25	8	0	9	0	4	1	2	0	1	4
2009	26	9	4	0	4	6	0	1	0	0	0	2
2010	35	10	6	0	10	1	2	1	4	0	0	2
2011	70	15	17	2	16	2	1	0	3	1	1	12

SCC=squamous cell carcinoma, BCC=basal cell carcinoma,
BSC=basosquamous carcinoma, MTE=malignant trichoepithelioma,
MP=malignant pilomatrixoma, AGC=apocrine gland carcinoma,
EGC=eccrine gland carcinoma,SGC=sebaceous gland carcinoma,
MGC=Meibomian gland carcinoma, CGC=ceruminous gland carcinoma,
MET=malignant epithelial tumors.

According to presented data, is shown that the most diagnosed malignant epithelial tumor throughout the entire study period was the squamous cell carcinoma, followed by basal cell carcinoma.
Cytological diagnosis of squamous cell carcinoma has a high degree of relevance, especially in poorly differentiated tumors. Depending on the degree of differentiation, cellular atypia may be discrete in the case of well-differentiated carcinoma (Fig. 1) or severe in the poorly differentiated case (Fig. 2). Tumoral cells can be isolated or clusters in which case the intercellular desmosomal connections are often obvious.
The nuclei appear small and hyperchrome in well-differentiated carcinoma (Fig. 1) and large, with obvious anisokaryosis and prominent nucleoli in the poorly differentiated (Fig. 2). In the poorly differentiated type, in the cytoplasm can appear vacuolisation, located mainly perinuclear (Fig. 2). Constantly in squamous cell carcinoma, inflammatory cells are present, especially neutrophils, the keratin is an induced element of the inflammatory process (4, 5, 7).
Histological aspects have a high degree of specificity, which allows to establish easily the diagnosis. The cords and islands of epithelial cells with

varying degrees of squamous differentiation from the epidermis penetrate the dermis, and the keratin appears as oxyphil clusters, concentric, known as "keratosic pearls" (Fig. 3 and 4). In the poorly differentiated squamous cell carcinoma, the keratosic pearls may be missing, in the cytoplasm of the tumoral cells can be seen oxyphil tonofilaments of keratin (Fig. 5).

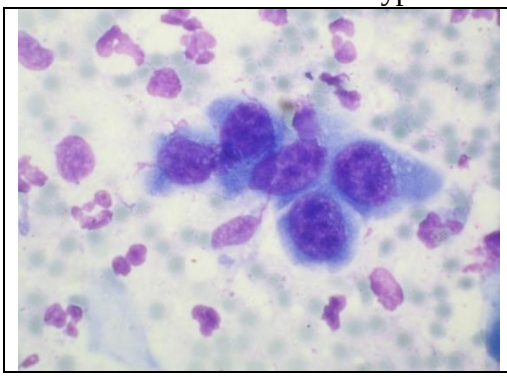

Fig.1.Poorly differentiated squamous cell carcinoma. Tumoral cells with severe anisokaryosis and obvious nucleoli. M-G G stain, 100x

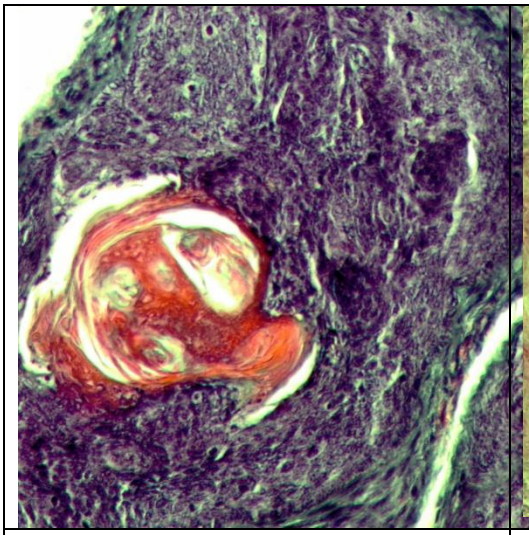

Fig. 2.Squamous cell carcinoma. Squamous cell proliferation and "keratosic pearls" inserted deep into dermis. Masson trichrome stain, 20x.

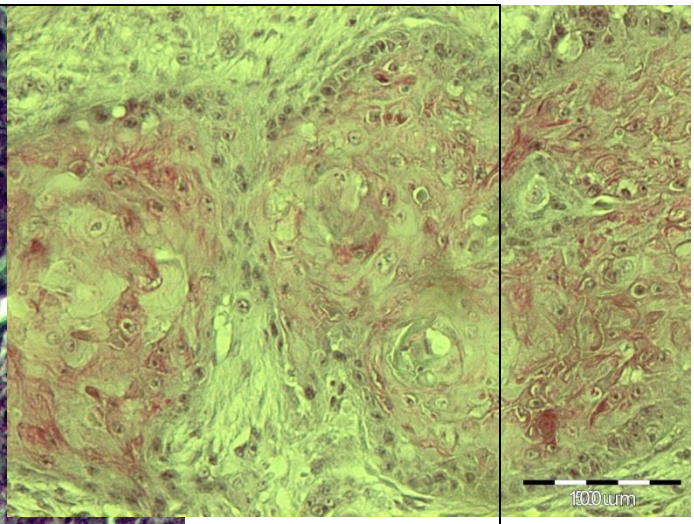

Fig. 3. Poorly differentiated squamous cell carcinoma. Anaplastic cells with intracytoplasmic keratine tonofilaments. Masson trichrome stain, 20x.

Basal cell carcinoma is an epithelial tumor with low degree of malignancy which can be differentiate either from the epidermis, or from the hair follicle epithelial structures.
In cytologically terms is characterized by cohesive sheet or ribbon of palisading epithelial cells, uniformly in shape and size, with N:C ratio generally 1:1, and only rarely dysplastic or with a reduced anaplastic grade (Fig. 6). Sometimes, the cells presents sebaceous differentiation, which makes difficult the differential diagnosis of this type of neoplasm and sebaceous gland tumors (2, 8). In these cases the histological examination allows to establish the correct diagnosis.

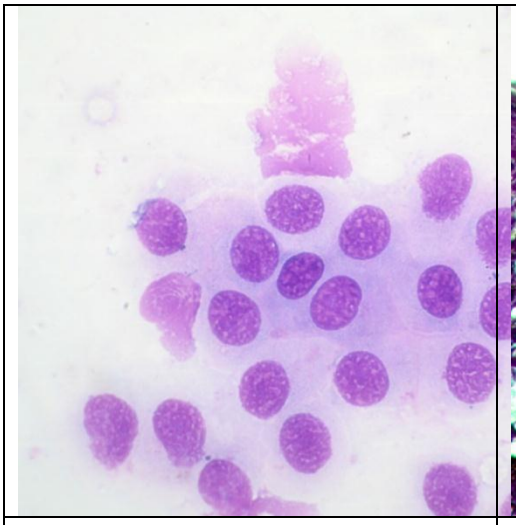

Fig. 6.Basal cell tumor. Epithelial cells of basal type, dysplastic and with a low anaplastic degree. M-G G stain, 100x

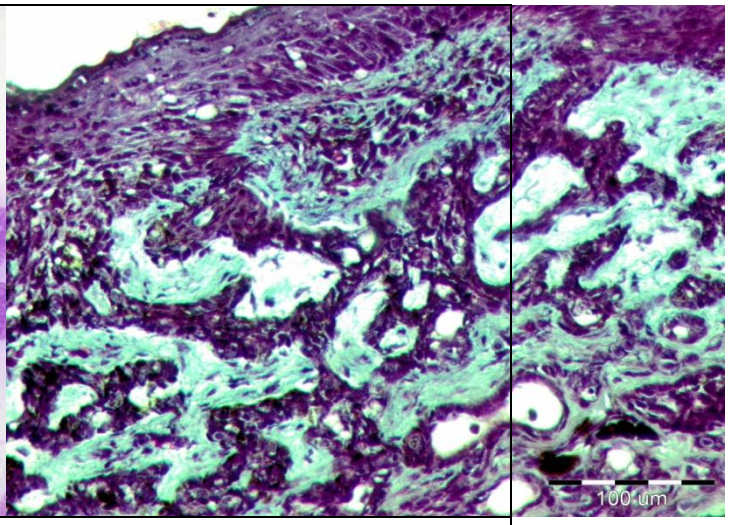

Fig. 7.Basal cell carcinoma - infiltrative type. Cords of epidermal epithelial cells infiltrated into the dermis. Masson trichrome stain, 20x.

Histological aspects encountered in basal cell carcinoma can be of two types: infiltrative or with clear cells (1, 4, 7). Histological examination had been established the diagnosis of basal cell carcinoma infiltrating type. Can be observed cords and tapes of basal epithelial cell type, with hyperchrome nuclei and reduced cytoplasm, which start from the epidermis to the dermis, inserting among its structures (Fig. 7).
Sebaceous gland carcinoma is a relatively rare tumor in dogs (2, 3). In cytologically terms is characterized by the presence of sebaceous cells with varying degrees of pleomorphism, with intracytoplasmic optical empty

vacuoles. Cytologically is difficult to differentiate the different types of sebaceous gland tumors (adenoma, epithelioma, carcinoma), and also differentiation of the sebaceous gland tumors of the basal cell tumors with sebaceous differentiation (2, 6, 8).
The histological examination allows to differentiate these lesions. Sebaceous gland carcinoma is characterized by the presence of sebaceous cells with moderate pleomorphism, with varying degrees of lipid charging of the tumoral cells (Fig. 8). The nuclei are large, with obvious nucleolus, sometimes atypical mitoses are detected.

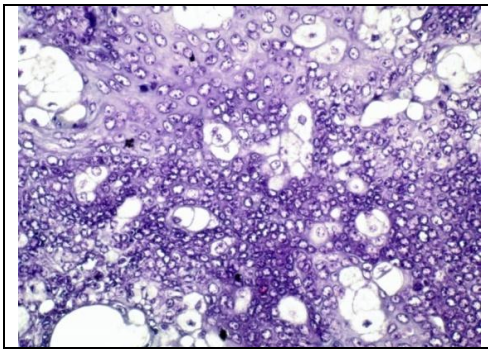

Fig. 8. Sebaceous gland carcinoma. Sebaceous epithelial cell type with moderate pleomorphism, with varying degrees of intracytoplasmic lipid charging, with large nuclei, with evident nucleolus. Masson trichrome stain, x40

Apocrine gland carcinoma develops from the epithelial secretory cells of apocrine glands. Are relatively common in dogs, are considered aggressive tumors with high metastatic potential. In histologically terms it may be encountered forms: solid, tubular and cystic. In our case has been detected the solid type, the most common type of apocrine gland carcinoma.
This type is characterized by proliferation of the epithelial cells, in the form of islands, separated by connective tissue. The nuclei are round or oval, pleomorphic, with evident nucleoli (Fig. 9).

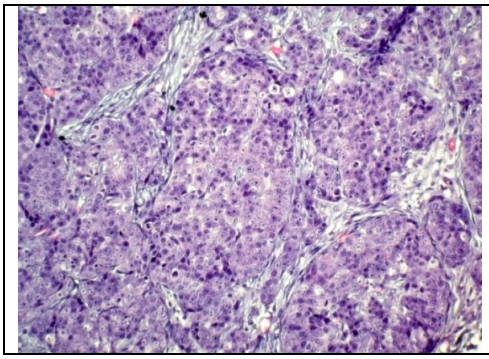

Fig. 9. Apocrine gland carcinoma.Proliferation of epithelial cells, with round or oval nuclei, pleomorphism with evident nucleoli, separated by connective tissue. Masson trichrome stain, 40x

CONCLUSIONS

Of the 1262 diagnosed dogs with cutaneous lesions, 224 (17.7%) presented malignant epithelial tumors.
The lowest incidence of malignant epithelial tumors was registered in 2009 - 12%, and the highest in 2007 - 22.7%.
The most affected breeds were Cocker, German Shepherd, Caniche, Rottweiler, but especially their crossbreeds.
The average age of diagnosed animals with MET was 9 years, maintaining constant throughout the 5-year study considered.
Males were more affected (56.69%) compared to females (43.30%).
The most common sites were on the torso, head and limbs.
The most utilized method was the cytopathology diagnosis through the fine needle aspiration puncture.
The most common diagnosed malignant epithelial tumors were squamocellular carcinoma (31.25%), basal cell carcinoma (21.42%), malignant trichoepithelioma (19.64%).

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MANAGEMENT OF DACRYOCYSTITIS IN A RABBIT

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Abstract

The rabbit's anatomy of the nasolacrimal system is unique, with a single lacrimal punctum and a tortuous nasolacrimal duct. Inflammation of the nasolacrimal duct, dacryocystitis, can be caused by primary infection with bacteria from the respiratory tract, secondary to infectious conjunctivitis or nasolacrimal duct obstruction. The latter can occur due to the rabbit's anatomy features, the tears' high content of lipid, dental pathology, as a result of chronic inflammations, foreign bodies, neoplasms and hyperparathiroidism. Further investigations to reach a diagnosis and to treat the condition may require general anaesthesia.

A 5 year-old Angora rabbit was presented with dacryocystitis. The nasolacrimal duct could not be flushed. Conjunctival bacteriological samples isolated Coryneform bacteria, Staphylococcus and Pseudomonas that were sensitive to tobramycine and gentamycine. Local treatment with tobramycine was initiated with no improvement. Radiographic investigations in order to assess dental malocclusion were declined. Clinical improvement was achieved with acetylcysteine nebulization therapy and regular flushing of the nasolacrimal duct followed by topical instillation of aqueous antibiotic solution.

Key words: *acetylcysteine, dacryocystitis, nasolacrimal, nebulization, rabbit.*

INTRODUCTION

Ocular diseases of rabbits are important to diagnose and treat early as some of them are part of the clinical signs of systemic diseases with serious implications. Genetic defects, infections, congenital malformations, nutritional deficiencies, environmental and management factors are of importance in rabbit's eye disorders (Williams, 2007).

Dacryocystitis' ethiopathogenesis is correlated with the anatomical particularities of the nasolacrimal system (Marini, 1996). There is a single large nasolacrimal punctum located in the ventromedial fornix and a long and tortuous nasolacrimal duct that passes through the lacrimal and frontal bones, taking two flexures and emerging behind the dental roots to the nostril (Marini, 1996, Morera, 2005, Williams, 2007). The duct mucosa folds in some area and is lined up with columnar stratified or pseudostratified epithelium and the submucosa is formed by connective

tissue very rich in vessels which may contribute to progression of the inflammation at this site (Morera, 2005).
The nasolacrimal duct can be affected either by a primary infection either by a partial or complete obstruction caused by the underlying tooth roots elongation that leads to secondary infection and altered drainage (Morera, 2005, Williams, 2007). Tooth root elongation is the most common dental disease in pet rabbits that are fed mostly commercial food instead of fresh and dried grasses (Harcourt-Brown 2002, Williams, 2007). Elongated roots can penetrate the bone and emerge through the periosteum (Harcourt-Brown, 2002).
Clinical signs of dacryocystitis vary with the etiology and the degree of obstruction and include marked epiphora, serous then purulent white, creamy ocular discharge (Figure 1), conjunctivitis, nasal discharge, blepharitis, periocular dermatitis and alopecia, secondary keratitis and dental disease (Florin et al., 2009, Morera, 2005, Williams, 2007).

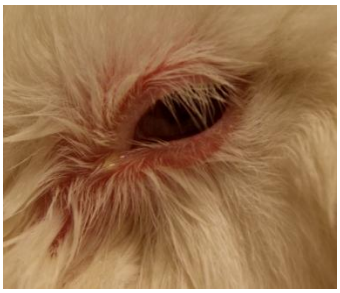

Figure 1.Epiphora with mucoid ocular discharge, blepharitis, periocular dermatitis (Enache original)

In a study of dacryocystitis in 28 rabbits the mean age was 4.4 years old, 89% was unilateral, 35% had an unknown cause, 50% had underlying dental diseases and 7% had nasal discharge (Florin et al., 2009).
Of 344 rabbits examined at the Veterinary Medical Hospital, Davis, California only 10% had ocular diseases, of which 73% had clinical signs of dacryocystitis (Burling, 1991).
Another research study performed on 586 New Zealand white rabbits showed an incidence rate of the ocular diseases of 9.6% and the most common condition was blepharitis and only 0.2% of the rabbits had dacryocystitis (Jeong et al, 2005).

MATERIALS AND METHODS

A 5 year-old Angora rabbit with a history of ocular discharge (Figure 2) presented at the Ophthalmology Department of the Faculty of Veterinary Medicine Bucharest for ophthalmic examination.

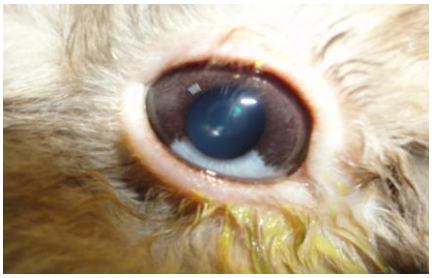

Figure 2.Ocular discharge (Ionascu original)

Focal illuminator, a direct and indirect ophthalmoscope were used for examination. Schirmer tear test strips and fluoresceine solution were part of the diagnostic procedures.
The oral cavity was also inspected for dental diseases but no macroscopic abnormality was detected. Further investigations by imaging the dental roots were declined. Bacteriological samples of the conjunctival discharge were collected and treatment was initiated based on the antibiogram.
Oxybuprocaine 0.4% was instilled into the inferior conjunctival sac as local anaesthesia to allow nasolacrimal duct irrigation. A syringe of two mL and a 21 G intravenous catheter with the stylet removed were used to approach the nasolacrimal punctum and to inject warm saline. A portable pediatric nebulizer device had ensured nebulization of the acetylcysteine solution mixed with saline prior to catheterization.

RESULTS AND DISCUSSIONS

The rabbit presented with intermittent periods of ocular discomfort as having epiphora, conjunctivitis with white, creamy ocular discharge and palpebral edema of both eyes. Clinical examination revealed nothing abnormal with no history of disease in the past.
Ophthalmic examination (Figure 3) showed good sight of vision, symmetric eyes, iridal heterochromia of both eyes and the presence of copious creamy

material at the medial canthus that could be expressed by digitally pressing the skin area of the nasolacrimal duct.

Figure 3.Rabbit ophthalmic examination (Enache original)

The Schirmer tear test values were over 20 mm, not due to excessive tearing, but the deficit in the tears' drainage (Figure 4). The fluoresceine test was negative for corneal erosions and the solution did not pass through nasolacrimal duct (Figure 5).

Figure 4.Schirmer tear test (Enache original)

Figure 5.Fluoresceine test (Ionascu original)

Conjunctival swab samples were obtained for bacteriologic culture at the Microbiology Department of the Faculty of Veterinary Medicine Bucharest and showed the presence of Coryneforms bacteria in the left eye and

Coryneforms, Staphylococcus and Pseudomonas in the right eye, sensitive to tobramycin, gentamicin and resistant to clindamycin.

Purulent ocular discharge with conjunctival hyperemia is a common sign of dacryocystitis but also to conjunctivitis. There have been several studies of the normal bacterial flora of the nasolacrimal system in rabbit (Cooper, 2011, Marini et al., 1996). The most common microorganisms isolated in the affected rabbit's conjunctiva were Moraxella sp., Oligella urethralis, Staphylococcus aureus, coagulase-negative Staphylococcus sp., and Streptococcus viridans and those isolated from the nasolacrimal duct flush fluid were Moraxella sp., S. viridans, and Neisseria sp (Marini et al., 1996). All microorganisms isolated were part of the normal conjunctival and nasolacrimal duct flora (Marini et al., 1996). An experimental chronic dacryocystitis was also obtained following inoculation of Staphyloccocus aureus in the lacrimal sac (Ishikawa et al., 2011, Snyder et al., 1976).

Treatment of dacryocystitis depends on the cause of the condition. Initially, daily periocular toilet and topical antibiotic instillation are performed for several weeks (Morera, 2005, Williams, 2007, Harcourt-Brown, 2002). If the ocular discharge is thick, acetylcysteine 1% can be used as a mucolytic along with repeated cannulation and flushing of the duct to remove the debris (Morera, 2005, Williams, 2007, Harcourt-Brown, 2002). The duct can be flushed with orofloxacin or gentamicin solutions or with an antibiotic based on the antibiogram but sometimes it requires repetition over several days to weeks (Williams, 2007). Severe cases require systemic antibiotics and pain relief and dental extractions if appropriate.

In this case, treatment was initiated with tobramycin instillations of one drop each eye twice daily. Tobramycin has been used for bacterial keratitis in a rabbit study (Bu et al, 2007).

The ocular signs persisted after four weeks of treatment, initial attempt of flushing the nasolacrimal duct was unsuccessful confirming the nasolacrimal duct obstruction. Revisit after one month of treatment had not shown significant improvement.

The second attempt of flushing the nasolacrimal duct was performed with acetylcysteine and saline solutions after inhalation of acetylcysteine by nebulization (Figure 6). This time the debris material could be flushed. Irrigation was performed under local anaesthesia with the conscious rabbit (Figure 7), with no undesirable reactions, although many authors recommend it under general anaesthesia (Morera, 2005, Williams, 2007). A 21G intravenous catheter was used, but 22 G or 24 G sizes can be used to minimize the risk of injuries (Morera, 2005, Harcourt-Brown, 2002).

Figure 6.Nebulization therapy (Enache original)

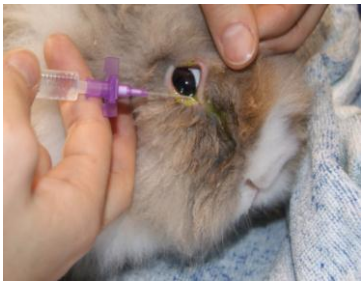

Figure 7.Nasolacrimal duct irrigation (Enache original)

Irrigation should always be performed slowly, without excessive pressure as there is a high risk of breaking the duct (Morera, 2005, Harcourt-Brown, 2002).
Acetylcysteine was proven to reduce the activity of the mucociliary system against tobramycin absorbtion and therefore to improve its action (Wang et al, 2000). Nebulization allows a higher bioavailability of the drug as the clearance of the small droplets is slower (Wang et al, 2000).
Treatment with local instillation twice daily of tobramycin and hyaluronic acid eye drops continued along with duct irrigations every 3 days, for two weeks. With irrigations and nebulization therapy the clinical signs improved, the palpebral edema and conjunctivitis disappeared (Figure 8).

Figure 8.Clinical aspect after treatment (Enache original)

Telephone updates were collected and there was no more eye discharge except occasional tearing when eating carrots.

CONCLUSIONS

Epiphora can be associated with early signs of dental disease, causing the blockage of the nasolacrimal duct.
Based on the ocular signs and the response to treatment, the nasolacrimal duct was either partially blocked with purulent material caused by a bacterial infection or partially obstructed by dental roots elongation.
Nasolacrimal duct is predisposed to obstruction due to infectious diseases, dental diseases or maxillary bone changes secondary to nutritional hyperparathyroidism.
As further investigations of dental roots were declined, a good management of this condition was achieved through repeated nebulization and flushing duct therapy along with local antibiotic treatment.

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PRELIMINARY DATA IN COMPARATIVE SERODIAGNOSTIC OF *NEOSPORA CANINUM* IN DOGS

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Abstract

For serological investigation of N. caninum infection in dogs more tests are available, including indirect fluorescent antibody test (IFAT), often considered as the reference test, and enzyme-linked immunosorbent assay (ELISA).
A total of 28 dogs were screened with a commercially multi-species indirect ELISA, including a subset of 9 samples previously tested by IFAT 1:50. A partial correlation was attempted between the two tests at the cutoff recommended by the manufacturer.
Seroprevalence on ELISA was 10.7% (3/28, $CI_{95\%}$=2.26-28.23), and all positive samples were also positive on IFAT. From previously tested samples by IFAT (8 positive and one negative), only 4 samples had the same result by ELISA (3 positive and one negative sample) and one sample was doubtful. Regarding the double tested samples, a poor agreement was found between the two tests (k=0.135) and difference between the prevalence obtained by the two techniques was statistically significant (p=0.05). Sensitivity and specificity were not determined because of the low number of samples tested so far, but is already planned in an outgoing experiment, as well as testing Neospora IFAT positive samples for Toxoplasma, to exclude false positive results.
It seems that IFAT is more appropriate than indirect ELISA for seroprevalence studies, and use of this indirect ELISA may require some techniques for adjustment of misclassifications.

Key words: *dogs, ELISA, IFAT, Neospora caninum.*

INTRODUCTION

Neosporosis was first described in puppies in Norway in 1984 (Bjerkas et al., 1984), but the causative organism, a protozoan parasite closely related with *Toxoplasma gondii*, was named *Neospora caninum* in 1988 (Dubey et al., 1988).
Although often *Neospora caninum* does not produce clinical signs of disease in adult dogs, this infection is epidemiologically important because the dog is the definitive host of the parasite (McAllister et al., 1998). Neosporosis is an important cause of abortion in cattle (Anderson et al., 1991), the most common intermediate host.

Diagnosis of *N. caninum* infection in dogs is based on serological assays such as the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assays (ELISA)(Silva et al., 2007), but IFAT is considered as a reference test in dogs naturally infected with *N. caninum* (Bjorkman and Uggla, 1999). Fewer data are reported for the use ELISA in detection of *N. caninum* infection in dogs.
The aim of this study was to compare two serological tests (IFAT and ELISA), frequently used for the diagnosis of *N. caninum* infection in dogs.

MATERIALS AND METHODS

A total of 28 dog sera, collected in 2011 and 2012, as a part of a larger seroepidemiological investigation, were included in this preliminary study. Dogs came from Bucharest and surroundings.
All sera were tested by indirect ELISA, but a subset of 9 samples was also tested by IFAT in a previous study (Mitrea et al., 2012). Some of tested dogs (n=3) showed neurological disorders (paresis, ataxia, myoclonus).
In order to detect the anti-Neospora caninum antibodies of the IgG class, two commercially available tests were used: IFAT (FluoNEOSPORA c., Agrolabo S.p.A., Italy) and a multi-species indirect ELISA (ID Screen Neospora caninum Indirect Multi-Species, ID-VET Lab., Montpellier, France).
The two tests were performed following exactly the manufacturer's instructions. The optical density values of indirect ELISA were read at 450 nm, using a spectrophotometer. The cutoff of the tests were S/P>50% (obtained by an equation provided by the manufacturer) for indirect ELISA and 1:50 dilution for IFAT. Only samples that demonstrated an apple-green fluorescence of the whole membrane of *Neospora* tachyzoites, using a fluorescence microscope, were considered positive for anti- *N. caninum* antibodies. For indirect ELISA, samples with S/P between 0.4 and 0.5 were considered doubtful.
Analysis of the data was performed using Fisher's exact test or Chi-square ($\chi 2$) test (Quantitative Parasitology 3.0 software. Statistical significance was assumed at $P \leq 0.05$. The test agreement was quantified by the Kappa (K) statistic, The K value can be interpreted as follows: < 0.20 poor; 0.21 - 0.40 fair; 0.41 - 0.60 moderate; 0.61 - 0.80 good; 0.81 - 1.00 very good (Altman, 1991).

RESULTS AND DISCUSSIONS

From a total of 28 dog serum samples tested by indirect ELISA, three were clearly positive (10.7%, $CI_{95\%}$=2.26-28.23) for *N. caninum* infection, and one was doubtful (3.6%).

From the 9 samples tested previously by IFAT, 8 were positive (88.9%). In the same set of samples, prevalence by ELISA was 33.3% (3/9). Difference between the prevalence obtained by the two techniques was statistically significant (*P*=0.05). All ELISA positive samples and the doubtful one were positive on IFAT for *N. caninum* infection.

In Europe, the seroprevalence rates of *N. caninum* infection varied between 0% and 51% in different countries and in different dog categories (Dubey and Schares, 2011).

In a preveously study conducted in south of Romania, specific antibodies were detected in 20.2% of dog sera by IFAT, with higher prevalence in cattle farm dogs (38.1%) (Mitrea et al., 2012).

No dog presenting neurological disorders was positive for *N. caninum.* This fact may sustain the asymptomatic evolution of *N. caninum* infection in dogs.

Performance of the indirect ELISA compared to IFAT was not as expected, especially in terms of positive samples (Table 1). The agreement between the two techniques at a confidence level of 95% was *k*=0.135 ($CI_{se(0)}$= -0.158, 0.428; $CI_{se(1)}$= -0.440, 0.709), which corresponds to a poor agreement (Altman, 1991).

Table 1. Correlation of results obtained by IFI and indirect ELISA

		ELISA			
		-	d	+	Total
IFAT	-	1	0	0	1
	d	0	0	0	0
	+	4	1	3	8
	Total	5	1	3	9

Test agreement:	
Kappa coefficient	0.135
Confidence interval for Kappa se(0)	-0.158, 0.428
Confidence interval for Kappa se(1)	-0.440, 0.709
Proportion of observed agreement	44.4%
Proportion of expected agreement	35.8%
Proportion of expected agreement minus hazard	8.6%
Maximum agreement not due to hazard	64.2%

For *N. caninum* infection, serological tests detects antibodies against surface antigens, more specific than intracellular antigens in *Apicomplexa* (Bjorkman and Uggla, 1999).
Dubey et al. (1988b) was the first to report the successfully use of an IFAT for detecting *N. caninum* infection in dogs, with a very little cross-reactivity with related protozoa and 100% sensitivity. This IFAT was based on whole in vitro grown tachyzoites as antigenic source. Almost the same succes was reported in a number of ulterior papers and this lead IFAT to be considered an almost perfectly specific diagnostic test for *N. caninum* infection.
On the other hand, for the screening of large numbers of sera, enzyme-linked immunoassays (ELISA) are usually cost effective and less time consuming (Lasri et al., 2004).
In the present study the ELISA test was evaluated for detection of *N. caninum* infection in dogs, with the IFAT considered as ”gold standard” at a confidence level of 95%. Table 2 presents the results from test evaluation.

Table 2. Evaluation of indirect ELISA test according to the gold standard

		Gold standard (IFAT)	
		Infected	Noninfected
Evaluated test (indirect ELISA)	Positives	3	1
	Negatives	5	0
Test evaluation:			
Sensitivity		37.5% (4.0%, 71.0%)	
Specificity		0.00% (0.00%, 0.00%)	
Positive Predictive Value		75% (32.6%, 117.4%)	
Negative Predictive Value		0.00% (0.00%, 0.00%)	
True Prevalence		88.9% (68.4%, 109.4%)	
Apparent Prevalence		44.4% (12.0%, 76.9%)	
Youden's J		-62.5% (-95.048%, -28.952%)	
Fiability		33.3% (2.5%, 64.1%)	

IFAT positive samples were classified according to the intensity of the fluorescence in positive samples (+) and intense positive samples (++). IFAT titers were not determined.
The results of ELISA and IFAT on individual samples are summarized in Table 3 and Figure 1.

Table 3. Intensity of response by IFAT and indirect ELISA

No of sample	Indirect ELISA		IFAT Cut-off 1/50
	S/P of sample	Clasification	
1.	68.48	Positive	+
2.	15.41	Negative	+
3.	74.22%	Positive	++
4.	3.47%	Negative	+
5.	39.98	Negative	++
6.	5.11	Negative	+
7.	43.58	Doubtful	+
8.	79.59	Positive	++
9.	0.94	Negative	-

+/++ Intensity of response

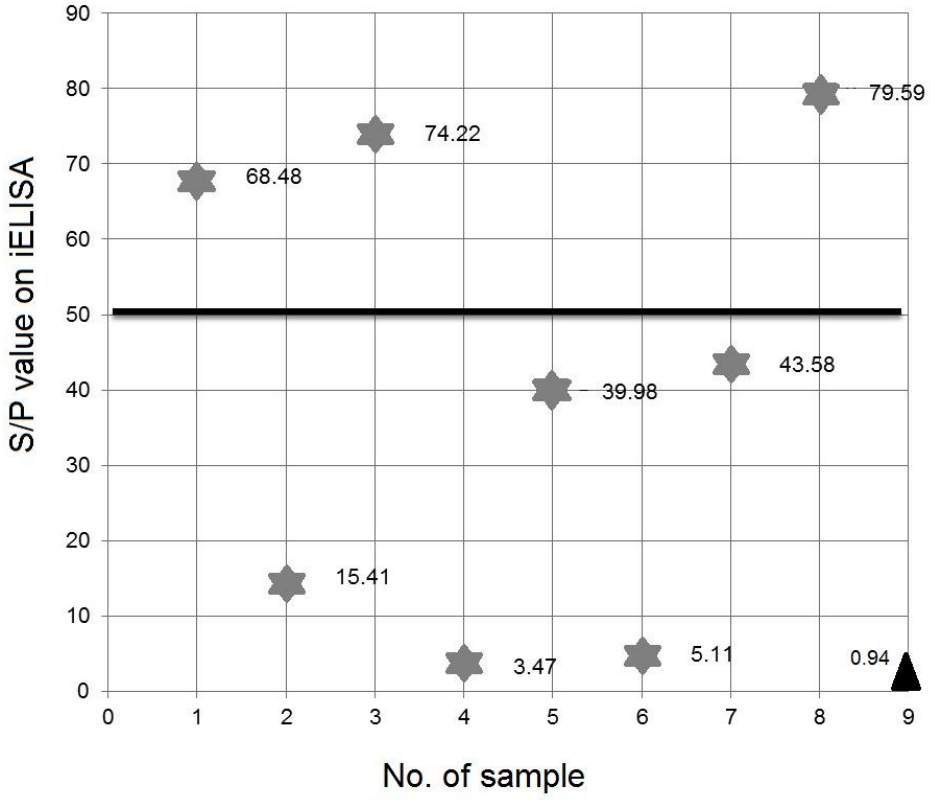

Figure 1. Procentual S/P ratio obtained in indirect ELISA: * - IFAT positive samples; Δ - IFAT negative sample.

Positive ELISA S/P values ranged from 68.48% to 79.59%. No intense positive reaction was observed by ELISA (S/P <100%). Negative and doubtful ELISA S/P values of IFAT positive samples ranged from 4.47% to 43.58% (Figure 1).

The negative sample on IFAT had the lowest S/P value on ELISA, and the 2 intense positive samples on IFAT had the highest S/P value on ELISA. From the intense positive samples (n=3) two were positive on ELISA and one was negative, but close to the doubtful zone (Table 3 and Figure 1).

Results from the present study were similar to those reported by others. Capelli et al. (2006) compared a competition ELISA and IFAT and concluded that cELISA is recommended for confirmation of clinical suspicion of neosporosis when high level of antibodies are expected. Lasri et al. (2004) found a poor positive but good negative agreement between IFAT and ELISA for the serodiagnosis of *N. caninum* infection in dogs. Silva et al. (2007) obtained a low kappa coefficient (k = 0.30), indicating a poor concordance between IFAT and an indirect ELISA results for *N. caninum* serology. In the same study, a good association was found regarding the negative agreement index (Pneg = 0.83) in contrast to the positive agreement (Ppos = 0.42) index.

According to the manufacturer, for the indirect ELISA used in the present study, correlation was found to be 100% between IFAT and ELISA on 17 dog serum samples (7 positive by IFAT), exept one serum which was negative by ELISA and positive by IFAT (the lowest titer from all IFAT tested samples, 1:80). As this serum was also ELISA-positive for *Toxoplasma*, it could be an IFAT false-positive, given that these parasites have epitopes in common. For use of this indirect ELISA in canine sera, analytical sensitivity is tested using an internal standard (pool of positive sera of different origins). Antigen used is purified *N. caninum* extract and the conjugate is an anti-multi-species IgG-HRP (Horseradish peroxidase conjugated).

Posible reasons for necorelation obtained between IFAT and ELISA in the present study are: low titer of antibodies in serum samples (not determined by IFAT), IFAT false-positive samples due to *T. gondii* cross-reactions or inappropriate cutoff value of the multi-species ELISA for dog sera.

According to Capelli et al. (2006), IFAT sensitivity of 100% assessed by Dubey et al. (1988b) was probably overestimated in the firs study about this method aplied in canine *N. caninum* infection, because was apreciated on clinical neonatal *Neospora* infections and in experimental infected animals.

Low titers of antibodies to *N. caninum* can be the expression of cross-reactivity to related parasites, particularly *T. gondii*, due to common tachyzoite and bradizoyte antigens (Bjerkas et al., 1994).

CONCLUSIONS

A poor agreement was found between indirect ELISA and IFAT (k=0.135) for detection of *N. caninum* infection in dogs.
In the same set of samples, IFAT classified more samples as positive (88.9%) than indirect ELISA (33.3%) and seems more appropriate for seroprevalence studies in asymptomatic dogs.
The discordance between the two tests was marked specially at less intense IFAT positive samples.
Further studies are planned in order to asses SE and SP of this indirect ELISA and a ROC analysis in order to verify if the cut-off recommended by the manufacturer corresponds to the highest sensitivity combined with a good specificity of indirect ELISA.
In addition, the identification of possible cross-reactions with *T. gondii* should be investigated.

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SEROSURVEILLANCE OF *NEOSPORA CANINUM* IN FARM AND COURTYARD CATTLE

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Abstract

Neospora caninum is an apicomplexan parasite related to Toxoplasma gondii, well known as an important cause of abortion in cattle. In live animals the routine diagnostic of infection is detection of anti-N. caninum antibodies in serum. This study aimed to investigate the exposure to N. caninum infection in cattle from south area of Romania, and to survey the seroprevalence in some herds. A total of 542 sera from dairy cattle (16 herds in 9 counties) were sampled at different intervals. In the first sampling only farm animals were included, but in the second sampling courtyard cattle were added (n=47). Anti-N. caninum antibodies were detected using an indirect ELISA test. Anti-N. caninum antibodies were found in all herds. A total of 189 samples (34.9%, $CI_{95\%}$=30.85-39.05) were positive, with 104/258 (40.3%, $CI_{95\%}$=43.27-46.58) in the first sampling and 85/284 (29.9%, $CI_{95\%}$=24.66-35.63) in the second sampling. The highest rate of infection was in Giurgiu County, with 18/29 infected animals (62.1%). Prevalence increased with age and abortions were more frequent in seropozitive caws (9/74, 12.2%) than in seronegative ones (5/83, 6%). Seroprevalence was significantly higher in farm (37.2%, 184/495) than in courtyard cattle (10.6%, 5/47). At the second investigation in the same farms prevalence decreased significantly, compared with the first one (35.1%, 46/131 vs. 49%, 47/96). N. caninum infection is widespread in dairy farms from the studied area and may be a cause of abortion in some herds. The prevalence decreased at the second investigation in the same farms.

Key words: *cattle, Neospora caninum, Romania, seroprevalence.*

INTRODUCTION

Neospora caninum is an apicomplexan protozoan recognized as an important cause of abortion in cattle. Mainly affected species are dogs and cattle (Dubey, 2003). The parasite was first isolated from dogs in Norway as a cyst-forming sporozoan closely related to *Toxoplasma gondii* (Bjerkas et al., 1984).

In cattle, the most frequent transmission pattern is vertical, from infected dams to their offspring with resulting lifelong infection, but postnatal infection is also possible, via ingestion of oocysts shed by infected dogs.

In cattle, neosporosis causes severe economic losses by reproductive disorders, especially abortions. Fetuses may die in utero, be stillborn, born alive with clinical signs, or born clinically normal but chronically infected (Dubey, 2003). The routine diagnosis of *N. caninum* infection in bovines is based on detection of specific antibodies in sera (Dubey and Schares, 2006). Serological studies show a widely varied prevalence between herds, regions, and countries. Prevalence of the infection in cattle can reach 90% in some herds (Dubey and Schares, 2011). This study aimed to investigate the exposure to *N. caninum* infection in cattle from south area of Romania, and to survey the seroprevalence in some herds.

MATERIALS AND METHODS

The serological survey was performed in 9 counties from the south of Romania: Ilfov, Giurgiu, Teleorman, Olt, Argeș, Dâmbovița, Prahova, Ialomița and Călărași. In 2010 and 2012, 542 blood samples were collected from 16 cattle herds randomly selected: 13 dairy farms (n = 495) and 3 villages (n = 47). Distribution of herds according to county is shown in Table 1.

Table 1. Distribution of herds according to provenience county

	Ilfov	Giurgiu	Teleorman	Olt	Argeș	Dâmbovița	Prahova	Ialomița	Călărași
Farms	A, B	C	D	E	F	G	H, I	J, K	L, M
Courtyard cattle	N	O	P	-	-	-	-	-	-

The herd's size varied within 23 – 840 cattle and the most common cattle breed was Holstein/Friesian. Animals were randomly sellected for individual sampling. Animals were divided in two groups: the first sampling (2010, n=258) and the second sampling (2012, n=284). In the first sampling only farm animals were included, but in the second sampling courtyard cattle were added. Three age categories were analyzed: 24 calves (12 days -15 months), 30 heifers (16 – 24 months) and 230 adult cows (25 months and over: 82 primiparous and 148 multiparous). Serological investigation was repeated in 4 farms (A, B, D and I, n=96, respectively A', B', D' and I', n=131) at 4 months – 2 years intervals between samplings. In addition to the 542 samples mentioned above, serum samples from two aborted fetuses were tested, one at 3 months of gestation and the other at almost 5 months of gestation. Mother of 5-month fetus was coming from farm M. Data on herd's size, age and reproductive history were obtained from herd owners' records, staff and local veterinarians.

Blood samples were taken from jugular, mammary, or caudal veins, using disposable needles and vacuum plane tubes. All samples were immediately

transported to the laboratory. Serum was removed after centrifugation at 2500 rpm for 10 min and stored at – 20ºC until use.
Sera were analyzed for anti-*N. caninum* antibodies using two commercially available indirect ELISA kits (HerdChek Neospora caninum Antibody Test Kit, IDEXX Lab. and ID Screen *Neospora caninum* Indirect Multi-Species, ID-VET Lab.) as per the manufacturer's instructions. Briefly, serum samples diluted 1:100 were analyzed for the presence of anti-*N. caninum* IgG antibodies. Plates were read at 620 nm (HerdChek test) or 450 nm (ID Screen test), and the test results were expressed as an S/P ratio obtained by an equation provided by the manufacturer. Samples with an S/P ratio equal or higher than 0.5, were considered positive. For ID Screen test samples with S/P between 0.4 and 0.5 were considered doubtful.
Data analysis was performed using Fisher's test and Chi-square test (Quantitative Parasitology 3.0 software). Statistical significance was assumed at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Seropositive animals were found in all examined herds, indicating a wide extending of *N. caninum* infection in cattle from analyzed area.
The average prevalence was 34.9% (189/542, $CI_{95\%}$=30.85-39.05), with 40.3% (104/258 $CI_{95\%}$=43.27-46.58) in the first sampling and 29.9% (85/284, $CI_{95\%}$=24.66-35.63) in the second sampling (p=0.012).
The highest rate of infection was in Giurgiu County, with 62.1% prevalence (18/29). The seroprevalence rates in different counties are mentioned in Table 2.

Table 2. Seroprevalence of *N. caninum* infection in cattle from the south of Romania

County	***Farms***				**Courtyard cattle**	***TOTAL***
	First investigation	***Second investigation***				
Ilfov	A	A'	B	B'	N	73/163, 44.8%
	19/27 70.4%	15/56 26.8%	13/19 68.4%	25/44 56.8%	1/17 5.9%	
Giurgiu	C	-			O	18/29, 62.1%
	16/20 80%				2/9 22.2%	
Teleorman	D	D'			P	8/67, 11.9%
	5/30 16.7%	1/16 6.3%			2/21 9.5%	
Olt	E	-			-	3/20 15%
	3/20 15%					

County	*Farms*			Courtyard cattle	*TOTAL*
	First investigation	***Second investigation***			
Argeș	F	-		-	7/52 13.5%
	7/52 13.5%				
Dâmbovița	G	-		-	8/20 40%
	8/20 40%				
Prahova	H	I	I'	-	28/70, 40%
	13/35 37.1%	10/20 50%	5/15 33.3%		
Ialomița	J	K		-	19/43, 44.9%
	16/20 80%	3/23 13%			
Călărași	L	M		-	25/67, 37.3%
	17/23 73.9%	8/44 18.9%			
TOTAL	104/258 40.3%	80/237 33.8%		5/47 10.6%	197/542 34.9%
	184/495 37.2%				

Overall, one county (Giurgiu) had a high prevalence of positive animals (over 60%), five counties (Ilfov, Dâmbovița, Prahova, Ialomița, Călărași) had a medium prevalence (between 30 – 60%), and three (Teleorman, Olt, Argeș) showed a low prevalence (less than 30%). The counties within the same group of prevalence had a grouped distribution on the map (Figure 1).

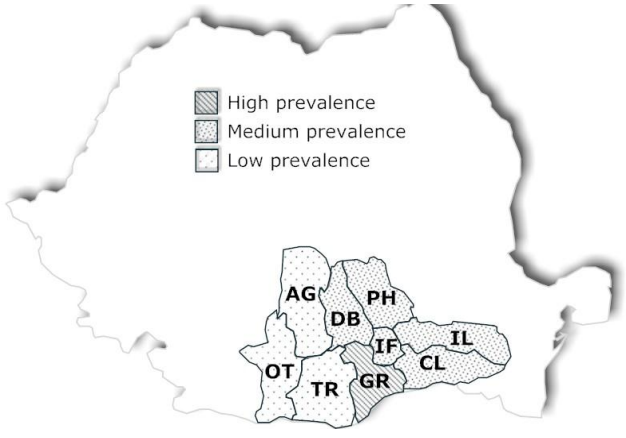

Figure 1. Grouped distribution of counties by prevalence category

In other regions of Romania, *N. caninum* infection in cattle was also reported, with 27.7% prevalence in west (Imre et al., 2012) and 34.6% prevalence in north-west and centre (Gavrea et al., 2011).

Prevalence increased with age (Figure 2), but there were no statistically significant differences between the age groups (p=0.792).

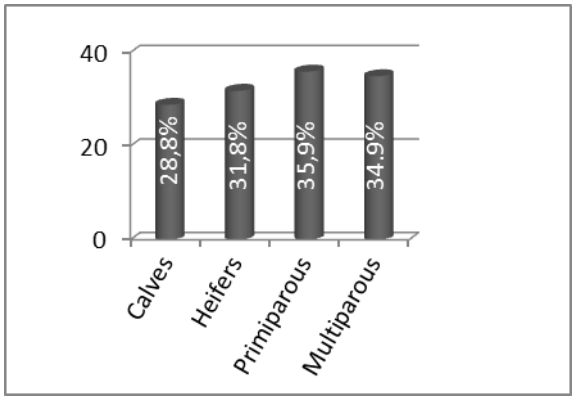

Figure 2. Prevalence of *N. caninum* infection by age groups

The presence of specific antibodies in clinically healthy calves may be caused by infection in the last period of gestation after development of their fetal immune system. The fetus begins to develop a specific immune response against the parasite in the fourth to sixth month of gestation; thus, if the fetus survives, the calf can be born clinically healthy, but congenitally infected (Innes et al., 2005). The extent of endogenous transplacental transmission is estimated to be 78.0–95.0% (Paré et al., 1996). It is also possible that calves were infected post-partum via pooled colostrum (French et al., 1999) or ingestion of oocysts, but unlikely because of their young age. We must consider the possibility that antibodies may be passively transmitted, giving false positive results for very young calves. Maternal antibodies in calves persist for 6 months (Paré et al., 1996). Hietala and Thurmond (1999) showed that, after 1 month, such passively acquired antibodies could still be demonstrated in 50% of sera from uninfected calves. However, in most of the calves, antibodies were not detected after 2 months, in the same study.

In two farms (A, B') where a reproductive history was available, abortions were twice more frequent in seropozitive caws (9/74, 12.2%) than in seronegative ones (5/83, 6%), although no statistical association was found (p=0.262).

The main symptoms of neosporosis in cows are abortion and stillbirth. Several studies have demonstrated that seropositive cows are more likely to abort than seronegative cows (Dubey et al., 2007). The risk of abortion is increased 2- to 4-fold for seropositive dams as compared with seronegative cows (Paré et al., 1997).
Seroprevalence was significantly higher (p=0.0001) in farm cattle (37.2%, 184/495) than in courtyard cattle (10.6%, 5/47). In farms, prevalence of *N. caninum* infection was 3.5 higher than in courtyard herds (Figure 3).

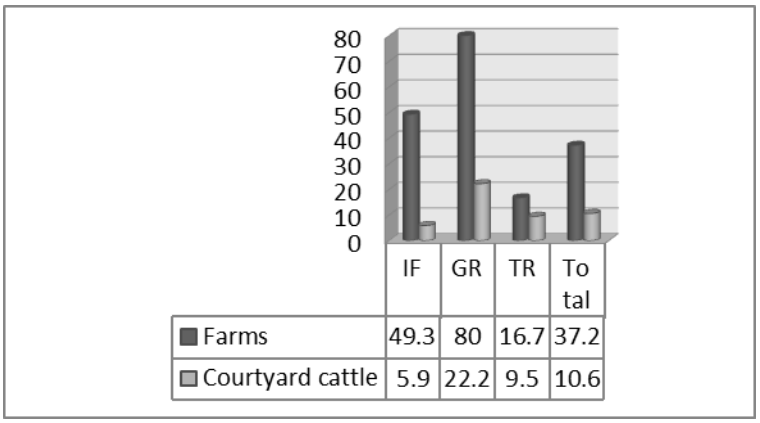

Figure 3. Prevalence of *N. caninum* infection in farm and courtyard cattle

The difference between breeding systems can be attributed to individual care for the animal. In addition, in the yards most dogs had restricted access. In pastures, oocysts contaminations caused by definitive hosts may be too low to pose a significant infection risk or oocysts may not survive during the summer months if they are very hot and dry (Dubey et al., 2007).
Major differences between breeding systems in our country have been also found in the north-west, where Gavrea et al. (2009) obtained a prevalence of 19.3% in a village from Cluj County, while in cattle from dairy farms in the northwest and center prevalence was 34.6% (Gavrea et al., 2011). In western Romania no correlation was found between seropositivity and cattle breeding system (Imre et al., 2012).
Most positive samples reacted intensely positive (Table 2). On this basis we can say that the specific antibody titer to *N. caninum* infection is high in herds in southern Romania. As the antibody titer is an indirect indicator of antigenic exposure to the immune system, an increase in antibody titer may reflect an increase in parasite activity and multiplication in the host (Innes et

al., 2005). Based on this, a high antibody titer, reflected by a high S/P ratio, may reflect a recent infection or reactivation of the infection.

Table 2. Classification of positive samples according to the intensity of color reaction expressed as S/P ratio

Positive samples	Low positive (0.5<S/P≤1) n (%)	High positive (S/P>1) n (%)
First sampling	27/104 (26%)	77/104 (74%)
Second sampling	39/85 (45.9%)	46/85 (54.1%)
TOTAL	66/189 (34.9%)	123/189 (65.1%)

In farms with repeated serological investigation (A and A', B and B', D and D', I and I'), the second set of samples revealed a significantly decreased prevalence (p=0.036) compared with the first one (35.1%, 46/131 vs. 49%, 47/96) (Table 3).

Table 3. Seroprevalence in farms with repeated serological investigation

Farm and sampling interval	First sampling			Second sampling		
	Prevalence	Mean S/P of positive	Intense positive reactions	Prevalence	Mean S/P of positive	Intense positive reactions
D/D' 2 years	5/30 (16.7%)	1.085	1/5 (20%)	1/16 (6.3%)	0.756	0/1 (0%)
B/B' 1 year	13/19 (68.4%)	1.113	8/13 (61.5%)	25/44 (56.8%)	1.21	16/25 (64%)
A/A' 10 months	19/27 (70.4%)	2.004	16/19 (84.2)	15/56 (26.8%)	0.901	6/15 (40%)
I/I' 4 months	10/20 (50%)	0.916	4/10 (40%)	5/15 (33.3%)	0.655	0/5 (0%)
TOTAL	47/96 (49%)	1.279	29/47 (61.7%)	46/131 (35.1%)	0.880	22/46 (47.8%)

Decreasing prevalence was sustained by decreasing mean S/P ratio of positive samples (from 1.279 to 0.880) and decreasing percent of high positive samples (S/P≥1) from the total of positive samples (S/P≥0.5) (from 61.7% to 47.8%). Same tendency was observed in every farm with repeated serological investigation, except farm B (Table 3). In the second sampling of farm B (B') more animals were included, compared with the first one, including primiparous and multiparous animals. These factors can be involved in decreasing prevalence of *N. caninum* infection associated with the increase of the mean S/P and percent of intense positive samples.

Some individual animals were repeatedly tested. In farm B, two animals remained negative in both investigations, but in farm I one animal remained negative, one remained positive with decreasing of antibody level at the second sampling (S/P decreased from high positive, 1.22 to low positive, 0.851), and the last one, low positive at the first investigation (S/P ratio=0.921) become negative at the second investigation (S/P ratio=0.274).

Decreasing prevalence may indicate a slowly decreasing tendency of *N. caninum* infection in the area, and that can be explained by culling of cows with reproductive failure or decreasing of dogs' number, but, however, these facts could not been verified. Another factor that can be incriminated is fluctuation of specific antibody level. In *N. caninum* infection levels of specific antibodies may persist for life, but fluctuate, and sometimes are below the detection limits of serological tests (Dubey and Scares, 2006). A policy of annual testing and culling of all seropositive cattle in one population reduced the seroprevalence from 12% to <1% in the first year of simulation (Häslet et al., 2006).

Results from the present study are different from those of others, regarding repeated serological investigation: Woodbine et al. (2008) did not observe strong temporal changes in a four year longitudinal seroepidemiological study of *N. caninum* infection in 114 herds, but Piagentini și col. (2012) observed an increse in seroprevalence at 3 years interval, comprising a total of 615 animals. In the second study the predominance of horizontal infection was demonstrated by testing caw-offspring pairs.

In the present study, since there was no significant association between seroprevalence and age of the animals tested, associated with significant decrease of seroprevalence in the second testing, it can be said that in these herds vertical infection was preponderent. Cows may transmit the infection to their offspring in several pregnancies (Fioretti et al., 2003). Congenital infection rates are high, varying from 80% in heifers, 71% in second parity cows, 67% in third parity cows and 66% in fourth parity and older cows (Dijkstra et al., 2003).

On the other hand, free walking dogs were observed on all examined farms. Also, the higher within-herd prevalence (Table 1) could be due to a greater external exposure to oocysts (French et al., 1999). In farm K a pair caw-calf was tested. The mother, primiparous, tested negative at serological investigation, but the calf, 5 months old, tested positive, with intense reaction. The other two positive samples from the same farm also presented an intense reaction, and came from animals of 17 and respectively 18 months. Based of these results, a horizontal source of infection can be suspected in this farm, either through oocysts shed by dogs, or by pooled

colostrum, including colostrum from infected animals. Feeding of pooled colostrum is a putative risk factor for seropositivity (Corbellini et al., 2006), but, however, cross-suckling of calves born to seronegative mothers on seropositive cows has not led to an infection (Davison et al., 2001).
Serum samples from the two aborted fetuses tested negative, but the mother of the older fetus tested positive, with intense positive reaction (S/P ratio=1.140). A low sensitivity was reported in several studies when serology is performed on aborted fetuses (Dubey and Schares, 2006), and these may be due to lack of fetal immunocompetence, a short interval between infection and fetal death or autolysis with degradation of immunoglobulins (Wouda et al., 1997). Thus, a negative serological result in an aborted fetus does not rule out *N. caninum* infection (Dubey and Schares, 2006).

CONCLUSIONS

N. caninum infection is widespread in cattle from the studied area (34.9% prevalence) and may be a cause of abortion in some herds.
Cattle from dairy farms are more exposed to *N. caninum* infection than courtyard cattle (37.2% vs. 10.6%).
The prevalence decreased at the second investigation in the same farms (35.1%), compared with the first one (49%).
Both horizontal and vertical infection may be suspected, depending on the analyzed herd.

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HYDROCEPHALUS IN DOGS

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Abstract
Anomalous conditions, such as hydrocephalus often result in early fetal or neonatal death. If animals survive birth, they may have significant alterations in consciousness and behavioral development.
Hydrocephalus can result in clinical signs due to loss of neurons or neuronal function alteration in ICP and all of its consequence.
In young dogs, prior to ossification of the cranial sutures, hydrocephalus may contribute to abnormalities of skull development such as a thinning of the bone structure, a dome-shaped or bossed appearance to the head or a persistent fontanelle.

Key words*: hydrocephalus, acetazolamide, dog*

INTRODUCTION

This paper presents the case of one dog that arrived in our clinic with the following symptoms: difficulty walking on the right front leg, loss of balance on the hind limbs. After taking the case history, the clinical and complementary exams, neurological exam, the diagnosis given was hydrocephalus and the appropriate treatment was started.

MATERIALS AND METHODS

These 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, neurological exam, blood exam and biochemistry exam.

RESULTS AND CONCLUSION

Case presentation

The patient is a female, Maltese, 9 months old. The owner came into our practice because he observed that the dog has difficulty walking on the right front limb. At the moment he came this symptom was present for a month.

On the general examination we found that the patient has a normal appetite, normal water intake, urination and defecation is also normal. The blood analyses were in the normal range.

We performed the neurological examination on which we found the following:

- Observation: loss of balance (to the right side), on the hind limbs, hypermetric on both front legs, more visible on the right front leg, tendency to walk on hind limbs like in the circus, as a general view of gait the patient has a “robot walk”.
- Hands on examination: Cranial nerves: no menace response on the right side, and slow response on the left side.
- Spinal reflexes: normal,
- Proprioception: normal except the right front limb which is slow on response.
- Posture: normal,

- Panniculus : normal,
- Perianal: present.
- VITAMIND D – anomalous.
- Neurological localization: forebrain and cerebellum.
- Differential diagnosis: hydrocephalus, herniation of the cerebellum, syringomyelia.

Hydrocephalus is the term used to describe a condition of abnormal dilation of the ventricular system within the cranium due to intracranial disease processes. Hydrocephalus can result in clinical signs due to loss of neurons or neuronal function alterations in ICP and all of its consequences. Occasionally, when hydrocephalus is associated with fourth ventricle enlargement, there may be vestibular dysfunction.

The diagnosis of hydrocephalus is aided by information obtained from **MRI, CT, and ultrasound examination.** In this case the diagnosis of hydrocephalus was confirmed with MRI.

After the diagnosis was established we decided the following:

The choice of treatment is generally dictated by physical status, age of the animal and cause of the hydrocephalus. Medical treatment may include general supportive care and medications

to limit CSF production and reduce intracranial pressure.

Glucocorticoids are used to decrease CSF production, thereby, limiting ICP and

further neurological injury.

Prednisolone at 0.25-0.5 mg/ kg is given orally twice daily, 14 days and continued at half a dose another 6 weeks. The dose is gradually reduced at

weekly intervals to 0.1 mg/ kg every other day. This dose is continued for at least 1 month. Then the medication is discontinued if possible.

We decided to give the prednisolone at 0.5 mg/ kg , orally twice daily, 14 days and continued at half a dose another 6 weeks.

Alternatively, dexamethasone may be given orally at 0.25 mg/ kg every 6 to 8 hours. The dose can be gradually reduced over 2-4 weeks. Acetazolamide a carbonic anhydrase inhibitor is thought to reduce CSF pressure by decreasing CSF production. Mannitol, hypertonic saline and furosemide may be administered to provide temporary decreases in ICP and are reserved for emergency situations.

Acetazolamide was given at 20 mg twice daily, permanent. Furosemide was given at 5mg/day , 20 days and after this period 5mg/48 hours permanent. Panangin : 5mg/48 hours, permanent. GABA – 62, 5 mg twice daily, permanent.

CONCLUSION

Hydrocephalus is considered to be anomalous in VITAMIND

Treatment is permanent

Clinical sings may or may not improve in time depending on the severity of the disease

Prognosis is reserved.

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HYPERTHYROIDISM IN CATS

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INTRODUCTION

Hyperthyroidism (thyrotoxicosis) is the most common endocrine disorder of the cat. It was first definitively diagnosed in 1979 and its incidence has increased dramatically since. It is unclear whether this is because it is truly a new disease or because it is being diagnosed more frequently as a result of improved awareness, a growing cat population, increased longevity or a combination of these factors.

MATERIALS AND METHODS

These 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, ultrasound, Rx, electrocardiography, blood exam, biochemistry exam and T4 evaluation.

RESULTS AND CONCLUSION

These 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, ultrasound, Rx, blood exam and biochemistry exam.
Level of T4 is measured in µg/dl and in nmol/L. To convert µg/dl to nmol/L, multiply by 12, 87. Normal level of T4 is: 1-4 µg/dl.

Case number	Age	Sex	Neutered	Breed	Weight loss	Polyphagia	Pu/Pd	Other symptoms and underlying diseases	T4 level (1-4µg/dl)	Blood exam

1. Mita	13	F	No	European shorthair	yes	no	no	Tachycardia Dyspnea Loss of appetite Dehydration Pale mucous membranes Depression	6.3 µg /dl	Normal
2. Miki	13	M	Yes	Burmese	yes	no	yes	Anorexia Dehydration Skin lesions Diabetes mellitus	4.2 µg/dl	Hyperglycemia
3. Misu	9	M	No	European shorthair	yes	no	yes	Tremors of head and legs Pulmonary edema Congestive cardiac failure	7 µg/dl	Lymphopenia ALT ALKP slightly elevated
4. Nae	14	M	No	Burmese	yes	yes	yes	Tachycardia Dyspnea Dehydration Palpable goiter	Very high Device couldn`t identify the level of T4	L/M WBC GRANS ALKP (elevated)

Hyperthyroidism is a disease seen almost exclusively in older animals. The average age at onset is 12-13 years. Almost all are in excess of 6 years and less than 5% are younger than 10 years at the time of diagnosis. There is no apparent sex predisposition.

Classically, affected cats are presented with a history of weight loss despite an increased appetite, polyuria/polydipsia and intermittent gastrointestinal signs of vomiting and/or diarrhea.

Notable findings on physical examination include tachycardia (heart rate in excess of 240 beats/min) with or without an audible systolic murmur. Goiter is apparent in over 95% of affected patients.

A small proportion (<10%) of cases presents with apathetic hyperthyroidism, where anorexia and depressions are the most significant features.
Treatment is aimed at controlling the excessive production of the thyroid hormones either by medical inhibition of thyroid hormone synthesis, surgical removal of affected thyroid tissue or destruction through radio ablation or local ethylene glycol administration.
Drugs that were used in treating hyperthyroidism: methimazole (1, 25-5mg/cat/day), carbimazole (2.5-5mg/cat/day), propranolol (2.5-5mg/cat, 3x/day), atenolol (3.125-6.25mg/cat, twice daily), L-carnitine (240 mg/cat/day)
Side effects of methimazole are**: facial excoriation** (localization is head and neck), **g.i. upset** (first 4 weeks of treatment, resolves with dose reduction), **hepatotoxicity, renal decompensation, coagulation abnormalities** (this reaction is rare but should be taken in consideration when the patient presents hemorrhage and is under treatment with methimazole), and **acquired myasthenia gravis**.

CONCLUSIONS

All patients with hyperthyroidism should come for periodic evaluation of T4, blood exam and physical exam
Patients must know that medication for this disease is for a long period of time, that cats with hyperthyroidism are aggressive and is difficult to administer oral medication.
If hyperthyroidism is diagnosed in time and the level of T4 is not too elevated than the patient should be fed with PD feline Y/D and in this case medication is not necessary.
Complications occur in chronic cases such as: pulmonary edema, congestive heart failure, neurological disorders and skin lesions.
Treatment is focused on the disease (specific treatment) and also on complications.

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PRRS DIAGNOSIS OBTAINED BY ELISA METHOD IN PROFFESIONAL AND HOUSEHOLD BREEDING UNITS FROM BRAILA

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Abstract

Porcine Reproductive and Respiratory Syndrome (PRRS) is an infectious disease produced by a virus and it is characteristic to swine. The disease produces important economic demages by reproduction and breathing problemes and with expensive cost by prevention and control of spreading (Rotaru, 2005).
This paper shows the results obtained using serological examination by ELISA method in big proffesional breeding swine units compared with household breeding swine units from Braila.
In the blood samples taken from different categories was observed a high incidence of antibodies PRRS to the swine growth in proffesional units compared with the results from swine growth in household units where the antibodies against PRRS were not presents.
In literature antibodies and virus detection may be a surprise because the clinical signs are not always present (Perianu, 2012).

Key words*: ELISA test, PRRS, swine.*

INTRODUCTION

The disease was initially confused with other diseases. At first it was called mystery swine disease (MDS) or infertility and respiratory syndrome (SIRS), blue ear disease (BEPD), epidemic abortion and respiratory syndrome of pigs (Pears). Currently run under three important names: in U.S.-infertility and respiratory syndrome (SIRS); in European countries - respiratory and reproductive syndrome of pigs (PRRS) porcine epidemic abortion and respiratory syndrome (Pears) (Zimmerman et al., 2006).
The virus that causes this syndrome was isolated and studied for the first time (1991), the Institute Lelystad in the Netherlands, for which it received initially name of Lelystad's virus. It is now included in the family Arterividae, like Arterivirus, along with other three pathogenic viruses to animals (Rotaru Elena, 2005; Zimmerman et al., 2006).

The clinical evolution could be acute or chronic marked by two main components respiratory and / or breeding (Rotaru Elena, 2005).
Respiratory disorders are present in all types of animals and manifests by: loss of appetite, moderate hyperthermia, cough and dyspnea (OIE, 2012).
Reproductive disorders are expressed by: late abortions, premature births or abnormal prolongation of gestation period, high neonatal mortality. At sows are reported: anestrous, agalactia and anorexia (OIE, 2012).

MATERIALS AND METHODS

Serum samples were collected from two commercial pigs units, from the following categories of pigs: piglets, young pigs, boars and sows, as well as from non- professional units in the surroundings.
Holdings are located at a distance of 30 km from each other and will be called in this paper farm A and farm B.
In the farm A, the disease was suspected based on clinical signs and lesions. The first clinical signs appeared in the maternity ward where the highest percentage of disease was reported in newborn piglets, weaned piglets and less to fatty pigs.
When an outbreak occurs serological examination was performed in alive and the dead animals.
From farm A were collected 50 samples of serums as follows: from piglets -10 samples; young pig-10 samples, fat pigs-10 samples, sows- 10 samples; boars-10 samples.
From same farm were collected 18 thoracic fluid samples from bodies as follows: 9 piglets bodies, 3 bodies of dead piglets, 3 bodies from yang pigs and 3 bodies fat pigs.
In the farm B disease appeared after the introduction of newly purchased lots of animals.
From this unit were tested a total of 25 blood samples as follows: piglets - 5 samples, young pigs-5 sample, fat pigs-5 samples; sows- 5 samples; boars -5 samples.
From farm B were collected 14 thoracic fluid samples from bodies as follows: 4 bodies piglets, 5 bodies of dead piglets, 3 bodies from yang pigs and 2 bodies fat pigs.
In this study were analyzed blood samples collected from non-professional growth units (households), at a distance of 1 km around the professional farms.

For detection of serum antibodies anti-virus PRRS was used immunoassay test (ELISA). PRRS virus antibodies can be detected by enzyme immunoassay in 7-14 days after infection and levels of antibody titers reached maximum at 30-50 days. Some pigs may become serumnegative within 3-6 months, but others remain serumpositive for longer. The maternal antibody can generally be detected until 4-10 weeks after birth, depending on the sows antibody titre (Yoon, 2002).
The HerdChek PRRS X3 test kit is an enzyme immunoassay for the detection of IgG antibody to porcine reproductive and respiratory syndrome virus (PRRSV) in swine serum and plasma samples. A microtitration format has been configured by coating recombinant PRRSV antigenes on the plate..
Studies were made using IDEXX PRRS ELISA kit (IDEXX Laboratories HerdCheck Switzerland AG.).
IDEXX PRRS ELISA is generally considered to have good specificity and sensitivity (OIE, 2012)
The kit components are as following:
-5 microtiter plates with 96 strips each lined with PRRS antigen virus,
-Sample diluent, phosphate buffer with protein stabilizers and sodium azide as a preservative,
-PRRSV-positive control, anti-PRRS antibody in phosphate buffer with protein stabilizers,
-PRRSV-negative control, not reacting to swine serum PRRS in phosphate buffer with protein stabilizers preserved with sodium azide,
-Anti-Porcine IgG conjugate labeled with peroxidase (HRPO),
- TMB substrate solution,
-Stop-solution (SDS),
- Wash-concentrate solution (10X).
After washing steps, unbound materials in these complexes are removed, then an anti-swine conjugate labeled with peroxidase is added and it will be bind to the antibodies wich are bind to antigens in the wells. Unbound conjugate is removed by washing stage and the next stage will add TMB substrate. The color that developed is directly proportional to the amount of PRRS specific antibodies present in the sample.
The first step was reprezent by sample preparation.
The sample is diluted 1:40 with sample diluent (e.g. by diluting 5 µl of sample with 195 µl of sample diluent). Do not dilute controls.
Washing solution
The wash concentrate should be brought to room temperature (18-25˚C) and mixed to ensure dissolution of any precipitated salts. The Wash Concentrate

must be diluted 10 fold (1/10) with distilled/ deionized water before use (e.g. 30 ml of concentrate plus 270 ml of water per plate to be assayed).
All reagents must be allowed to come to room temperature (18-25˚C) before use.

Figure 1. ELISA kit

Dispense 100 µl of undiluted Negative Control in the first two wells (A1, B1). Then it will be add 100 ml undiluted Positive Control in the next two wells (C1, D1).
Dispense 100 µl of diluted sample into two wells of the assay plate Incubate for 30 min at 18-25˚C. The plate was washed 3-5 times with 300 ml of washing solution, then beat on a filter paper to remove traces of liquid wich could be on well.
Add 100 ml each of swine anti IgG conjugated with peroxidase labeled (HRPO) to each well. Incubate at 18-25 ˚C, 30 minutes.
The plate will be wash 3-5 times with 300 ml of washing solution. Microtiter plate will be gently shake on a filter paper to remove traces of liquid wich could be on well. Next will be add 100 ml of TMB substrate solution into each well. Incubate 15 minutes at 18-25 ˚C.
Add 100 ml of stop solution to each well to stop the reaction. Measure and record the wavelength of A (650) samples and controls.

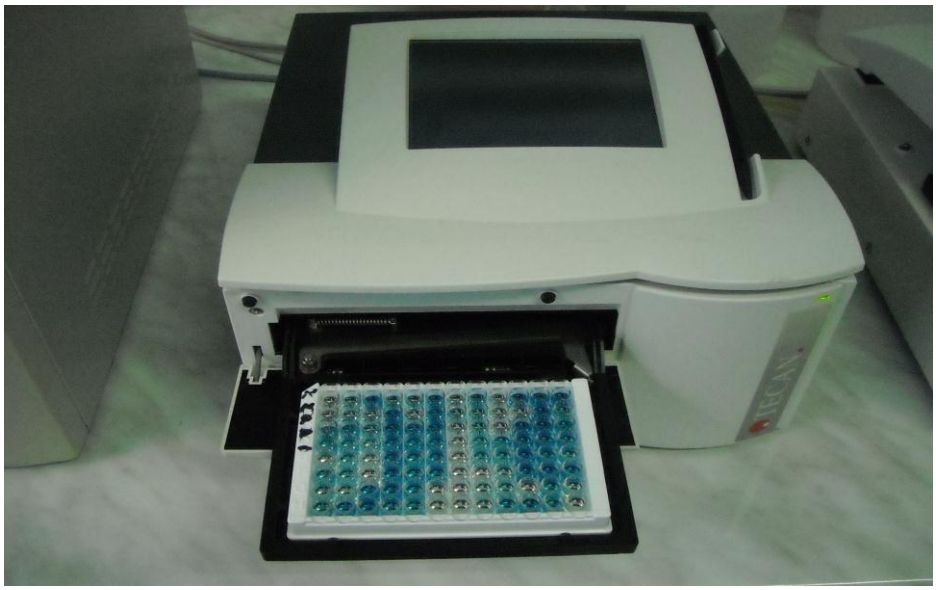

Figure 2. ELISA results

Validation test.
The test could be valid if the following specifications are found:
The positive control mean minus the mean of the negative control must be greater than or equal to 0.150. In addition the negative control mean (NCX) must be less than or equal to 0.150. For invalid assays, technique may be suspect and the assay should be repeated. The presence or absence of antibody to PRRSV is determined by calculating the sample to positive (S/P) ratio (Yoon, 2002).

Calculations
Calculation of negative control mean (NCX)

$$NCx = \frac{NC1\ (A650) + NC2\ (650)}{2}$$

Calculating of positive control mean (PCX)

$$PCx = \frac{PC1\ A\ (650) + PC2\ (650)}{2}$$

Interpretation of results
The presence or absence of antibody to PRRV is determined by calculating the S/P ratio for each sample. If the S/P is less than 0.40 the sample is considered Negative for PRRSV antibodies. If the S/P is greater than or

equal to 0.40 then the sample is considered Positive for PRRSV antibodies (Yoon, 2002).

RESULTS AND DISCUSSION

Of the 50 serum samples collected from pigs breed in farm A were obtained the following results presents in table 1.

Table 1. ELISA test results on serum of pigs from farm A

Category	No. samples	No. (%) samples		
		positive	dubioase	negative
suckling	10	10 (100%)	0	0
young pigs	10	5 (50%)	0	5 (50%)
fat pigs	10	3 (30%)	0	7 (70%)
sows	10	7 (70%)	0	3 (30%)
boars	10	4 (40%)	0	6 (60%)
Total	50	29 (58%)	0	21 (42%)

Following bloking ELISA immunoassay test at the 18 samples taken from the bodies, results were presents in table 2.

Table 2. ELISA test results of thoracic fluid from cadavers of pigs from farm A

Category	No. samples	No. (%) samples		
		positive	dubioase	negative
piglets farrowed dead	3	3 (100%)	0	0
suckling	9	9 (100%)	0	0
young pigs	3	0	0	3 (100%)
fat pigs	3	0	0	3 (100%)
Total	18	12 (66,66%)	0	6 (33,33%)

In farm B were obtained the following results presents in table 3.

Table 3. ELISA test results on serum of pigsfrom farm B

Category	No.samples	No. (%) samples		
		positive	dubioase	negative
suckling	5	4 (80%)	0	1(20%)
young pigs	5	5 (100%)	0	0
fat pigs	5	5 (100%)	0	0
sows	5	5 (100%)	0	0
boars	5	5 (100%)	0	0
Total	25	24 (96%)	0	1 (4%)

Following bloking ELISA immunoassay test for the 14 samples taken from the bodies, results were as follows in table 4.

Table 4. ELISA test results of thoracic fluid from cadavers of pigs from farm B

Category	No. samples	No. (%) samples		
		positive	dubioase	negative
piglets farrowed dead	4	4 (100%)	0	0
piglets bodies	5	5 (100%)	0	0
young pigs	3	3 (100%)	0	0
fat pigs	2	0	0	2 (100%)
Total	14	12 (85,7%)	0	2 (14,3%)

Samples were taken from non- professional breeding units were also made by enzyme immunoassay ELISA and the result was negative in all samples examined.

CONCLUSIONS

The presence of PRRS antibodies suggests the circulation of virus in the studied farms.
The risk of becoming an infected unit is directly proportional to the size of the flock, with the quarantine absence and frequency of introducing new animals in farm.
PRRS is a disease that occurs in the intensive system of breeding pigs due to growth technology: the introduction of new effective to cohabitation with the existing one, artificial insemination, etc.
In the farms studied, the biggest losses were recorded at piglets because the passive immunity transferred from sows to piglets is short.
ELISA test can detect antibody against PRRS virus and is very important to know which is the serum status.

ACKNOWLEDGMENTS

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EPIDEMIOLOGICAL RESEARCH CONCERNING THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME DURING 2011

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Abstract

The swine from the present study confront with serious breathing and reproduction problems specific to PRRS disease (Rotaru 2005).
This disease affects the immune system of pigs and has a concomitant evolution with another viral and bacterial infection.
*PRRS is recognized around the world by the economical impact that it produces in swine breeding units (*Perianu T. *et al 2005).*
It is a viral disease with endemic evolution characterized by reproduction alert signs to sows and by breathing signs to young pigs(Benfield, 1999).
In most of the cases of evolution the multiple ethyology includes the following bacterium: Mycoplasma hyopneumoniae, Pasteurella multocida, Actinobacillus pleuropneumoniae and viruses: virus Aujeszky virus, porcine reproductive and respiratory syndrome, influenza virus, transmissible gastroenteritis virus and virus respiratory coronavirozei, low conditions of maintenance will overtake the protection mecanism of the body (Benfield, D 1999).
In this paper are presented the epidemiological researches achieved during 2011 in two proffesional swine units from Braila.

Key words: *epidemiological indicators, PRRS.*

INTRODUCTION

Respiratory disorders and reproductive syndrome (PRRS), is a swine disease recognized worldwide by the economic impact which it produces in pigs industry (Stănuică, 2005).
It is a viral disease, with endemic evolution, characterized by reproductive disorders in gilts and sows, and respiratory signs at weaned piglets (Răpunteanu, 2002).
It is occurred almost simultaneously in the United States (1987) and Canada (1988), and in late 1990 was described in Germany. Since 1991, he quickly released in the Netherlands, Belgium and Spain, causing panic among pig farmers from Europe (Răpunteanu, 2002).

Existence of the disease in Romania was confirmed by laboratory methods by a team of researchers, led by Stănuică, at the Pasteur Institute in 1998 (Stănuică, 1999).

MATERIALS AND METHODS

In 2011 were conducted epidemiological investigates in two units of pig husbandry industry.
The company consists of two separate farms located at a distance of about 30 km from one another.
First unit work called "Farm T" has a capacity of 31,989 pigs and farm is organized as closed circuit consisting of the following areas: pregnancy, maternity, youth, fat pig.
"Farm B" has a capacity of 120,000 pigs and is divided into three farms as follows:
- Farm 1 (pregnancy - maternity-youth)
- Farm 2 (youth and pork fat)
- Farm 3 (closed circuit farm produce F2 for Farm 1).

This paper aims conducting an epidemiological study in a PRRS outbreak which occurred in 2011 in the 2 swine farms. Data were obtained by collating more information about movement of livestock (new animals introduced, births, birth rates, fecundity, morbidity and mortality) and the clinical episode of PRRS in flocks under study. Epidemiological researches followed up on the following parameters:
- Birth,
- Prolificacy,
- Abortions in sows and gilts.

In farms with open-circuit, the risk of contamination is directly proportional with: herd size, frequency of introduction of new animals and application of prophylactic quarantine. In geographic areas with several swine farms, the major risk factor is the represented by the density of pigs. Other factors depend on the virulence of strains, breeding technology, hygiene, stress, quality of feed and the presence of bacterial diseases (Benfield, 1999).
In PRRS syndrome, the epidemiological investigation leads to a presumptive diagnosis and at the same time allows: identifying sources of infection, dissemination of disease in farm livestock, virus dissemination outside the farm and identify contributing extrinsic factors (Benfield, 1999).

RESULTS AND DISCUSSION

Disease began shortly after the entering of newly acquired lots of animals.
In farm T, during the year were buying a total of 23 boars in the following months: February (7 boars) May (6 boars) and July (10 boars).
First signs of disease appeared in the maternity unit when animals were found dead and reduced viability piglets, piglets after farrowed in short time (within 10 days) have respiratory distress, reduced viability, digestive disorders and skin bruising (Albina, 1997).
Morbidity was extended to youth and fat pigs.
The highest percentage of disease was reported in newborn piglets, weaned piglets and less at fatty pigs. It seems that these categories (newborn piglets and weaned piglets) are most susceptible to infection because passive immunity transferred from sows to piglets is short (Albina, 1997).
A possible source of infection may be represented by introducing boars in February.
In farm B during the year were purchased gilts and boars with unknown situation on PRRS syndrome as: January 1100 gilts, February 1020 gilts, March 1200 gilts and 8 boars, April 1100 gilts, May 1300 gilts and 7 boars, June 1050 gilts.
The first signs of disease were found in pregnant sows manifested by: decreased appetite, pyrexia, dyspnea, dead piglets, reduced viability, rarely agalactia, sometimes cyanosis of teats and vulva. (Rotaru 2005).
Clinicaly the disease manifested a period of about 25 days during which were observed abortions complicated by bacterial infection of the uterus.
Analyzing the evolution of birth in 2011, at B farm, there is an obvious decline in February (at 72.3% from 88.0% value recorded in august) due to the PRRS evolution during January. So the animals that were between 80 to 100 days of gestation, during the disease progression they are lost products of conception due abortion.

Table 1. Evolution of birth in B farm

Birth	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
%	73,2	**72,3**	74,2	75,3	74,4	73,6	78,1	**88,0**	86,0	81,5	81,5	74,6

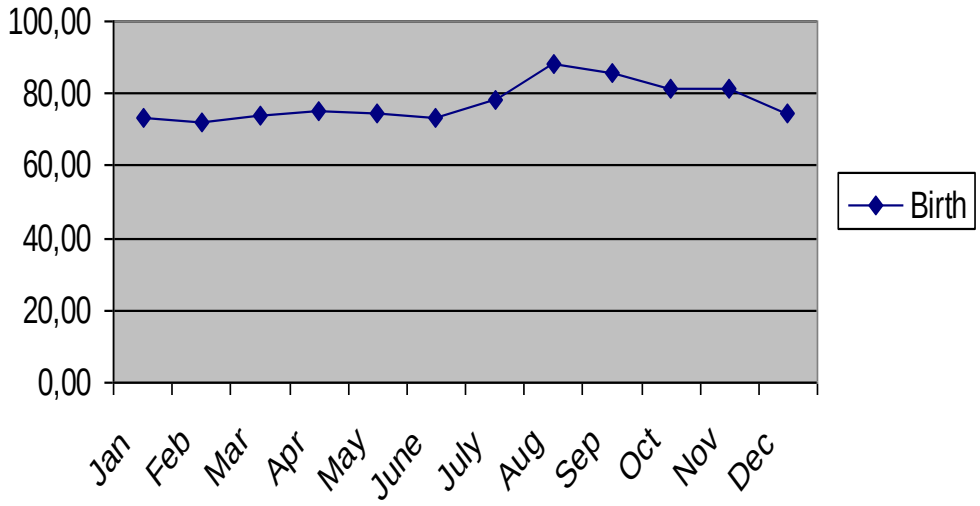

Figure 1. Dynamic of birth in B farm

By analyzing how has evolved birth at T farm, can conclude the presence of the syndrome in September, when there was a decrease in the value of this parameter (69.0%) compared with other months (November 83.2%).

Table 2. Evolution of birth in T farm

Birth	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
%	79,8	79,5	81,2	75,6	82,5	87,9	80,0	83,9	**69,0**	78,6	**83,2**	90,8

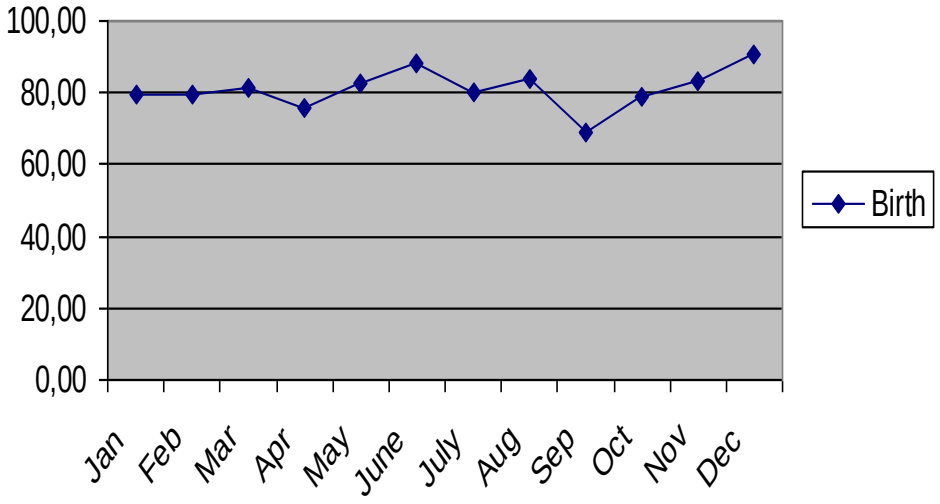

Figure 2. Dynamic of birth in T farm

Another parameter studied was prolificacy.
In T farm, this indicator has high values in June, July and August after which values decreased in the next months.

Table 3. Evolution of prolificacy in T farm

Prolificacy	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
%	9,7	9,4	9,8	9,6	9,5	9,9	9,8	9,9	9,5	9,5	9,5	9,5

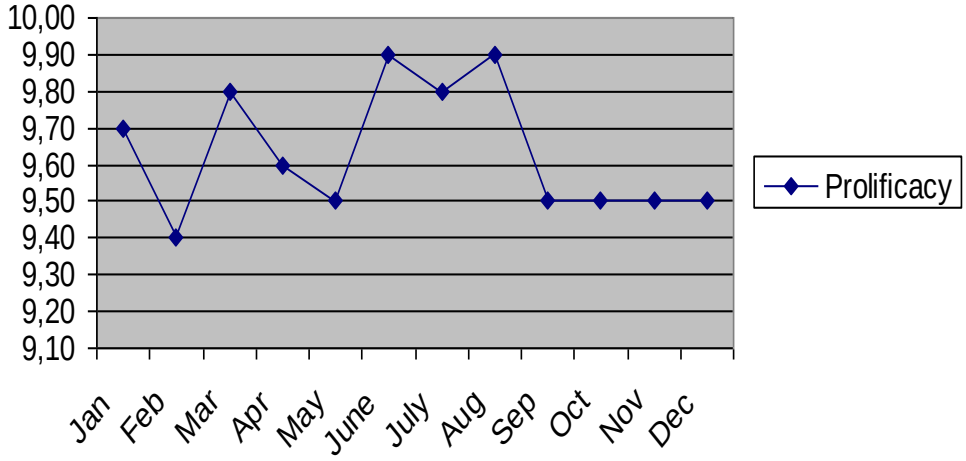

Figure 3. Dynamic of prolificacy in T farm

Declines of this indicator in T farm, at September, October and November are due to the increased number of dead piglets at farrowing.

In B farm evolution of this indicator is presented in Table 4.

Table 4. Evolution of prolificacy in B farm

Prolificacy	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
%	8,9	8,6	8,8	9,0	8,8	8,3	8,6	7,8	8,5	8,5	8,1	9,6

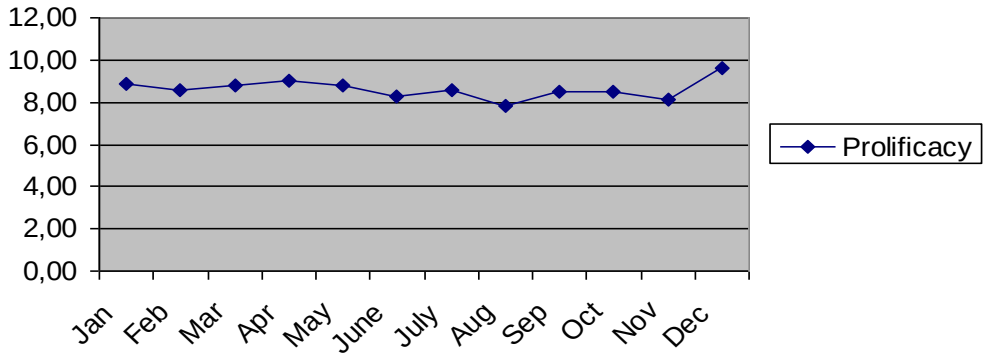

Figure 4. Dynamic of prolificacy in B farm

Evolution of PRRS virus in T farm generated an excessive increase in abortions. However, the slightly high level of abortions from July (compared with previous months) reveals that this virus had a influenced in pregnant sows and gilts. Presence of abortion in B farm has a high incidence than in T farm. In this farm was predominant the genital form at sows.

Tabelul 5. Situation of the abortion in farm T and B

Month	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Farm T	3	3	3	3	3	4	5	3	2	4	1	3
Farm B	4	4	8	2	5	5	2	3	8	7	5	3

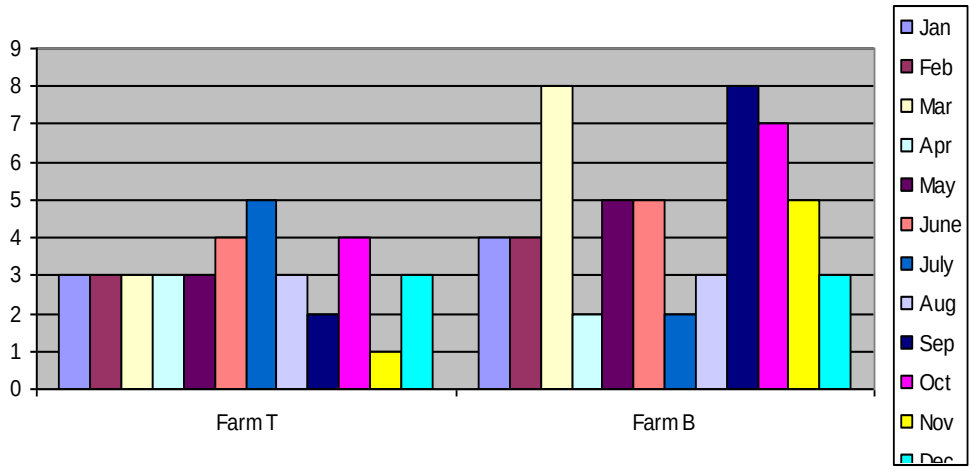

Figure 5. Dynamic of the abortion in farm T and B

CONCLUSIONS

The disease appeared in farms after the acquisition of new effective of animals without respecting the prophylactic quarantine.
The birth in B farm showed a clear decrease in February (72.3%).
The values of prolificacy obtained recorded a fall in these months: August, September and October.
In T farm the birth rate recorded value was 69% in September compared to other months (November 83.2%).
Prolificacy in T farm recorded high values in June, July and august after that the values decreased in the coming months.
The highest number of abortions occurred in B farm.

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The work of Filip Sorina Eugenia was performed as part of the project POSDRU/107/1.5/S/76888 *"Doctoral Scholarships for high quality training for young Researchers in the field of agronomy and veterinary medicine"*, co-financed by European Social Fund with the Operational Program Human Resources Development 2007-2013, area of intervention: 1.5. Doctoral and Post-doctoral Programs to support the research.

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PRESENTATION OF A CASE OF OSTEOSARCOMA IN A ROTTWEILER BREED DOG

Ionut Cristian Garjoaba , Andrei Tanase

ABSTRACT

We hereby present an osteosarcoma case in a Rottweiler breed dog, the osteosarcoma being located in the epiphyseal - metaphyseal area of left humerus.
The dog has been submitted successively to clinical, radiologic and cytology investigations, the last one showing the existence of a type of osteoblastic osteosarcoma.
Osteosarcoma is a malignant tumour of the bone tissue usually striking the Canidae, affecting in particular medium and large breeds of dogs, more frequently located at the level of the appendicular skeleton, in the epiphyseal – metaphyseal area of long bones.
The diagnosis in the case of osteosarcoma is found further to a cytology investigation, the sample being taken by means of X-ray guided bone biopsy.
***Key words**: osteosarcoma, cytology investigation, bone biopsy, X-ray guiding.*

INTRODUCTION:

Osteosarcoma is a malignant oncological disease which appears in the bone tissues and is particularly frequent in medium and large breeds of the Canidae (1).
Information in the professional literature concerning the frequency of osteosarcoma diagnosed in Canidae according to the breeds (2), are as follows, large to medium size breeds: German Shepherd (11,29%), Rottweiler (16,94%), Great Dane (8,87%), Boxer (6,45%), *Deutsche Bracke* / German Hound (4,03%). The small size breeds are less frequently affected by osteosarcoma: Pointer (0,81%), Dachshund (1,61%).
Regarding the location of osteosarcoma: they are located both at the level of the axial skeleton (skull, vertebral column) and, particularly, at the level of the appendicular skeleton, as well as there are extraskeletal osteosarcoma (3).
Concerning the frequency of osteosarcoma occurrence there is a a significant difference, namely, 89,51% have been found at the level of appendicular skeleton, and fewer at the level of the axial (8,87%) and even fewer, osteosarcoma with extraskeletal location (1,62%),(4). Regarding location of osteosarcoma in limb bones, a 33,87% frequency was found at the level of long bones metaphysis , 42,74% at the level of long bones epiphysis and 12,9% at the level of long bones diaphysis. (4).

Concerning the osteosarcoma diagnosed Canidae according to gender, professional literature data show the 54,83% are male and 45,17%) are female dogs.
Concerning the age distribution in the development of osteosarcoma, data show that 23,39% of Canidae were between 5 and 7 years old, 3,22% of the Canidae were between 12 and 18 years old, and 73,39% were between 8 and 11 years old.
From the point of view of histomorphology, osteosarcoma may be: osteoblastic osteosarcoma, fibroblastic differentiation osteosarcoma, condroblastic differentiation osteosarcoma and telangiectatic type sarcoma(5).

MATERIAL AND METHOD:

The case presented is that of a female Rottweiler brought to the veterinary consulting–room because of a tumefaction in the left scapulo-humelar area, having a painful response at the thorough palpation of the tumefied area. (fig.1.)
The case-history would indicate that the tumefaction in the scapulo-humeral area occurred two weeks before but, functionally, no limping or movement break was noticed.
The clinical examination was followed by a radiological one, and an alternation was observed between bone lysis areas and those of bone proliferation at the proximal epyphiseal–metaphyseal level of the left humerus. (fig.2.)
After the radiological examination, the dog was anaesthetised by means of neuroleptanalgesia (acepromazina=0,3mg per kg i.m,dillution ketamina – NaCl 0,9% 1mg per 1ml i.v.)
After having the specific effects of anaesthesia in place, the bone biopsy was performed on the anatomy correspondent of the area affected, radiographic film being used for guidance in order to have a tissue sample from the areas with moderate radio transparency. (fig.3.)

RESULTS AND DISCUSSIONS

The result of the cytology investigation show a cito-morphological aspect typical for osteoblastic osteosarcoma (fig.4). In order to have reliable cito-morphological aspects, the sample should be removed and investigated for cytology in compliance with the essential rules of X-ray guided bone

biopsy in order to have samples from the areas of moderate radio-transparency and not from those with increased radio-transparency or radio-opacity.

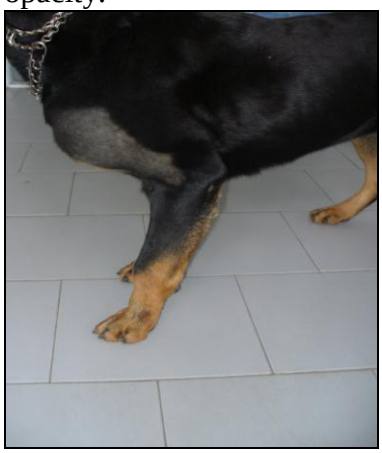

Figure 1. Swelling of the left scapulohumeral region

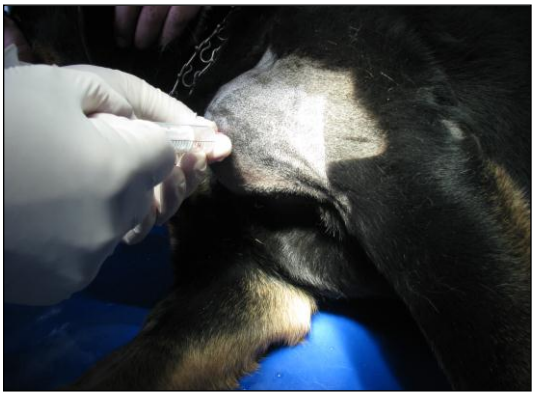

Figure 2. Performance of fine needle aspiration Biopsy

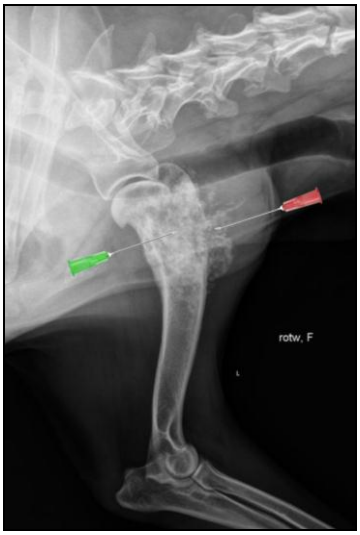

Figure3. Osteolytic areas alternate with zones of bone proliferation in the epi-metaphyseal region of the bone.

Recommended (green needle) and inadvisable (red needle) biopsy site.

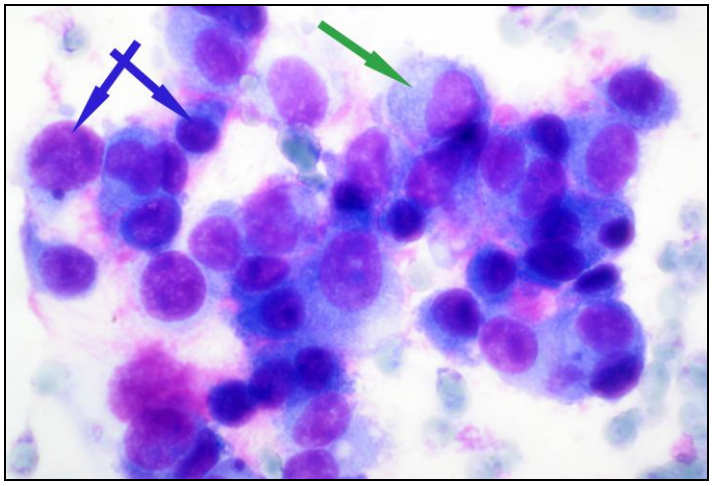

Figure 4. Group of tumor cells with a dysplastic appearance, exhibiting anisokaryosis (blue arrows) and lightly basophilic cytoplasm (green arrow). Cytologic features are typical of osteoblastic osteosarcoma. (May-Grünwald-Giemsa stain, 100x.).

CONCLUSIONS

Osteosarcoma occur in medium and large breed dogs
Osteosarcoma occur more frequently in the epiphyseal – metaphyseal area of long bones
Bone aspiration biopsy is performed successfully by means of X-ray guidance .
The sampling of the material for cytology investigation is made from areas shown on the X-ray film as having moderate osteolysis.

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ASSESEMENT OF MINERAL NUTRIENTS, HEAVY METALS AND PESTICIDES IN POULTRY LIVER USING INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract

Poultry liver is considered to be one of the most important sources of mineral nutrients in humans' diet, but due to its specific structure tends to bind chemical contaminants such as heavy metals and pesticides. The aim of this study was to perform a short characterization of mineral nutrients concentration and heavy metals and pesticides contamination in commercial poultry liver samples and their possible effect on food safety.
Eleven commercial poultry liver samples coming from 3 different Romanian slaughterhouses were submitted to analysis. Heavy metals (Cd, Pb, Al) and mineral nutrients (Ni, Cr, Mn, Cu, Fe, Zn, Ca, Mg, K, Na) were determined by Inductively Coupled Plasma-Mass Spectrometry. For organochlorine and organophosphorus pesticides quantification Gas Chromatography/Mass Spectrometry was used.
The concentrations for heavy metals ranged from 0.008 to 0.03 mg/kg Cd, 0.02 to 0.06 mg/kg Pb, 0.001 to 0.002 mg/kg Hg and 0.09 to 0.6 mg/kg Al. For all samples, the values of organochlorine and organophosphorus pesticides were under the limit of detection. For mineral nutrients, concentrations ranged from 0.02 to 0.07 mg/kg Ni; 0.02 to 0.09 mg/kg Cr; 0.5 to 1.32 mg/kg Mn; 0.9 to 2.6 mg/kg Cu; 12.9 to 48.7 mg/kg Fe; 3.7 to 7.7 mg/kg Zn, 35.6 to 62.3 mg/kg Ca, 86.6 to 167.2 mg/kg Mg, 1555.9 to 1668 mg/kg K, 593.51 to 1127.8 mg/kg Na.
Although it is known that people ingest heavy metals and pesticides from animal products, the concentrations obtained in this study showed that there is no risk for human health linked to the consumption of poultry liver.

***Keywords**: Gas Chromatography-Mass Spectrometry, heavy metals, Inductively Coupled Plasma-Mass Spectrometry, pesticides, poultry liver.*

INTRODUCTION

Poultry liver is considered to be one of the most important sources of mineral nutrients in humans' diet, but due to its specific structure tends to bind chemical contaminants such as heavy metals and pesticides.
Indeed, the liver, a major organ involved in metabolic processes, is considered to be one of the most eloquent witness of any disturbance in the body, as it is the subject to different types of etiologic attacks: infectious, toxic, metabolic, nutritional and traumatic (Doneley, 2004).
Offal consumption is not negligible in European Union. Analyzing the data extracted from the «Comprehensive European Food Consumption Database; Concise Data Base summary statistics - Total Population », it can be seen that the consumption of edible offal, including poultry liver, is between 1 g/day in Ireland and 26.1 g/day in Poland, with an average of 7.12 g/day for the European Union, considering the countries that participated to the survey (European Food Safety Authority, 2011).
In particular, poultry liver consumption needs a special attention. Indeed, poultry liver is considered to be an important source of nutrients, such as vitamins, macro elements and microelements, in some countries, it is used in pregnant women diet and in nutritional disorders.
For food from animal origin, one of the possible causes of exclusion from consumption because of a risk for public health is the contamination with chemical substances, such as heavy metals and pesticide which are contaminants tending to accumulate in poultry liver.
According to the Commission Regulation 1881/2006, the maximum tolerance levels for heavy metals in liver, including poultry liver, are established only for cadmium (Cd) and lead (Pb), respectively 0.5 ppm (mg/kg) for both (European Union Regulation (EC) No 1881/2006).
Interactions between toxic and essential metals are central to mineral balance and the antioxidant defense system in mammals and birds (Lopez-Alonso et al., 2007; Pappas et al., 2010).
Most of the studies show that residues of aluminum, cadmium and lead are the most frequent heavy metals to be determinate in poultry liver (Goyer, 1997; Jihen et al., 2008).
The residues of pesticides have become a factor for the environmental pollution and their toxic effects have been observed in humans and animals.
Organochlorine and organophosphorus pesticides are fat-soluble components which bioaccumulate through food chain.

The acute health risks of pesticides, their long persistence and tendency to accumulate in body tissues have raised a great concern about possible human health impacts due to low but chronic exposure (Salem et al., 2009). Using of Inductively Coupled Plasma-Mass Spectrometry for heavy metals and mineral nutrients monitoring and Gas Chromatography-Mass Spectrometry for pesticides quantification are one of the most commonly known techniques applied in animal production, including poultry industry. The aim of this study was to perform a short characterization of mineral nutrients concentration and heavy metals and pesticides contamination in commercial poultry liver samples and their possible effect on food safety.

MATERIALS AND METHODS

Eleven commercial poultry liver samples coming from 3 different Romanian slaughterhouses were submitted to analysis. The livers were from industrial intensive indoor rearing birds that were slaughtered at 40 days of age.
For heavy metals and mineral nutrients analyze, using Guirlet and Das (2012) protocol , approximately 500 mg of each poultry liver sample were digested with 2 ml concentrated nitric acid, 5 ml deionized water and 1 ml H_2O_2, in microwave pressure digestion system, type Berghof speed wave MVS-3 (Table 1).

Table 1. Parameters of sample digestion for heavy metals and mineral nutrients analyze

eating stages	Time (min)	Power (W)	Temperature (°C)
1	5	300	120
2	5	500	160
3	5	600	190
4	10	400	190
5	5	Pause	Pause

Samples were diluted to 25 ml with ultrapure water and analyzed by an Inductively Coupled Plasma-Mass Spectrometer (ICPMS, Perkin Elmer, Sciex, DCR 2) to determine heavy metals (Cd, Pb, Al) and mineral nutrients (Ni, Cr, Mn, Cu, Fe, Zn, Ca, Mg, K, Na).

An internal standard (^{103}Rh, CertiPUR®, Merck) was added to each sample and calibration standard solutions.
For organochlorine and organophosphorus pesticides quantification GC/MS was used. The following sample preparation protocol was used: 5 to 10 grams of chopped livers were sonicatied in 20 mL methylene chloride. The extract was filtered through glass wool and sodium sulfate to remove water and any particulate. After solid-liquid extraction the extract was concentrated to 1 or 2 ml. An amount of 1 or 2 ml of clean-up extract was analyzed by PolarisQ – Quadrupole Ion Trap GC/MS.
An internal standard (Pestanal, Riedel de Haen – Fluka) for organochlorine pesticides (lindan, aldrin, dieldrin, DDT, ά-HCH) and organophosphorus pesticides (malation, paration, parathion-metil, fention, dimetoat, ethion, phorate) was added to each sample.

RESULTS AND DISCUSSIONS

The results are presented (ppm or mg/kg) as mean values of a triplicate analysis of the sample extract and statistical analyses were performed with SPSS software version 19 for Windows.
The Inductively Coupled Plasma-Mass Spectrometer's limit of detection (LOD) for each heavy metal and mineral nutrient is presented in Table 2.

Table 2. LOD of ICP-MS for heavy metals and mineral nutrients

Chemical element	LOD (ppt)
Cd 114	0,08
Pb 208	0,07
Al 27	0,05
Ni 60	0,10
Cr 52	0,12
Mn 55	0,17
Cu 63	0,05
Fe 56	0,12
Zn 64	0,45
Ca 40	0,10
Mg 24	0,08
K 39	0,27
Na 23	0,14

The concentrations for heavy metals ranged from: 0.008 to 0.03 mg/kg for Cd, 0.02 to 0.06 mg/kg for Pb and 0.09 to 0.6 mg/kg for Al (Figure 1-3).
For mineral nutrients, concentrations ranged from: 0.02 to 0.07 mg/kg for Ni; 0.02 to 0.09 mg/kg for Cr; 0.5 to 1.32 mg/kg for Mn; 0.9 to 2.6 mg/kg for Cu; 12.9 to 48.7 mg/kg for Fe; 3.7 to 7.7 mg/kg for Zn, 35.6 to 62.3 mg/kg for Ca, 86.6 to 167.2 mg/kg for Mg, 1555.9 to 1668 mg/kg for K, 593.51 to 1127.8 mg/kg for Na (Figure 4-13).

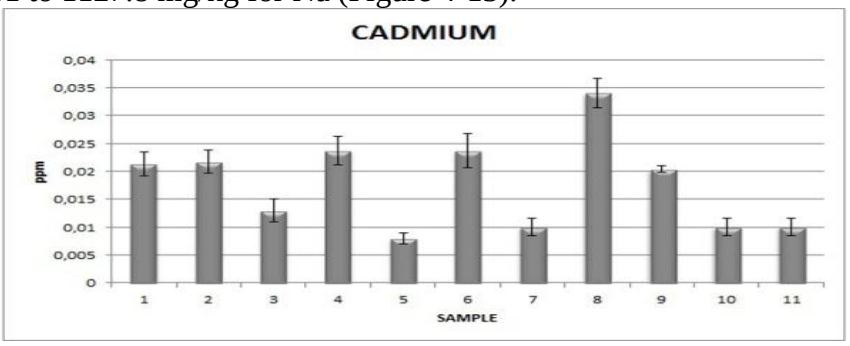

Figure 1. The content of cadmium (mean ± S.D. in mg/kg) in liver samples

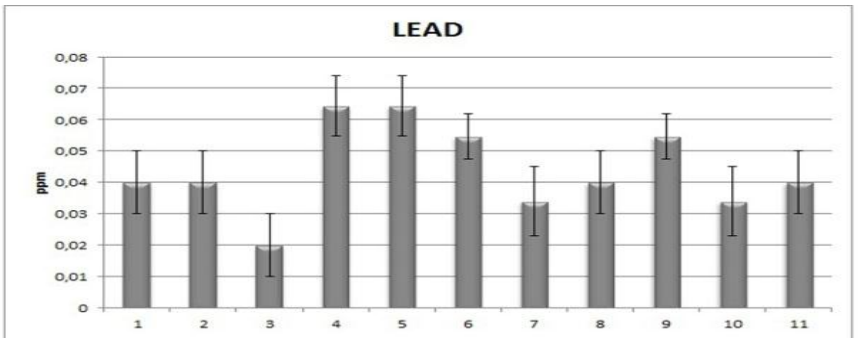

Figure 2. The content of lead (mean ± S.D. in mg/kg) in liver samples

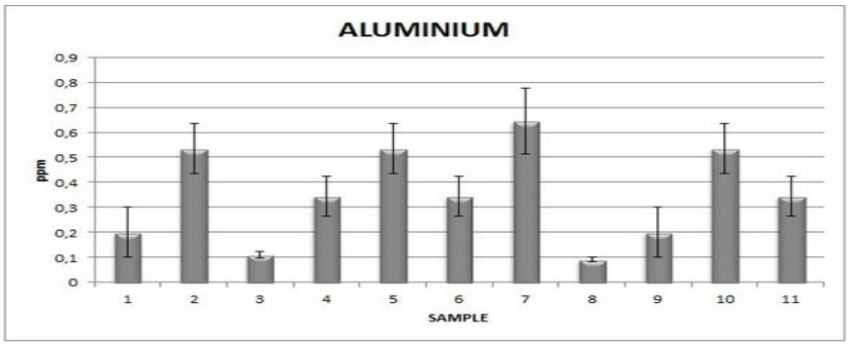

Figure 3. The content of aluminum (mean ± S.D. in mg/kg) in liver samples

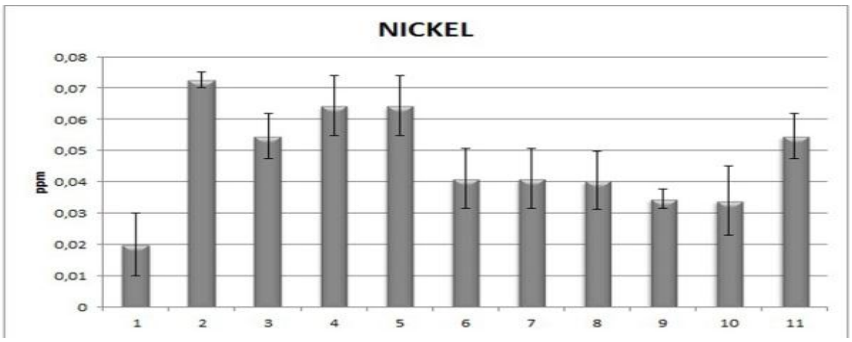

Figure 4. The content of nickel (mean ± S.D. in mg/kg) in liver samples

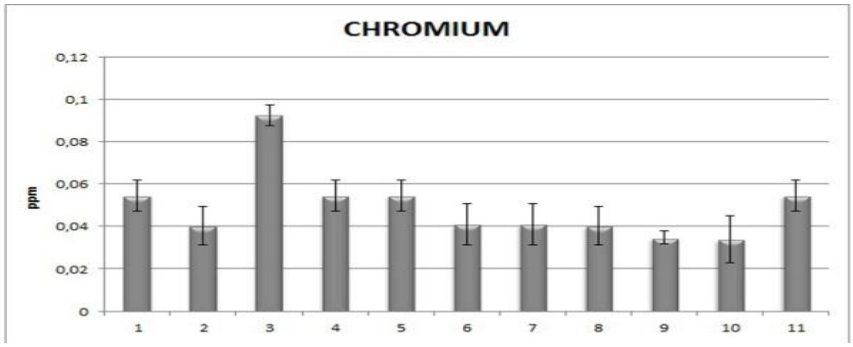

Figure 5. The content of chromium (mean ± S.D. in mg/kg) in liver samples

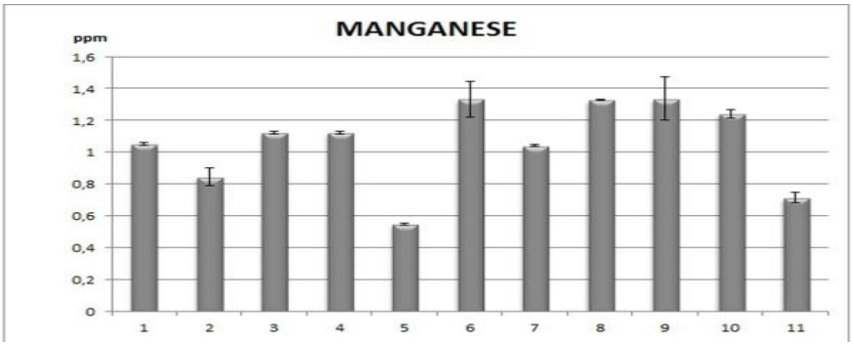

Figure 6. The content of manganese (mean ± S.D. in mg/kg) in liver samples

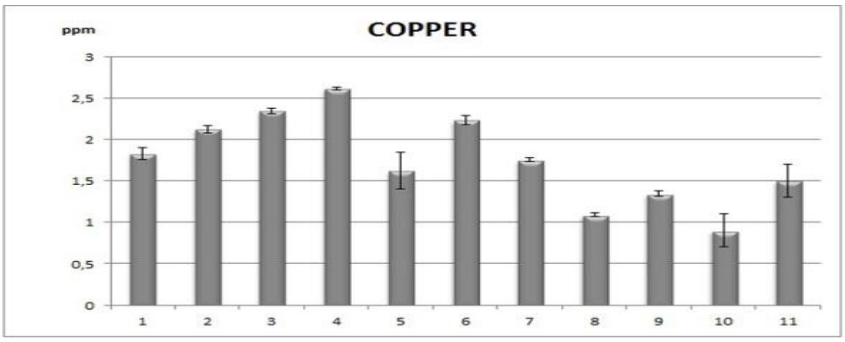

Figure 7. The content of copper (mean ± S.D. in mg/kg) in liver samples

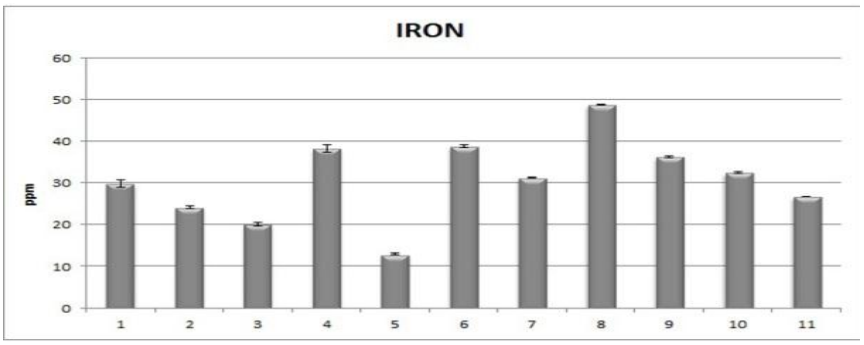

Figure 8. The content of iron (mean ± S.D. in mg/kg) in liver samples

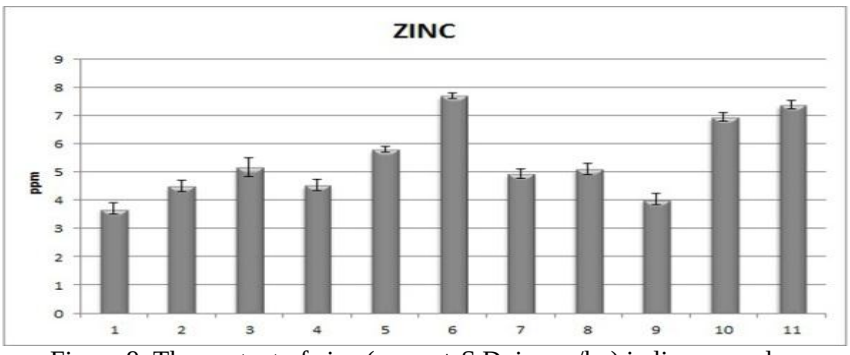

Figure 9. The content of zinc (mean ± S.D. in mg/kg) in liver samples

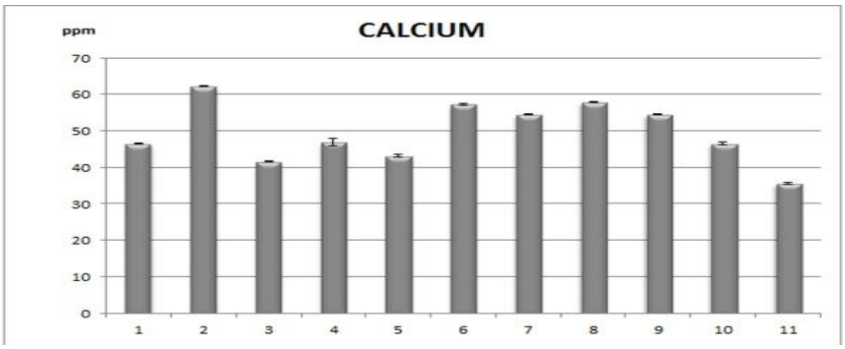

Figure 10. The content of calcium (mean ± S.D. in mg/kg) in liver samples

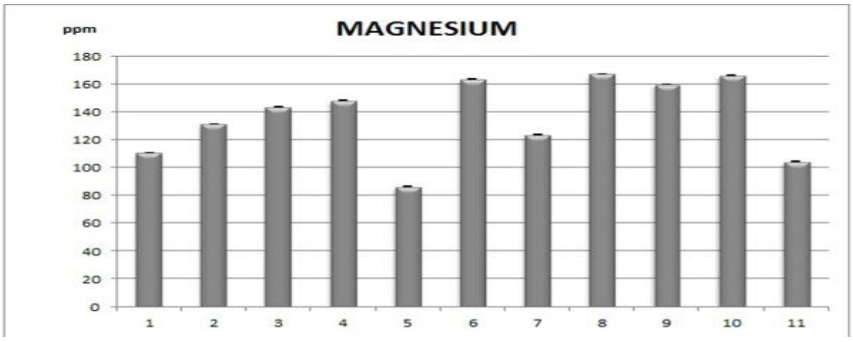

Figure 11. The content of magnesium (mean ± S.D. in mg/kg) in liver samples

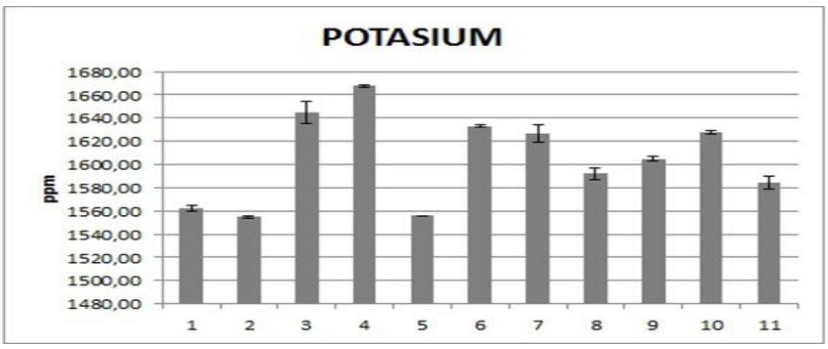

Figure 12. The content of potassium (mean ± S.D. in mg/kg) in liver samples

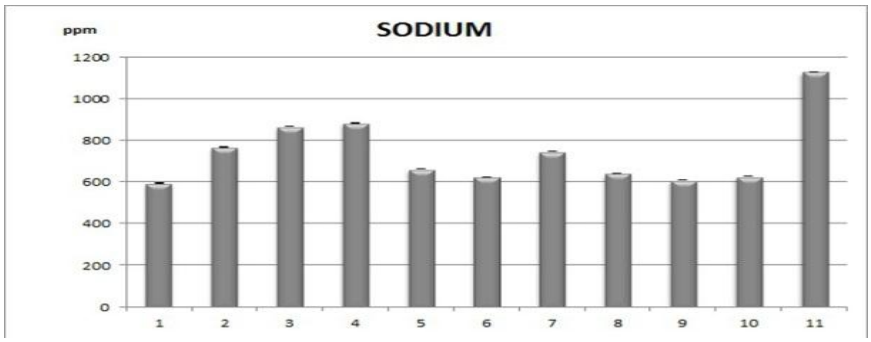

Figure 13. The content of sodium (mean ± S.D. in mg/kg) in liver samples

For all samples, the values of organochlorine and organophosphorus pesticides were under the limit of detection of the Gas Chromatography-Mass Spectrometer.

All poultry liver samples were under the maximum tolerance levels for heavy metals in liver, including poultry liver, which were established only for cadmium (Cd) and lead (Pb), respectively 0.5 ppm (mg/kg) for both (European Union Regulation (EC) No 1881/2006).

In this research, the concentrations of heavy metals and pesticides were not measured for water and feed of these poultry, but from literature it is known that the main source of heavy metals in chicken and turkey meat arises from contamination of poultry feed and drinking water (Baykov et al., 1996; Okoye et al., 2011).

Other sources of contamination can be dirty slaughter places and packaging.

CONCLUSIONS

Although it is known that people can ingest heavy metals and pesticides because of background levels especially in animal products, we show here, from a very limited study, that this intake from liver is most probably not exceeding the current total daily intake and thus it can be considered that there is no risk for human health linked to the consumption of poultry liver.

However, in case of accidents, when large amounts of these chemical substances are spread in the environment, heavy metals and pesticides contamination represent a real risk for the consumer health, because of their bioaccumulation thought the food chain and a risk assessment should be performed, in order to quantify the exposure of humans to that precise contamination.

AGNOWLEDGEMENTS

This study is part of the POSDRU project 88/1.5/S/52614 "Doctoral scholarships for high quality training for young researchers in the field of agronomy and veterinary medicine" and it is part of the PhD thesis "Correlations between liver pathology in broiler chickens and food safety"- Oana-Mărgărita Ghimpeţeanu.

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THE IMPORTANCE OF INTRAOPERATIVE RETROBULBAR BLOCK ON ANESTHETIC MANAGEMENT OF ENUCLEATION

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Abstract

One of the major goals of anesthesia is control the pain. For far too many years the veterinary profession has considered analgesia and pain management to be of little interest with practitioners as quoted outdated aphorisms as " animals feel less pain than humans", or "pain is beneficial because it limits movement"and "analgesia mask clinical degeneration ". Now we know that all these are totally false and that pain affects life and rehabilitation of patients in a tremendous way.

The aim of this article is to clarify specific conditions that occur in anesthesia following retrobulbar block using as reference parameters : pupillary reflex and position of the eyeball, before and after the block but also the heart rate and non invasive blood pressure.

***Key words**: analgesia, eye enucleation, retrobulbar block.*

INTRODUCTION

Pain has been defined by International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage ".

Local anesthetics are extremely useful in surgery procedures for providing analgesia and pain management, increasing the benefits in anesthesia protocol .

Retrobulbar block represents a regional anesthetic nerve block into the retrobulbar space, providing akinesia of extraocular muscles by blocking cranial nerves II, III ,IV, V (ophtalmic and maxilary branches) and VI, thereby preventing movement of the globe; it is a very useful procedure for enucleation surgeries, being an important tool for managing anesthetic

safety and patient comfort especially in old animals less tolerant of higher anesthetic levels.

MATERIALS AND METHODS

The animals that have been used for the study and proposed for enucleation were examinated at Faculty of Veterinary Medicine, Department of Ophtalmology, both dogs and cats developing clinical conditions such as: glaucoma and buphtalmia (Figure 1), penetrating corneal wounds with lack of substance, descemetocele, avulsion of the eyeball, intracameral neoplasms, uveitis and phthisis bulbi, especially encountered in viral feline rinotracheitis, etc. Contraindications for this tehnique were: inflammation, retrobulbar processes or abscesses.

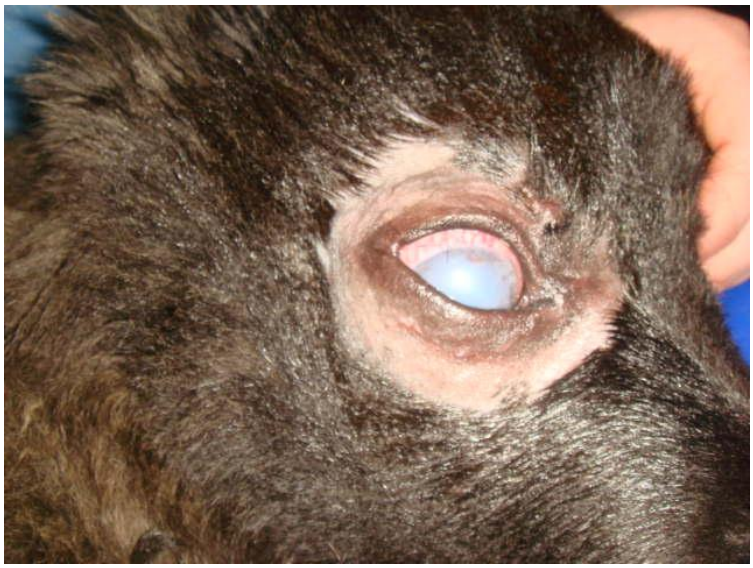

Figure 1. Buphtalmia before retrobulbar block

Retrobulbar block was performed under general anesthesia using both, total intravenous anesthesia (TIVA) or inhalatory tehnique. There have been used different protocols of anesthesia for TIVA protocol, according to ASA, such as: in premedication Acepromazine 10-40 µg/kg, ± an nonsteroidal Anti-inflamatory drug (Onsior, Metacam, Rymadyl, and

Tolfedine) followed by placing a catheter for induction and maintenance using BLK cocktail (in 500 ml NaCl 0.9%, Butorphanol 10 mg, Lidocaine 120mg, and Ketamine 500 mg). Another example for TIVA protocol was premedication with Acepromazine 10-40 µg/kg, ± an nonsteroidal Anti-inflamatory drug, than for induction and maintenance using Diazepam and Ketamine iv mixture 1:1. Inhalation anesthesia was performed with Acepromazine 10-40 µg/kg and Tramadol 2-5 mg/kg, ± an nonsteroidal Anti-inflamatory drug in premedication, followed by induction with Propofol 4-6 mg/kg, and maintanence with Isoflurane 1-2%.

Local anesthetics used for the surgeries were Lidocaine 2% 2-4 mg/kg and Bupivacaine 0.5% 1-2 mg/kg, mixed into the same syringe. The doses for lidocaine was halved in cats.

Retrobulbar block in our study was performed using both inferior temporal palpebral, and superior temporal palpebral injection techniques (Figure 2); it appears to be an intimidating-looking procedure, but it is really easy and safe. There are few steps to be followed :

- Hair clipping and skin disinfection of the affected eye.

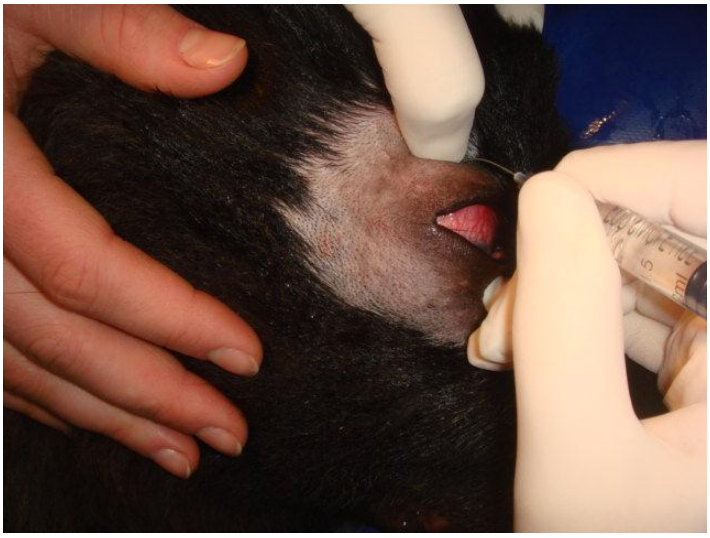

Figure 2. Retrobulbar block by palpating the superior orbital rim

- The conjunctiva is anaesthetized using topical eye anesthetics such as Benoxycaine (Figure 3).

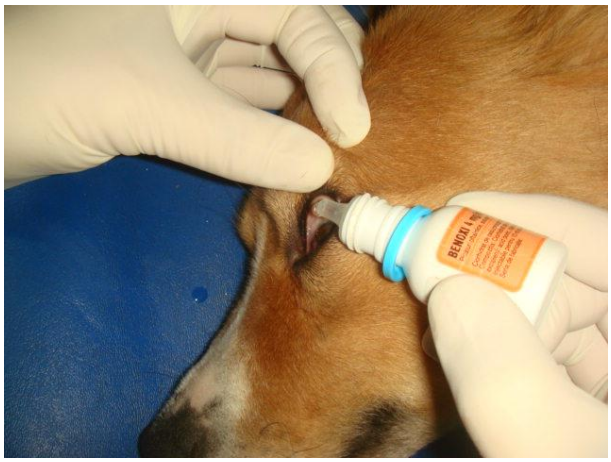

Figure 3. Topical eye anesthetics such as Benoxycaine.

- Use a 22, 23 standard needle wich is slightly bend (15- degree) in its middle.
- Place the needle perpendicular to the skin, directed medially toward the retrobulbar area.
- Advance the needle in the caudomedial direction. With the slight bend in the needle, advancing it will naturally follow this course.
- Once placed, aspirate. If there is no blood into the syringe, inject the local anesthethic (Figure 4).

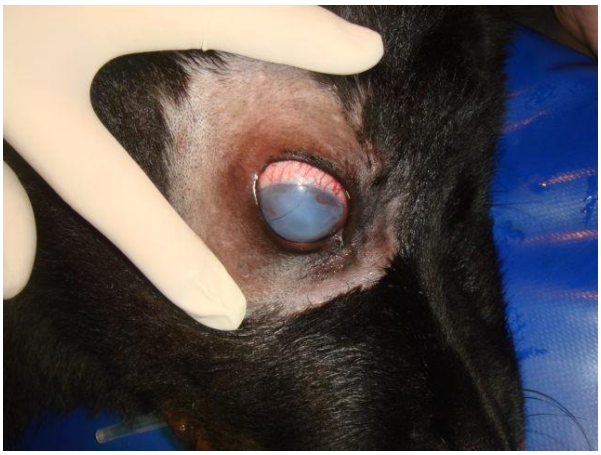

Figure 4. Exposure of the globe after retrobulbar block

- For enucleation, after closure of the lids, administer a peri-incisional infiltration of bupivacaine because the skin of the face would not have been blocked by the retrobulbar local anesthetic.

Complications associated with this block are either local or systemic. Local ocular include hematoma formation, which is the most common complication seen because of inadvertant puncture of vessels within the retrobulbar space, perforation of the globe and neuritis.

Systemic complications include oculocardiac reflex, local anesthetic toxicity and brainstem anesthesia.

Oculocardiac reflex represents a decrease in pulse rate following eye manipulation during the procedure. The reflex is mediated by nerve connections between the trigeminal cranial nerve and the vagus nerve of the parasymphatetic nervous system. There were pacients which developed the reflex in which heart rate suddenly dropped between 30 to 50 beats per minute. In some of them did not use anything and they successfully recovered by avoiding manipulation and for the others, there have been used atropine 0.02-0.04mg/ kg.

RESULTS AND DISCUSSIONS

Effectiveness of injection with local anesthetic (lidocaine2% and bupivacaine 0.5%) into retrobulbar space following eye enucleation has shown two aspects: first one is that provides akinesia of the extraocular muscles by blocking cranial nerves II,III and VI, thereby preventing movement of the globe and providing comfort for the surgeon's technique and the second one is that requires less anesthetic substance conferring additional analgesia and reducing the need for additional intra or postoperative analgesics.

CONCLUSIONS

Retrobulbar block acts as a part of polymodal analgesia of intraoperative anesthetic protocol for enucleation surgeries and can greatly improve our ability to provide complete and compassionate care.

Maintaining good analgesia makes practice more enjoyable for us, and definitely improves the quality of our patients' lives.

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www.vasg.org Veterinary Anesthesia & Analgesia Support Group; Practical Advice for the Compassionate Veterinary Practitioner

www.wikipedia.org Retrobulbar block.

THE CHARACTERISTICS OF LAMENESS IN DAIRY COWS

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Abstract

Lameness is considered one of the most important aspects of the animal health besides breeding problems and mastitis. This evaluation is based probably partially on the incidence of this clinical sign and the economical importance of accidental diseases.
In another sense lameness could have even a greater importance. Well established control protocols can be implemented regarding both mastitis and breeding problems, but not in the case of bovine lameness problems where such protocols need specification depending on the numerous factors such as raising conditions, forage management, the capacity of farmers to understand that high production is achieved with the price of functional efficiency of the animals.
In addition to the multitude of risk factors we have the permanent evolution of nutrition practice and management systems. The latter serve the best interest of man rather than cow comfort.
On the farm where the study has been developed cow lameness was around 20%, the conditions that generated lameness were the following: 5% sole ulcer, 5% heel erosion, 4% necrobacilosis, 2% interdigital dermatitis, 3% interdigital phlegmon, 1% claw deformity. The high percentage of lame cows suggest the presence of subclinical laminitis in the herd, and it is advisable to note these conditions in special records, and develop a more extensive investigation, including monitoring the nutrition management.(1)
Other painful conditions such as renal colic or abomasum dysplasia are not as frequent as lameness.

Key words: *attitudes, bovines, gait, lameness, leg*.

INTRODUCTION

Cheston said that the veterinary who faces lameness issues in a bovine farm needs to examine the management problems of the farm than to treat a long series of individual cases. (2)
Lameness is a clinical sign and should not be regarded as disease or disorder.
The main reader that is in view here is the veterinary who practices bovine surgery, but also the farmer who is concerned with the health of his herd and the financial performances of the farm.

The pictures are an important component of the communication process with those whom this article is addressed to, as they are suggestive in presenting the clinical cases.

MATERIALS AND METHODS

Bovines in a dairy herd half breed Holstein – Friesian, inspection, palpation.

RESULTS AND DISCUSSIONS

Starting from the function of the limbs lameness can be divided according to the two phases of the stride: lameness in the phase of suspension and lameness in the phase of support. We can add to these the mixt lameness that pertain to both suspension and support.
In bovines, the most frequent lameness occurs in the support phase. These dominate the symptomatic frame of pedal disorders and have a significant value of anatomoclinical diagnosis.(3)
The intensity of lameness vary from a hindrance in walking, to walking with three legs. Bovines are very resistant to pain, and because of this, pain has to be excruciating so that a cow should limp on a soft surface.
Therefore, it is highly recommended that lameness in bovines should be diagnosed on firm, level and non-slippery ground surface.
A lame cow prefers decubitus (Figure1), and when a cow swings its limb away from the body it is attempting to relieve pain in the outside claw (Figure 2).

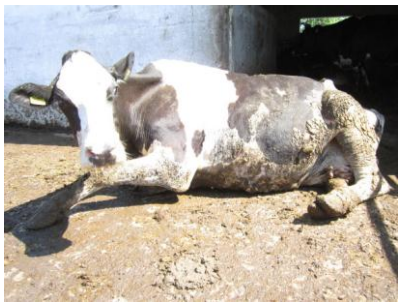

Figure 1. Cow prefers decubitus

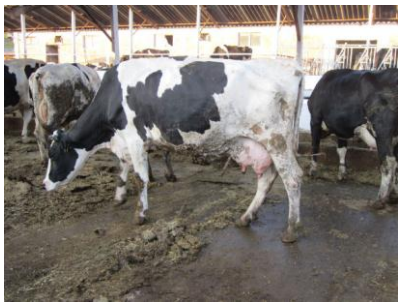

Figure 2. Cow swings its limb away from the body

The gait with the sick limb is hesitant, as it is out of support, and the back is arched more or less, according to the intensity of the pain. (Figure 3)

Figure 3. Hesitant gait and arched back

Determining the seat of lameness and its nature has required the systematic exam of all the anatomical components of the sick limb, starting with the emergence with the body and finishing with the examination of claws. (Figure 4)

Figure 4. Examination of claws

In order to diagnose lameness a cow should be observed from each side: from the front and behind, when standing quietly and when walking. Than it should be observed when it turns to the left and then to the right.
Normal stride has three phases:

1. Weight-bearing phase
2. Forward phase (Protraction)
3. Backward phase (Retraction)

During the weight-bearing phase, the bearing surface of heel-bulb junction of a hind limb normally touches the ground first. This is followed by the abaxial part of the weight-bearing margin of the wall and then the toe. Finally, the bearing surface of the claw will slide forward to an extent, depending on the friction generated by the ground surface.
The protraction phase is when the limb swings forward and then back to weight-bearing.The retraction phase is the backward swing of the limb.
A healthy cow walks with a level spine and places her hind feet almost exactly onto the same spot as the fore feet.
According to the place of the pain there can be observed different attitudes of the bovines while walking.
Thus in acute laminitis the cow can protract the hind limbs (camping forward), and the forelimbs are retracted under its body (camping back) – Figure 5.

Figure 5. Camping forward and camping back

The pain in the heel bulbs or posterior region of the sole determines the cow to retract its hind limbs. Such a situation can occur in hip arthritis (Figure 6)

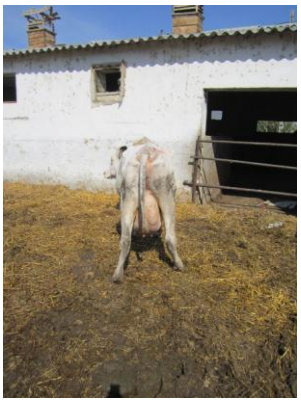

Figure 6. The pain in the heel bulbs

A lame cow will hold its head lower than normal, will spend less time in bearing the weight on the affected limb and the stride will be shorter. (Figure 7).

Figure 7. A lame cow will hold its head lower than normal

”Cow Hock” Posture either in the forelimbs or the hind limbs is an indicator of severe pain (Figure 8)

Figure 8. ”Cow Hock” Posture

Hanging leg lameness is a reaction to extreme pain as would occur from septic arthritis of the pedal joint or a fractured pedal bone (Figure 9).

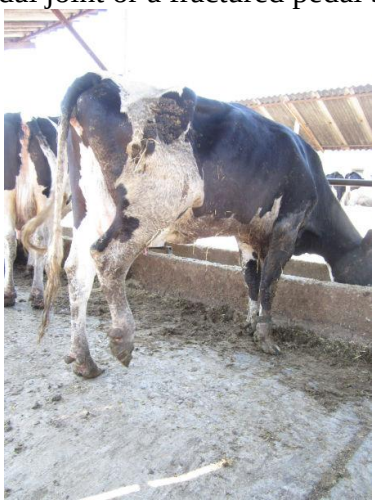

Figure 9. Hanging leg lameness is a reaction to extreme pain

If an animal stands or walks with its feet close together it is „walking narrow." This is often the sign of the seat of lameness being located in the inside (medial) claw. Cattle with subclinical laminitis mainly in the medial claws will walk narrow. (Figure 10)

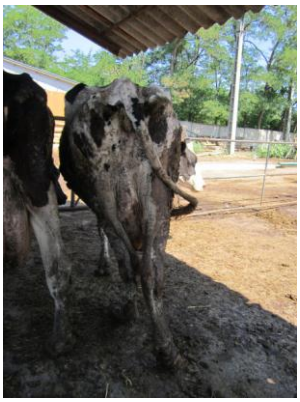

Figure 10. Walking narrow

In order to diagnose lameness in cows it has been designed a score system from 1 to 5 recommended for current use on the farm (2):

Locomotion score 1
Clinical Description
Normal
Description: Stands and walks normally. All feet placed with purpose.
Back Posture Standing: Flat / Back Posture Walking: Flat
Locomotion Score 2
Clinical Description
Mildly Lame
Description: Stands with flat back, but arches when walks. Gait is slightly abnormal.
Back Posture Standing: Flat / Back Posture Walking: Arched
Locomotion Score 3
Clinical Description
Moderately Lame
Description: Stands and walks with an arched back. Short strides with one or more legs.
Back Posture Standing: Arched / Back Posture Walking: Arched
Locomotion Score 4
Clinical Description
Lame
Description: Arched back standing and walking. Favoring one or more limbs but can still bear some weight on them.
Back Posture Standing: Arched/ Back Posture Walking: Arched
Locomotion Score 5
Clinical Description
Severely Lame
Description: Arched back, refuses to bear weight on one limb. May refuse or have great difficulty moving from lying position.
Back Posture Standing: Arched / Back Posture Walking: Arched

CONCLUSION

The incidence of lameness in dairy herds probably ranges from 0 to 60%. An annual incidence of over 10% should be regarded as a problem herd and foot health should be monitored very closely. If the incidence of sole ulcer, toe ulcer and white line disease together exceeds 5-10% in a herd, this would be a strong indication that subclinical laminitis is present and a comprehensive investigation is justifiable on economic grounds.
Scoring the Severity of Lameness.

The severity and duration of a lameness should be recorded in the health records of any heard with a claw health problem.

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EVALUATION OF THE MULTILINEAR CAPACITY OF CANINE MESENCHYMAL STEM CELLS

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Abstract

Interest of the medical world towards regenerative therapy using mesenchymal stem cells has become increasingly prominent, given the many recent successes. Dogs are ideal candidates for testing the methods of isolation, cultivation and differentiation of mesenchymal stem cells into multiple cell lines and their use in regenerative therapy. The aim of this paper was to test the multipotence and multilinearity of mesenchymal stem cells derived from canine bone marrow and umbilical cord blood. Mesenchymal stem cells were obtained from a total of 12 dogs following medular aspiration or by collecting cord blood during caesarean section. Samples were processed using Histopaque 1077 and then cultured in α-MEM supplemented medium. In order to assess the stemness and multipotency of mesenchymal cells isolated from canine bone marrow and umbilical cord blood, their phenotype was characterized by assessing the Oct4 gene expression followed by the evaluation of their differentiation potential towards bone, cartilage, fat and nerve cells. Canine bone marrow and umbilical mesenchymal stem cells expressed the Oct4 gene. This gene expression was not identified after differentiation, however was shown in cells grown in propagation medium. Osteogenic, chondrogenic, adipos and nervous differentiation was demonstrated by identifying specific morphology, specific stainings and by assessing the gene expression of genes of interest. Canine mesenchymal stem cells have a high multilineage capacity, being able to differentiate towards osteogenic, chondrogenic, adipogenic and nervous lines, These properties can be exploited in order to use this type of cell therapy in homologous, heterologous and even xenogenic regenerative therapies.

Key words: *canine, mesenchymal, stem cells, differentiation.*

INTRODUCTION

The study of multipotent mesenchymal stem cells and their differentiation potential towards various cell lines, like bone, cartilage adipose tissue or neural tissue, represent an extremely important research topic. (Zucconi et al., 2010; Pall et al., 2009; Malgieri et al., 2010). Interest of the medical world towards regenerative therapy using mesenchymal stem cells has

become increasingly prominent, given the many recent successes (Martin et al., 2002; Livingstone et al., 2003; Krampera et al., 2007). Dogs are ideal candidates for testing the methods of isolation, cultivation and differentiation of mesenchymal stem cells into multiple cell lines and their use in regenerative therapy (Bianco et al., 2001; Cancedda, 2003; Baertschiger, 2005). The aim of the present study was to test the multipotence and multilinearity of mesenchymal stem cells derived from canine bone marrow and umbilical cord blood.

MATERIALS AND METHODS

Mesenchymal stem cells were collected from 12 dogs by medular aspiration as well as collection of cord blood during caesarean section. Samples were processed using Histopaque 1077 and then cultured in α-MEM medium. In order to o assess the stemness and multipotency of mesenchymal cells the following protocols were used: assessment of Oct4 gene expression and evaluation of differentiation potential towards bone, cartilage, fat and nerve cells by evaluating the specific morphology of cells, performing specific staining and assessing the gene expression of genes of interest. Cellular differentiation was performed by culturing cells in specific culture media, as follows:

- For osteogenic differentiation
  - DMEM –LG (Sigma-Aldrich™);
  - 10 mM ß glycerophosphate (Sigma-Aldrich™);
  - 300 μM ascorbic acid (Sigma-Aldrich™);
  - 10% FCS (Gibco®);
  - 1% antibiotic-antimycotic mix (Gibco®).
- For chondrogenic differentiation:
  - DMEM –LG (Sigma-Aldrich™);
  - 10-7 mol/l Hydrocortison (Sigma-Aldrich™);
  - 100 μM Ascorbic acid (Sigma-Aldrich™);
  - 1% ITS (Sigma-Aldrich™);
  - 2% HS (horse serum) (Gibco®);
  - 1% antibiotic-antimycotic mix (Gibco®).
- For adipogenic differentiation

- RPMI medium (Gibco®);
- 5% FCS(Gibco®);
- 100 μM ascorbic acid (Sigma-Aldrich™);
- 0.5 mM Isobutyl-xanthine (Sigma-Aldrich™);
- 1% antibiotic-antimycotic mix (Gibco®).

– For nervous differentiation:
- NEUROBASAL medium (Invitrogen™).
- 2 mM glutamax (Sigma-Aldrich™);
- 10-6 M retinoic acid (RA) (Sigma-Aldrich™)

The specific differentiation markers identified by PCR are shown in table 1:

Table 1. The specific differentiation markers identified by PCR

Markers	**Gene**	**Primer sequence (5'-3')**
Stemness	OCT4	Forward GAGTGAGAGGCAACCTGGAG
		Reverse GTGAAGTGAGGGCTCCCATA
Bone	*OSTEOPONTIN*	Forward CATATGATGGCCGAGGTGATAG
		Reverse CAAGTGATGTGAAGTCCTCCTC
	OSTEOCALCIN	Forward GAGGGCAGCGAGGTGGTGAG
		Reverse TCAGCCAGCTCGTCACAGTTGG
Cartilage	COL2A	Forward GAAACTCTGCCACCCTGAATG
		Reverse GCTCCACCAGTTCTTCTTGG
Nervous	BIII TUBULIN	Forward GCACACTGCTCATCAACAAG
		Reverse TCTTGCTCTCCTTCATGGA
	GFAP	Forward CGAGTTACCAGGAGGCACTA
		Reverse TCCACGGTCTTTACCACAAT
	NESTIN	Forward GAGAACCAGGAGCAAGTGAA
		Reverse TTTCCAGAGGCTTCAGTGTC

RESULTS AND DISCUSSIONS

Canine medullar and umbilical mesenchymal stem cells have shown OCT4 gene expression. This gene expression was no longer identified after differentiation, but was shown in cells cultivated on propagation medium (Figure 1).

For osteogenic differentiation, at the end of cultivation the mineralized cellular matrix was noticed as well as the osteogenic nodules. The apparition

of calcium deposits was proven by Alizarin-Red coloration (Figure. 2). The cells expressed the osteopontin and osteocalcin markers (Figure 3).
Regarding the chondrogenic differentiation, the cells presented a flattened morphology with aggregation tendency (Figure 4). The chondrogenic phenotype was proven by showing the expression of COL2A marker (Figure 5).

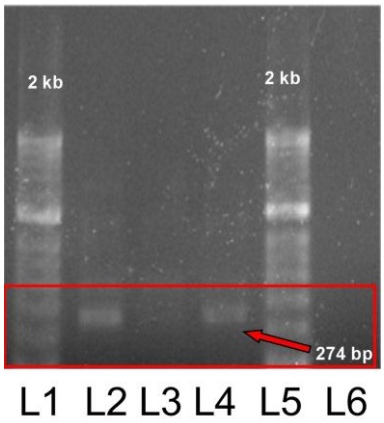

Figure 1 Oct4 expression before and after differentiation
(L1, L5 – ladder, L2 – BM MSCs, L4 – UCB MSCs, L3, L6 – osteogenic differentiation)

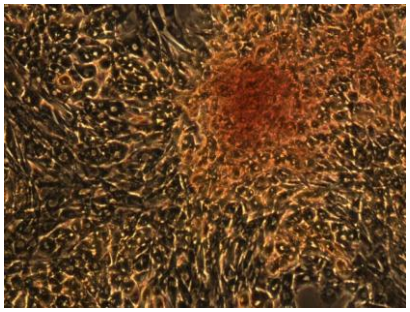

Figure 2 Alizarin red staining
-extracellular calcium deposits

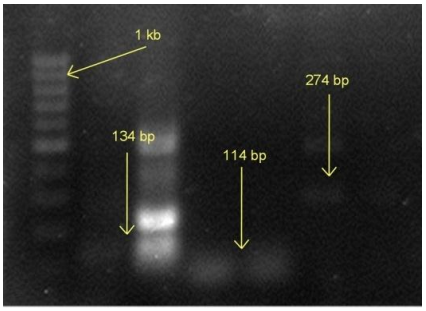

Figure 3 Osteocalcin 134 bp and
osteopontin 114 bp expression

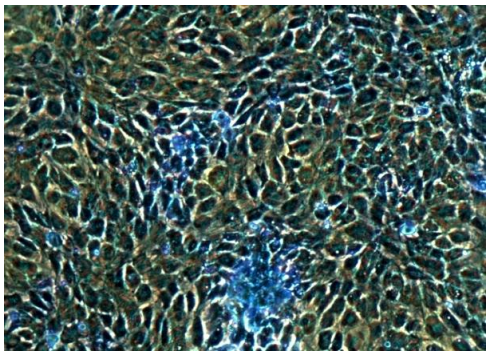

Figure 4 Alician blue staining - glycosaminoglycans deposits

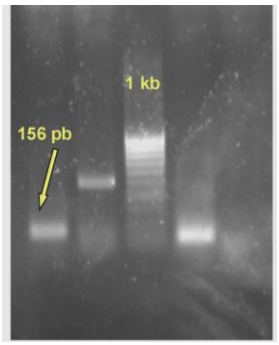

Figure 5 Col2A 156 bp expression

For adipose differentiation, lipid vacuoles were noticed shown by Oil Red coloration (Figure 6, 7).

Nervous differentiation showed a remarkable rounding of cells accompanied by increase of cell size (Figure 8). We also showed Nestin 328 bp and GFAP 277 bp expression for early differentiation as well as B3 tubulin for late differentiation (Figure 9).

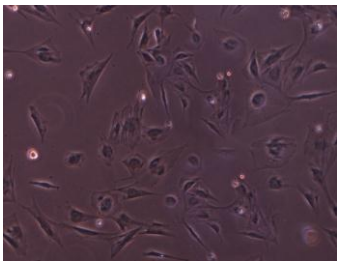

Figure 6 Lipid droplets

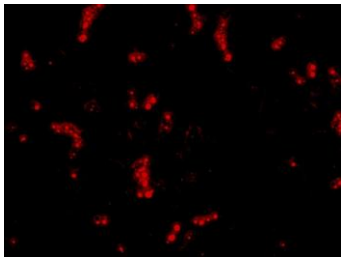

Figure 7 Oil Red staining

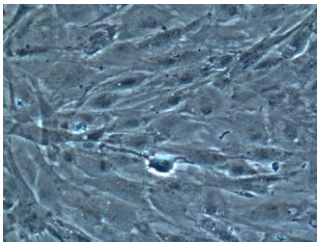

Figure 8 Intercellular filaments

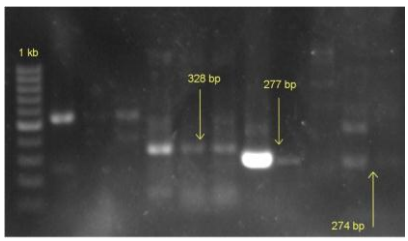

Figure 9 Nestin, GFAP and B3 tubulin

CONCLUSIONS

Canine mesenchymal stem cells have a high multilineage capacity, being able to differentiate towards osteogenic, chondrogenic, adipogenic and nervous lines.

These properties can be exploited in order to use this type of cell therapy in homologous, heterologous and even xenogenic regenerative therapies.

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POST-TRANSPLANTATION DISTRIBUTION of CD44+ HUMAN MESENCHYMAL STEM CELLS IN a MOUSE model

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Abstract

Mesenchymal stem cells (MSCs) are playing an important role in tissue engineering. Because of their properties to differentiate in multiple lineages, these cells became promising materials for the treatment of different types of degenerative disease, including bone disorders. In order to evaluate the distribution of xenogeneic MSCs engraftment, the aim of our study was the screening of human CD44+ MSCs distribution after intraperitoneal transplantation in a mouse model for osteoporosis. Human MSCs were harvested from the palatal subepithelial connective tissue. The cells were grown in DMEM/F12 (Sigma Aldrich) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. After i.p. transplantation of 1,1x10^6 CD44+ hMSCs in a mouse model, the screening of donor cells engraftment from blood samples was assessed at 4 and 11 days post-transplantation. The mice were euthanized by cervical dislocation at 14 days, followed by human MSCs engraftment assessment in blood, bone marrow and spleen samples. Results were quantified by immunophenotypic characterization with FACS Canto II flow cytometry system (BD Biosciences, San Jose, CA, USA). Our data confirmed the special homing characteristic of human MSCs in a mouse xenograft model. At 4 days post injection, in blood samples was found a percentage of 0,5% CD44+ cells and at 11 days, a percentage of 0,1% of CD44+ cells. At 14 days, a percentage of 0,1 % CD44+ human MSCs was found in blood as well as in bone marrow, but all spleen samples were negative.

Key words: *human MSCs, mouse model, osteoporosis, engraftment.*

INTRODUCTION

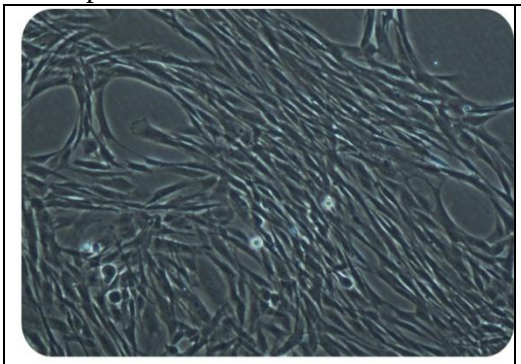
The National Institute of Health resources is defining the stem cells as ‘‘an undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ’’(Dominici et al., 2006, Groza et al., 2009, Diptiman et al., 2010). During the past decades, the multipotent potential property of MSCs was showed in a large number of studies were the culture expended MSCs were differentiated into phenotypic different

lineages, including osteocytes (Bruder et al., 1998), cartilage (Johnstone et al., 1998), adipocytes, muscles (Pittenger et al., 1999) and tendon (Young et al., 1998). Currently, MSCs are most frequently isolated from the bone marrow (Moustapha et al., 2004), but recent studies demonstrated that the mesenchymal stem cells derived from the human palatal subepithelial connective tissue (PDLSCs) and placenta have the same properties as the bone marrow derived MSCs (Pall et al., 2009, Hidefumi et al., 2011).
Because of their unique properties, MSCs are playing an important role in tissue engineering. These cells became promising materials for the treatment of different types of degenerative diseases, including bone disorders due to their capacity to differentiate into osteoblasts (Bruder et al., 1994). It is known that the increased life expectancy leaded to an increased prevalence of post-menopausal osteoporosis and related fractures (Cho et al., 2009). This progressive skeletal disorder, characterized by the reduction of the bone mass as a result of diminished osteogenesis, presents a slow response to therapeutic agents, reason why it is necessary to develop alternative treatments (Kim et al., 2006).
The therapeutic efficacy of transplanted MSCs depends on their mobilization from the place of injection and trafficking through the circulation to the injured tissue. The mechanism of homing after systemic transplantation requires an understanding of the distribution dynamics of injected MSCs (Diptiman et al., 2010).
In order to evaluate the distribution of xenogeneic MSCs engraftment, the aim of our study was the screening of CD44+ human MSCs (hMSCs) distribution after intraperitoneal (i.p.) injection in a mouse model for osteoporosis.

MATERIALS AND METHODS

Human MSCs were harvested from the palatal subepithelial connective tissue with informed consent approved according to the procedures of the institutional review board. The cells were cultured-expanded in DMEM/F12 (Sigma Aldrich) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. The multipotency of cultured CD44+ hMSCs was confirmed by immunophenothypic evaluation performed with the BD FACS Canto II flow cytometer.
The enzymatic treatment with 0.25% trypsin-EDTA solution (Sigma-Aldrich) was followed by cell count with a hemocytometer. The hMSCs were centrifuged and suspended at a concentration of $1,1x10^6$ cells/ 0,1ml

PBS. The obtained cells suspension was injected in the intraperioneal cavity (i.p.) in a total volume of 0.1 ml in a mouse model with induced osteoporosis.



A

B

Figure 1. A – Stabile line of hMSCs CD44+
B – Intraperitoneal transplantation of hMSCs

The mechanism of homing after transplantation was evaluated by screening of donor cells engraftment level in blood samples at the 4th and the 11th day post injection. The blood was collected from the tail vein on heparin coated MiniCollect tubes (Greiner). The serum was recovered by centrifugation at 2500 rpm for 10 minutes at room temperature. Samples were stored at -80°C until analysis.

At the 14th day post injection, the mice were euthanized by cervical dislocation and organs, including spleen and long bones, were harvested together with blood samples. The bone marrow was isolated from the femur by removing epiphysis and flushing the shaft with 1 ml PBS after insertion of a 27 gauge needle. The spleen was minced into 2-4 mm pieces using a scalpel blade. An appropriate amount of trypsin EDTA solution was added and the obtained suspension was incubated at 37°C for 30 minutes. The cells were dispersed by gentle pipeting and subsequently were filtered using a cell strainer to eliminate the debris. The cell suspension was centrifuged at 1500 rpm for 4-5 minutes, at 4°C and the supernatant was discarded.

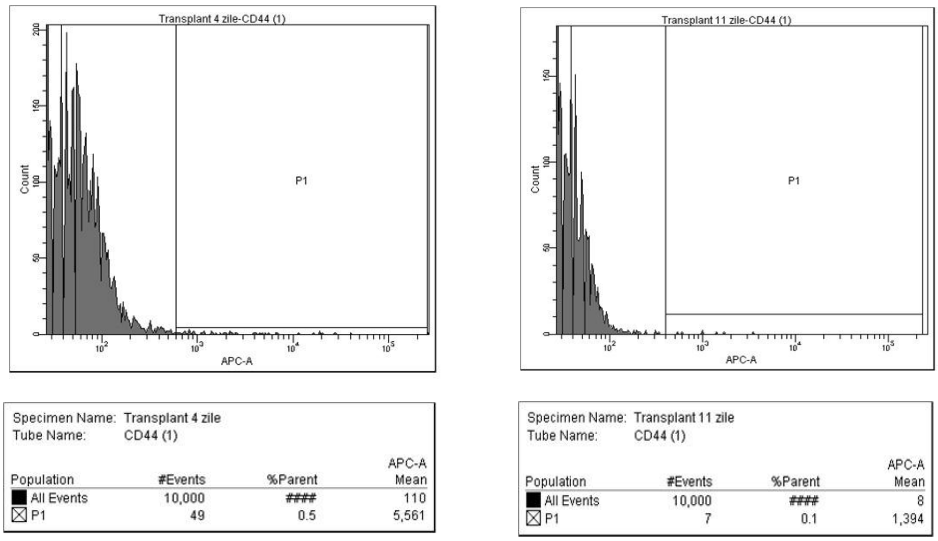
From all cell sources, a final concentration of 2x10^{7} /ml cells was used for immunophenotypic characterization. Anti CD44 antibody (BD Biosciences, San Jose, CA, USA) was used and the quantification was performed with the BD FACS Canto II flow cytometry system.

RESULTS AND DISCUSSIONS

CD44 is a cell surface glycoprotein, being involved in epithelial cell adhesion, reason why is considered to be involved tissue remodeling and stimulation of cell proliferation and migration. Recent studies demonstrated the importance of CD44 expression on MSCs, not only because is a robust marker of pluripotency, but also because plays important functions in cell-matrix interaction, mechanism of homing and apoptosis resistance (Sally et al., 2012; Zoller, 2011).

The percentages of the hMSCs cells in the blood were clinically screened on day 4, 11 and 14 after the i.p. injection, end the engraftment in bone marrow and spleen was evaluated at the 14th day of the study.

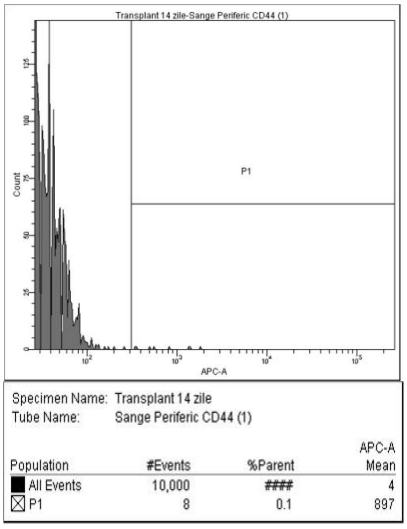
As it is showed in figure 2.A, the circulating number of CD44+ hMSCs peaked at the 4th day post-injection, in the blood samples being found a percentage of 0.5%. The percentage of donor-derived cells had decreased at the 11th day after the treatment, a level of 0.1% CD44+ hMSCs being detected (figure 2.B).



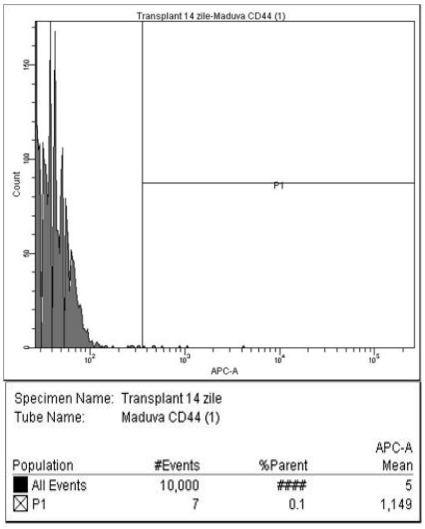
A B

Figure 2. A – CD44+ hMSCs kinetics at 4th day post injection
B - CD44+ hMSCs kinetics at 11th day post injection

When analysing the blood and organ samples on the 14th day, 0.1 % CD44+ hMSCs were found in blood (Figure 3.A), indicating that the maintained level is similar to day 11. The most important finding was the presence of 0.1% hMSCs in bone marrow. (Figure 3.B). All spleen samples were negative (Figure 3.C).



A



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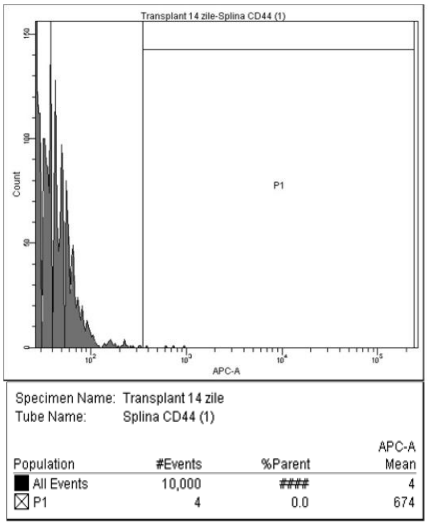

C

Figure 3. A – CD44+ hMSCs kinetics at 14th day in blood
B - CD44+ hMSCs engraftment at 14th day in bone marrow
C - CD44+ hMSCs engraftment at 14th day in spleen

Our data confirmed the special homing characteristic of human MSCs in a mouse xenograft model. The representative results of the FACS profiles which confirm the kinetic data are present in table 1.

Table 1. FACS profile of hMSCs

Screening day	Percentage of hMSCs (%)		
	Blood	Spleen	Bone Marrow
Day 4	0,5%	-	-
Day 11	0,1%	-	-
Day 14	0,1%	0%	0,1%

This data indicate that intraperitoneal injection is an optimal method to realize the bone marrow engraftment of donor derived MSCs after i.p. injection. Compared with other studies, in which the peak of circulating retroviral transducted MSCs was found at 7 days post-injection (Kim et al., 2006), we had the highest percentage at 4 days, which indicate the early engraftment of hMSCs cells after *in vivo* transplantation in an osteoporotic mouse model. There are many reports which suggest that injected MSCs have the potential to differentiate into multiple tissues after transplantation in different animal models (Prockop, 1997; Pereira et al., 1998; Azizi et al., 1998), also being reported that MSCs do not arrive in bone marrow after systemic transplantation (Kim et al., 2006; Murry et al., 2004, Balsam et al., 2004,). Contrary to this findings, in our experiment a percentage of 0.1% donor-derived MSCs were found, which suggest hMSC engraftment in the bone marrow after systemic transplantation via intraperitoneal cavity. The most important aspect of this finding consist in bone marrow engraftment of transplanted MSCs, that have the property to initiate the proliferation of donor hematopoietic stem cells along side with donor derived stromal cells (Kushida et al., 2001).

CONCLUSIONS

Our data confirmed the special homing characteristic of human MSCs in a mouse model with induced osteoporosis, the injection via intraperitoneal cavity being a facile and non-invasive method for engraftment of donor derived cells.

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IS THE CHROMATIC PUPILLARY RESPONSE (CPR) A "FOOTHOLD" IN THE DIAGNOSIS OF OPHTHALMOLOGICAL AND NEUROLOGICAL DISORDERS?

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Abstract

Recent studies have shown that a light stimuli of a certain intensity and wavelength can influence the pupillary response. The aim of this research is to evaluate the CPR and to establish its role in the differential diagnosis of ophthalmological and neurological disorders.
CPR is part of the patients' ophthalmological clinical examination protocol brought to the clinic of the Bucharest Faculty of Veterinary Medicine. These patients (dogs and cats) had one or more ophthalmological symptoms (progressive loss of vision, mydriasis, anisocoria, blindness) and/or neurological symptoms (nystagmus, torticollis, circling, ataxia, proprioceptive deficits).
CPR examination was performed in the darkroom, using the IRIS-VET device.
CPR is positive in healthy animals, represented by miosis.
Negative, delayed or incomplete CPR (mydriasis) to the red light (630 nm, 200 kcd/m^2) reveals an impairment of the photoreceptor cells of the retina, lesions encountered in retinal detachments, retinal dysplasia or progressive retinal atrophy.
CPR absence to the blue light (480 nm, 200 kcd/m^2) provides information about the optic nerve and retinal ganglion cells.
Negative CPR both to the red and blue light was present in glaucoma and optic chiasm disorders.
The study showed that CPR is a fast and easy method to differentiate between ophthalmological and neurological disorders.

Key words: *chromatic pupillary response, melanopsin, neurological disorders.*

INTRODUCTION

The pupillary light response (PLR) is a reflex that controls the diameter of the pupil, in response to the intensity of the light. It is an objective parameter in assessing the retinal, optic nerve andoculomotor nerve function. (Grozdanic, 2007)

Traditionally, clinical testing of PLR activity is performed with nonchromatic white light stimuli of different light intensities.
The pupil's response to light stimuli of a certain intensity and wavelength is chromatic pupillary response (CPR). The CPR was evaluated based on the response of the photoreceptors, the melanopsin-containing retinal ganglion cells and the optic nerve to the white, red and blue light stimuli.
The discovery of the melanopsin-containing retinal ganglion cells and their mediation of the PLR has allowed better understanding of the neural input to the PLR and the conditions of light stimulus affecting it. (Grozdanic, 2007)
The melanopsin-containing retinal ganglion cells represent a small subset (~1-3%) of the retinal ganglion cells.
They play a role in synchronization of the biological clock with the light-dark cycle, contribute to photic regulation of the hormone melatonin from the pineal gland. Recent information show that intrinsically photosensitive retinal ganglion cells (ipRGCs), using the photopigment melanopsin, respond directly to light to drive pupillary constriction. These cells project and innervate the superior colliculus and dorsal lateral geniculate nucleus, retinotopically organized nuclei mediating object localization and discrimination. Thus, ipRGCs can support spatial visual perception. (Ecker, 2012; Hattar, 2002; Kardon, 2009; Markwell, 2010)
The peak spectral sensitivity of the receptor is between 460 and 484 nm. (Grozdanic, 2007; Markwell, 2010)
Taking those information into consideration it is possible using PLR to differentiate diseases affecting the outer retina from those affecting the inner retina and optic nerve based on properties of the light stimuli, such as wavelength and intensity.(Grozdanic, 2007)

MATERIALS AND METHODS

The study was conducted on healthy patients, on patients with ophthalmological signs, and/or with neurological signs.
To rule out the possible presence of ocular and neurological disease, the healthy patients, with age between 3 months and 7 years, were carefully examined, using direct ophthalmoscopy, intraocular pressure measurement, and neurological exams.
The evaluation of CPR was performed using the IRIS-VET device (Biomed Vision Technologies). It tests the retina and optic nerve function based on the evaluation of spectral properties of the pupil light reflex and is

characterized by powerful light sources with very precise light intensity output (200 kcd/ m^2) and very precise wave lengths (ultra-bright red diode source – 630 nm; ultra-bright blue light diode source – 480 nm), which can be used to elicit different spectral components of the pupil light reflex (Figure 1).

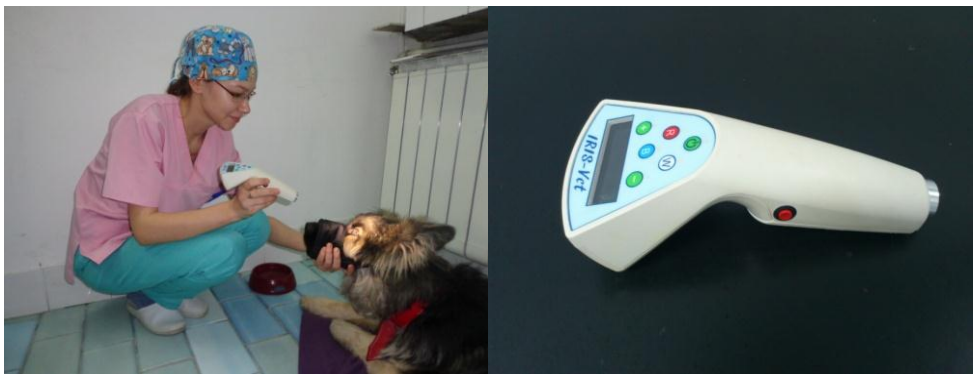

Figure 1. The IRIS VET device

The patients were examined in a dark room, after 15 seconds, in order to achieve the dark adaptation.
The right eye was illuminated using white light from a distance of 5 cm, for approximately 10 seconds and the pupillary changes were observed. If complete pupil constriction was achieved in less than 10 seconds, the light source was turned off. After 15 seconds, when dark adaptation is achieved again, the left eye was illuminated with white light from a distance of 5 cm and changes in the pupil diameter were observed.
After CPR to white light stimuli was assessed and after the dark adaptation was achieved, each eye was stimulated using red light stimuli(200 kcd/ m^2, 630 nm) and blue light stimuli (200 kcd/ m^2, 480 nm), following the above protocol.
The white light was used for the overall assessment of the eye, the red light provided information about the photoreceptors' (rods and cones) function, and the blue light assessed the ipRGCs and optic nerve function.
A normal CPR is represented by pupillary constriction to the light stimuli. Decreased or absent pupillary constriction when stimulated with red light revealed retinal damage. No response (mydriasis) to blue light was recorded when damage of the ipRGCs and optic nerve was present (Figure 2).

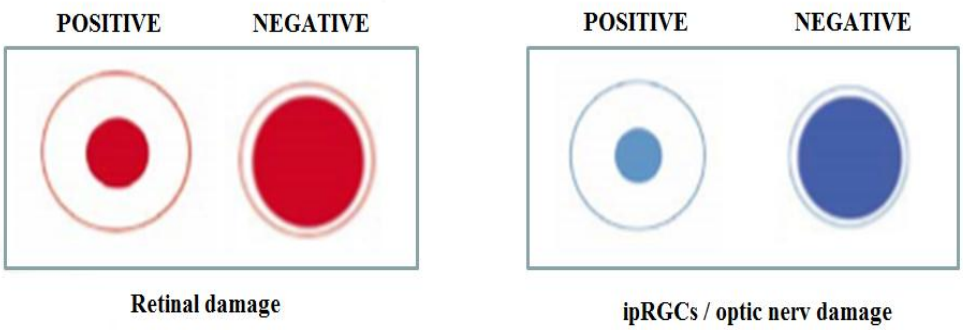

Figure 2.The CPR to red and blue light stimuli

After all pupillary responses were observed and recorded, we used the obtained results in combination with other parameters obtained during clinical examination and complementary tests to establish the final diagnosis.

Electroretinography was used to establish diagnoses of progressive retinal atrophy (PRA) in two dogs. The patients displayed a complete absence of retinal electrical activity during ERG recording.

Ultrasound confirmed the diagnosis of retinal detachment in all affected patients.

RMN was used to establish the diagnosis of hemorrhage located near the optic chiasm in one French Bulldog, age of 1 year, with no neurological or ocular signs, and with relatively normal CPR.

RESULTS AND DISCUSSIONS

All healthy patients presented miosis to all light stimuli.

The blind patients presented to the clinic with mydriasis of one or both eyes, anisocoria, absent pupillary light reflex, absent menace response, normal or increased intraocular pressure. The neurological signs of some of the patients included nystagmus, torticollis, circling, ataxia, proprioceptive deficits.

IRIS VET was used to differentiate between a neurological and an ophthalmological disorder in the first stage of the clinical examination and to guide the further diagnostic approach.

The fundus ophthalmoscopic examination revealed the diagnosis: retinal detachment. The pupil light reflex was usually incomplete, slow or sometimes absent (Figure 3).

Due to the photoreceptor (outer segments) damage, the photoreceptor-mediated pupil light response (red light) is usually absent, while melanopsin-mediated response (blue light) is present or decreased. Retinal detachment older than 1month sometimes resulted in the complete loss of the red response and incomplete blue response.

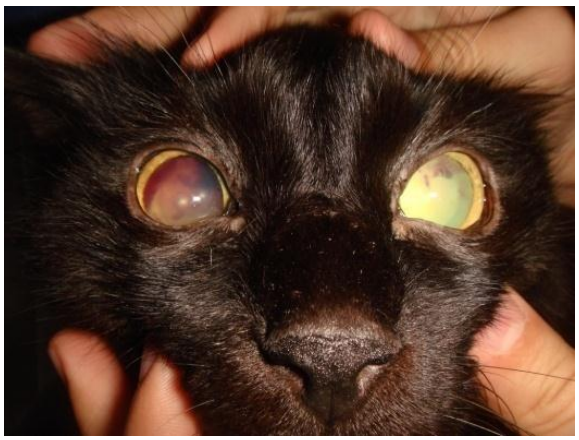

Figure 3. Retinal detachment in a cat – bilateral mydriasis

Progressive retinal atrophy is characterized by progressive loss of vision, hyperreflectivity of the tapetum lucidum (Figure 4). Stimulation of the pupil light reflex in PRA patients with red light does not provide any response (mydriasis). Stimulation with blue light provides immediate and complete miosis due to activation of melanopsin-containing retinal ganglion cells (ipRGCs).

PLR to blue light (melanopsin-mediated response) is usually normal in early retinal degeneration or decreased with pupillary escape in advanced retinal degeneration, which is suggestive of the inner retina structural and organizational remodeling and retinal ganglion cell degenerative changes. Electroretinography was used in 2 cases in order to confirm the diagnosis.

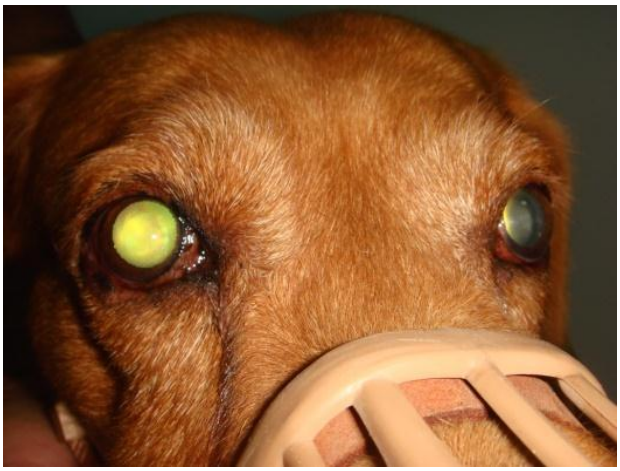

Figure 4. Progressive retinal atrophy in a dog (mydriasis, hyperreflectivity of the tapetum lucidum)

Glaucoma affects both the retina and the optic nerve. The patients had increased intraocular pressure (>20 mmHg), mydriasis and no PLR. In one case, the only clinical signs were increased intraocular pressure (27-30 mmHg) and mydriasis. Using CPR was crucial because it indicated an ophthalmological disease, specifically a retinal damage: the photoreceptor-mediated CPR (red light stimuli) was absent, while melanopsin-mediated CPR (blue light stimuli) was slightly decreased or with pupillary escape, indicating that it was not an inner retina or CNS disorder. An ultrasound was performed and the final diagnosis was retinal detachment with secondary glaucoma.

In secondary glaucoma, where the retinal and optic nerve function were damaged due to increased intraocular pressure and ischemia, CPR was absent (mydriasis).

Disorders manifested with blindness, anisocoria, mydriasis, blindness, normal or decreased CPR and without neurological signs were further investigated with RMN. The results confirmed neurological disorders such as: metastatic brain tumor, optic chiasm tumor or hemorrhages involving the optic chiasm (Figura 5).

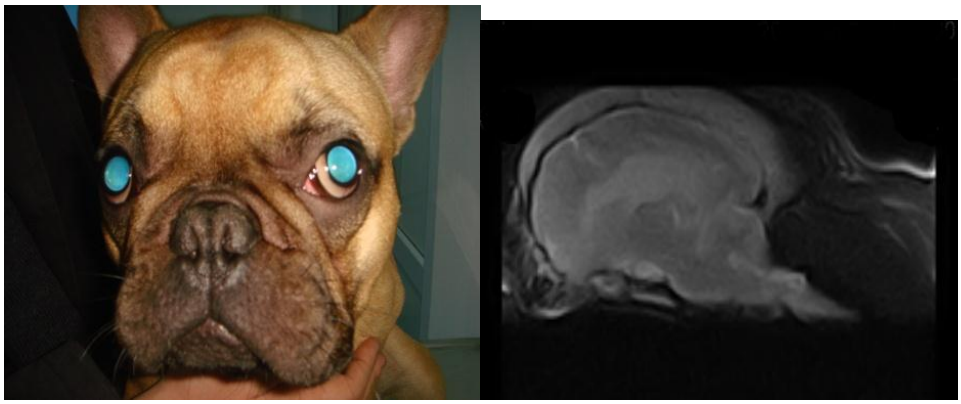

Figure 5. 1 year old dog with bilateral mydriasis, blindness, with no other neurological/ophthalmological signs. RMN: optic chiasm hemorrhages

CONCLUSIONS

Using light stimuli of different wavelength (red and blue) it is possible to easily differentiate between rod-cone mediated pupillary response (outer retina) and melanopsin-mediated pupillary response (inner retina).
CPR is a fast and easy way to separate the disorders affecting the outer retina from those affecting the inner retina. Decreased, incomplete or absent CPR to red light stimuli but normal CPR to blue light stimuli suggests a photoreceptors' damage, whereas an absent CPR to blue light stimuli suggests a melanopsin-containing retinal ganglion cells, or optic nerve damage.
The chromatic pupillary response (CPR) proved to be a "foothold" in the diagnosis of ophthalmological disorders (retinal detachment, progressive retinal atrophy) and neurological disorder (glaucoma, brain tumor, optic chiasm tumor) before complementary expensive tests were performed.

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PRELIMINARY DATA ON SEROLOGICAL SURVEY OF EXPOSURE TO ARTHROPOD-BORNE PATHOGENS IN STRAY DOGS FROM BUCHAREST, ROMANIA

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Abstract

Canine vector-borne diseases (CVBDs) represent an important group of illnesses affecting dogs around the world. In addition to their veterinary importance, some CVBD-causing pathogens are of major zoonotic concern, with dogs potentially serving as reservoirs and sentinels for human infections. The present study aimed at assessing the seroprevalence of some selected arthropod-borne pathogens (*Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi sensu lato*, and *Anaplasma phagocytophilum*) in stray dogs from Bucharest's areas, using point-of-care assays: SNAP® Heartworm test (n=16) and SNAP 4DX (n=75), IDEXX Laboratories, Westbrook, ME. The SNAP heartworm detects only *D. immitis* antigen, whereas the SNAP 4DX detects *D. immitis* antigen and antibodies against *E. canis*, *A. phagocytophylum*, and *B. burgdorferi*. All animals displayed no clinical signs at the physical examination, therefore they were assumed as clinical healthy. Overall, 30.77% (28/91) of the dogs were serologically-positive to one or more of the tested pathogens. The prevalence of positive test results was as follows: *D. immitis*, 18.68% (17/91), *E. canis*, 4.00% (3/75), *A. phagocytophilum*, 16.00% (12/75). Three dogs (4.00%) were co-exposed to *D. immitis* and *A. phagocytophilum* and one (1.33%) was co-exposed to *E. canis* and *A. phagocytophilum*. There was no evidence for *Borrellia* infection. This study provides on insight of exposure to certain pathogens infecting stray dogs in some areas of Bucharest, emphasizing high risks for vector-borne diseases.

Key words: *arthropods, stray dogs, pathogens, vector.*

INTRODUCTION

Dogs are competent reservoir hosts of several zoonotic agents and can serve as a readily available source of nutrition for many blood feeding arthropods. The explosion of the canine population, and their increasingly close relationship with humans in both urban and rural areas pose new concerns for human public health (Otranto et al., 2009a; Genchi et al., 2011a).
Canine vector-borne diseases (CVBDs) represent an important group of illnesses affecting dogs around the world. These diseases are caused by a

diverse range of pathogens, which are transmitted to dogs by different arthropod vectors, including ticks and insects (fleas, mosquitoes, phlebotomine sandflies) (Otranto et al., 2009b). In addition to their veterinary importance, some CVBD-causing pathogens are of major zoonotic concern, with dogs potentially serving as reservoirs and sentinels for human infections.
The growing medical interest in canine vector-borne diseases CVBDs) is directly related to both animal welfare and public health (Beugnet and Marié, 2009).
Stray dogs (free-roaming) are often present in urban areas representing an increasing public health concern (Slater et al., 2008). Despite of the great concern worldwide on vector-borne diseases generally (Knols and Takken, 2007), and on CVBDs particularly, little is known about the occurrence and prevalence of vector-borne pathogens in dogs in different areas of Romania. There is only one recent epidemiological study on the prevalence of vector-borne pathogens in dogs in Romania (Mircean et al., 2012), in which Bucharest' area was not included. Therefore, the present study aimed at assessing the seroprevalence of some selected arthropod-borne pathogens (*Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi sensu lato*, and *Anaplasma phagocytophilum*) in stray dogs from Bucharest's areas.

MATERIALS AND METHODS

We evaluated the prevalence of arthropod-borne pathogens in stray dogs from Bucharest's areas using point-of-care assays: SNAP® Heartworm test (n=16) and SNAP 4DX (n=75), IDEXX Laboratories, Westbrook, ME. The SNAP heartworm detects only *D. immitis* antigen, whereas the SNAP 4DX detects *D. immitis* antigen and antibodies against *E. canis*, *A. phagocytophylum*, and *B. burgdorferi*. Stray dogs (n=91), originated from two different areas of Bucharest (in southeastern Romania), which were subjected to the sterilization procedure, were included in the study. All animals displayed no clinical signs at the physical examination, therefore they were assumed as clinical healthy.

RESULTS AND DISCUSSIONS

The seroprevalence of infection or exposure and co-exposure to several arthropod-borne pathogens in stray dogs in Bucharest's area are displayed in Table 1. Overall, 30.77% (28/91) of the dogs were serologically-positive to one or more of the tested pathogens.

In decreasing order, the seropositivity was as follows: to *D. immitis*, 18.68% (17/91), *A. phagocytophilum*, 16.00% (12/75), *E. canis*, 4.00% (3/75). Three dogs (4.00%) were co-exposed to *D. immitis* and *A. phagocytophilum* and one (1.33%) was co-exposed to *E. canis* and *A. phagocytophilum*. There was no evidence for *Borrellia* infection in this study.
These findings strongly indicate that dogs from the studied area are potentially at risk of major canine vector-borne diseases some of them of zoonotic concern.

Table 1. **Seropositivity (number positive and percentage) of stray dogs from southeastern Romania to some selected arthropod-borne pathogens**

Location	Samples positive/samples tested (percentage)					
	***D.im.* (Ag)[a]**	***E.c.* (Ab)[b]**	***A.ph.* (Ab)[c]**	***B.b.* sl (Ab)[d]**	***D.im.* + *A.ph.***	***A.ph.* + *E.c.***
Area A	10/59	1/44	10/44	-	3	-
Area B	7/35	2/31	2/31	-	-	1
Total	**17/91** (18.68%)	**3/75** (4.00%)	**12/75** (16.00%)	-	**3/75** (4.00%)	**1/75** (1.33%)

[a]Antigen of *Dirofilaria immiitis*; [b]Antibody to *Ehrlichia canis*; [c]Antibody to *Anaplasma phagocytophilum*; [d]Antibody to *Borrelia burgdorferi* sensu lato.

In a similar study, Mircean et al. (2012) have been reported lower values of seroprevalence of *A. phagocytophilum* (5.5%), *D. immitis* (3.3%), and *E. canis* (2.1%). However, focal regions were found in the southeast of Romania for all these pathogens, with the highest prevalence, up to 31.00% for *D. immmitis*, 17.00% for *E. canis*, and 10.3% for *A, phagocytophilum*, respectively (Mircean et al., 2012).
These findings can be explained by the particular ecological conditions (climate, biotopes) associated with the distribution and abundance of vector competent arthropods, ticks (for *A. phagocytophilum, E. canis*) and mosquitoes (for *D. immitis*) in the studied areas (southeastern Romania, Bucharest's area included). Moreover, stray dogs are at high risk of acquiring vector-borne pathogens, mainly because they are often untreated against ectoparasites, thus, representing an easy feeding source for them. In addition, the general conditions of these animals (e.g., poor nutrition) may contribute to susceptibility to some VBDs. Likewise, when infected, stray dogs are often neither monitored nor treated against vector-borne pathogens (Otranto and Dantas-Torres, 2010).

A serological study of selected vector-borne diseases in shelter dogs in central Spain using also point-of-care assays reported similar data for *A. phagocytophilum* (19.0%), but lower for *E. canis* (5%) (Couto et al., 2010).
In Portugal, Cardoso et al. (2012) had reported high risks of healthy dogs, serological tested, for CVB-pathogens, like *D. immitis* (3.6%), *E. canis* (4.1%), *B. burgdorferi* (0.2%), *Anaplasma* spp. (4.5%),
Similarly, in a study in Germany, 41.9% (26/62) of healthy dogs were found to be seropositive for *A, phagocytophilum* (Jensen et al., 2007).
The prevalence of *E. canis* infection in dogs in Italy, estimated by serological surveys varied from 14.9% in southern Italy (Otranto et al., 2008) to 46.7% in Sardinia (Cocco et al., 2003), emphasizing some varieties among foci according to local factors (e.g., vector population density and activity patterns).
Prevalence rates/ranges (%) reported for *D. immitis* in some European countries, were very different, like: from 0.6 to 80% in Italy, 0.6 to 6.8% in France, 1.6% in Switzerland, 6.2% in Serbia, from 10 to 34% in Greece (as reviewed by Traversa et al., 2010). Moreover, canine dirofilariosis by *D. repens* has been considered for a long time to be mainly diffused in southern regions of Italy, while *D. immitis* is considered endemic in northern regions with prevalence rates ranging from 22 to 80% in dogs untreated with prophylactic drugs (Rossi et al., 1996; Genchi et al., 2001; Genchi et al, 2011b).
Although positive serological results may suggest prior exposure and not necessarily disease, they can alert veterinarians to take into consideration further clinical and diagnostic evaluation of individual dogs (Carrade et al., 2011). Many dogs infected with vector-borne agents remain asymptomatic for months or even years, but diagnosis of subclinical infection is important (Ionita et al., 2012), as those animals might still serve as reservoirs of pathogens to other hosts including humans. Therefore, especially in areas of endemicity, an annual serological screening would be recommended to promote early detection and treatment (Otranto et al., 2009).
Travelling of dogs from arthropod-borne diseases endemic areas into non endemic areas and *vice versa* poses a risk for the introduction and dissemination of exotic pathogens if competent vectors are present (Otranto and Dantas-Torres, 2010).
In Germany, some of CVB-pathogens, like *Babesia* spp., *Leishmania* spp., *D. immitis* or *E. canis* have repeatedly been recorded in travelling and imported dogs (including from Romania) (Hamel et al., 2012).
The introduction of non-endemic pathogens, and sometimes their vectors, by dogs is documented also in Austrian dogs (Leschnik et al., 2008).

The above phenomenon highlights the importance of establishing effective surveillance systems to avoid the importation of infected animals into and from different regions. A future risk may arise from an increasing number of imported dogs, carrying vectors that may be host to various pathogens, to areas still free of those pathogens. A further problem is the probability, that these vectors may become native when climate conditions are going to be favorable to them (Daugschies, 2001; Deplazes et al., 2006).

CONCLUSIONS

This study provides on insight of exposure to certain pathogens infecting stray dogs in some areas of Bucharest (southeastern Romania), emphasizing high risks for vector-borne diseases, some of them of zoonotic concern. Therefore, the findings are expected to serve as a reference for future investigations and control actions in order to protect dogs and limit the risk of transmission of vector-borne agents to humans.

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CLINICAL, ULTRASOUND AND LABORATORY CHANGES IN CUSHING SYNDROME IN DOGS

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Abstract

Dogs` adrenal pathology is dominated by Cushing syndrome, mostly by iatrogenic origin or neoplasic glandular lesions.
Clinical evaluation were performed following the classic screening protocol and additional hematological and biochemical investigations (liver/kidneys function), hormonal determinations (basal cortisol or after stimulation tests, i.e. suppression) and ultrasound exams. In our study were included 18 dogs with Cushing syndrome (hyperadrenocorticism). From clinical point of view the main registered clinical signs were bulimia, polyuria-polydipsia syndrome, abdominal ptosis, hepatomegaly, calcinosis and/or cutaneous hyper pigmentation, bilateral symmetrical alopecia). The biochemical blood profile registered changes (increased ALT and AST activity, hyperlipidemia, decreased serum urea levels, hyperglycemia) and urinary (diluted urine, proteinuria).
Ultrasound reveals in case of affected adrenal glands appears as distinct structures, flattened shape, appearance lobe, located cranio-medial kidney, caudal of the mesenteric and celiac artery and cranial of the renal artery and the right (lower) prior to renal vein and cranial right kidney. According to their topography, size and structure the ultrasound changes were very useful for the diagnosis of the diseases related to adrenomegaly and changing their echogenicity and echostructure.

Key words: *adrenal glands, Cushing syndrome, dogs*.

INTRODUCTION

The adrenal pathology in dog's pathology is mainly dominated by Cushing syndrome, in most cases by iatrogenic origin or tumoral glandular changes (Syme et al., 2001).
In dogs, Cushing syndrome with pituitary origin (center) is the most common cause of spontaneous Cushing, representing over 80% of cases (Feldman, 2005; Galac, 2010).
Cushing syndrome encompasses a variety of clinical and biochemical abnormalities resulting from chronic exposure to high concentrations of glucocorticoids, in addition with many clinical and parenchymatous functional changes.

In such cases if clinical changes are obvious, next step is the adrenal ultrasound evaluation, using higher transducers and functionally testes by dosing cortisol, basal, or using specific tests (ACTH stimulation/suppression with dexamethasone).
As in human medicine, canine hyperadrenocorticism (Cushing syndrome) has various pathophysiological origins but all share one common denominator, the chronic excess cortisol systemically.

MATERIALS AND METHODS

In our study were included 18 dogs with Cushing syndrome (hyperadrenocorticism), initially suspect by the specific clinical and paraclinical changes.
Clinical evaluations were performed following the classic screening protocol and additional hematological and biochemical investigations (liver/kidneys function), hormonal determinations (basal cortisol or after stimulation tests, i.e. suppression) and ultrasound exams (Witt and Neiger, 2004).
In Cushing's syndrome (hyperadrenocorticism), adrenal impairment is accompanied by morphological and eco-structural alterations, appreciable ultrasound method that ranks priority in terms of relevance and specificity (Hoerauf and Reusch, 1999; Wood at al., 2007; Codreanu et al., 2009). Adrenals ultrasound was performed using high frequency transducers (8 -18 MHz).

RESULTS AND DISCUSSIONS

From clinical point of view the main registered clinical signs were bulimia, polyuria-polydipsia syndrome, abdominal ptosis, hepatomegaly, calcinosis and/or cutaneous hyperpigmentation, bilateral symmetrical alopecia), results presented in Table 1.
The biochemical blood profile registered changes (increased ALT and AST activity, hyperlipidemia, decreased serum urea levels, hyperglycemia) in 68.75% and urinary (diluted urine, proteinuria) in 81.25%.
Was dosed the basal cortisol and then were given 2.2 IU / kg Cortrosyn, after which was dosed the cortisol, after administration (Gould et al., 2001; Bosje et al., 2002). Following dosing basal cortisol and cortisol after ACTH stimulation was possible to confirm Cushing (Cushing's syndrome), results are shown in Chart 1.

Table 1. Pooled data for establishing the diagnosis of Cushing syndrome

CLINICAL CHANGES	No. and % of patients		NOTES ON PHYSICAL EXAMINATION	No. and % of patients	
	No.	*%*		*No.*	*%*
Polyuria/ Polydipsia	18	100	Alopecia	10	55,5
Polyphagia	9	50	Dermatosis	4	22,2
Respiratory changes/dyspneea	3	16,6	Comedones	3	16,6
Abdominal ptosis	12	66,6	Cutaneous calcification	5	27,7
Dorsal bilateral alopecia	14	77,7	Decreased muscular tonus	10	55,5
Weakness	9	50	Hepatomegaly	7	38,8
Lethargy	10	55,5	Increased limphonodes	3	16,6
PARACLINICAL MAIN CHANGES					
Biochemical altered parameters : Alkaline Phosphatase: 284-584 U/l ALT: 66-134 U/l AST: 64-91 U/l Cholesterol: 744-980 mg/dl					
URINE EXAMINATION					
Specific gravity in all tested samples < 1,013					
ACTH STIMULATION TEST					
Excessive cortisol response in tested dogs after ACTH administration > 24,9 □g/dl (24,9-33,6 □g/dl)					

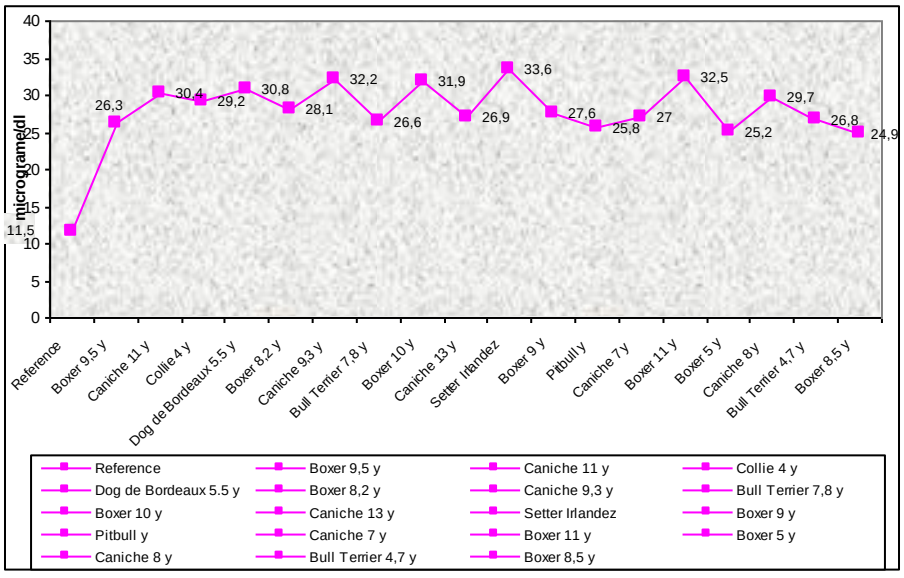
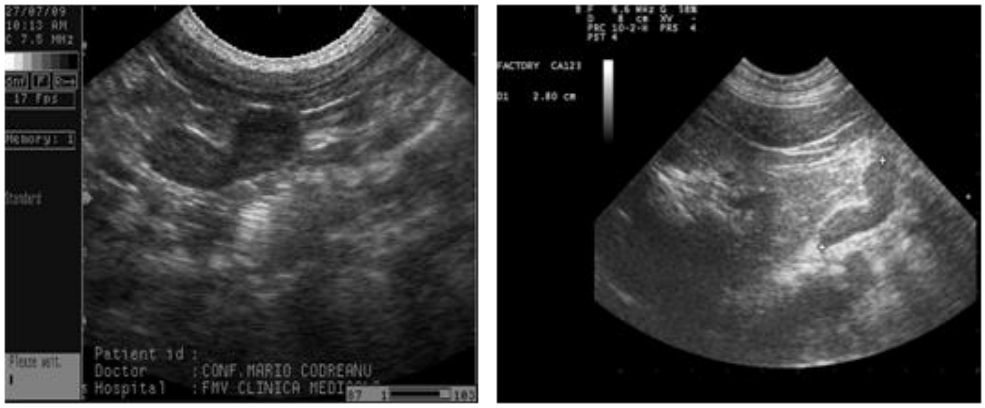
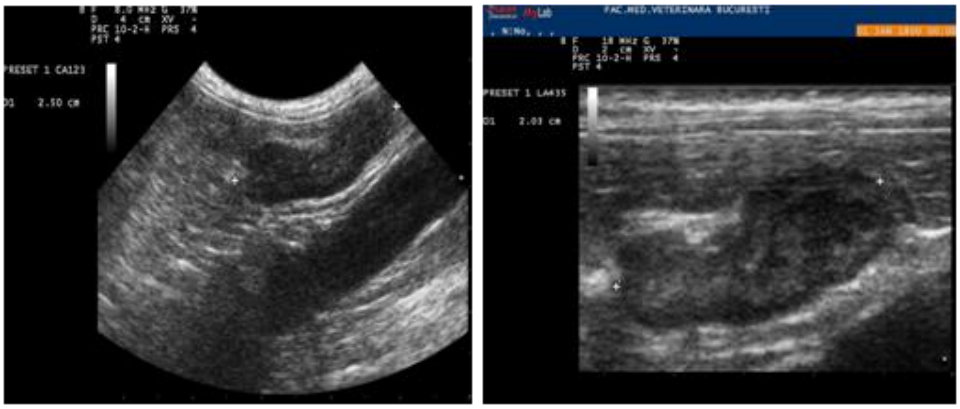


Chart 1. Results of cortisol level after stimulation (ACTH), which confirm the diagnosis of hyperadrenocorticism in selected dogs

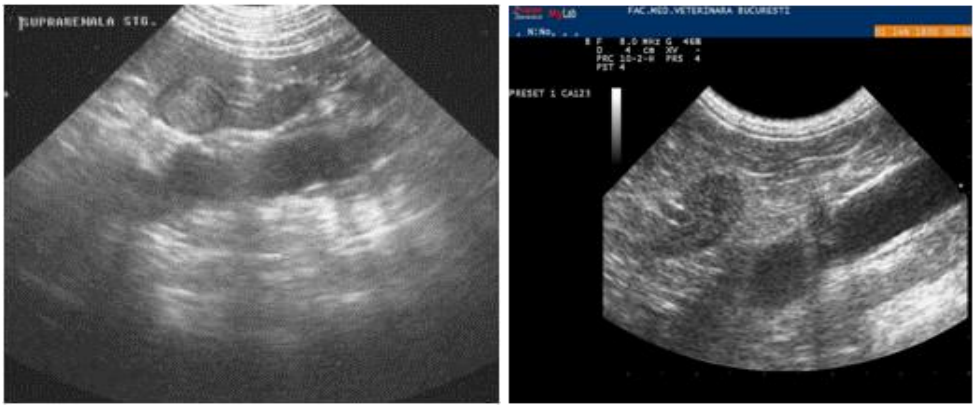
Ultrasound reveals in case of affected adrenal glands appears as distinct structures, flattened shape, appearance lobe, located cranio-medial kidney, caudal of the mesenteric and celiac artery and cranial of the renal artery and the right (lower) prior to renal vein and cranial right kidney (Figures 1-6).
In Cushing syndrome the most important ultrasound changes were represented by diffuse bilateral in 68.75% (Figures 1-4), unilateral (18.75%) adrenomegaly and local ultrasonographic changes of irregular shape, different echostructure and echogenicity in 12.5%, of nodular aspect (Figures 5-6).



Figures 1 - 2. Diffuse adrenomegaly - normal echostructure



Figures 3 - 4. Localised adrenomegaly-cortical-medular normal ratio



Figures 5 - 6. Nodular changes of different echostructure and echogenicity

CONCLUSIONS

In establishing and confirming the diagnosis of hyperadrenocorticism in dogs from our investigation and for recommending fair and effective therapeutic measures, we have performed very cautious anamnesis corroborating data with results of clinical examination, additional laboratory investigations, obtaining thus an insight into the context which seeks, accurate differentiation of this syndrome of different pathological processes similar events.

Increased levels of basal cortisol and the cortisol excessive response (statistically significant) after administration of ACTH in all dogs of group (from 24.9 to 33.6 μg / dl), confirm the diagnosis of hyperadrenocorticism.

According to their topography, size and structure the ultrasound changes were very useful for the diagnosis of the diseases related to adrenomegaly and changing their echogenicity and echostructure.

When can be visualized both adrenal glands, and their size is relative similar, the most probably can be the expression of the hyperadrenocorticism, and when their size, echostructure and echogenicity is very different, the diagnosis with a high degree of accuracy.- is adrenal tumor.

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REVIEW PAPER
ISSUES IN FISH CORTISOL MEASUREMENT

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Abstract

This paper represents a review of the stress response in fish with emphasis on cortisol, different methods of sampling, advantages and disadvantages. Cortisol is one of the most common measured stress indicator hormone in fish. The reasons why this hormone is often addressed by researchers are: Cortisol can be rapidly and accurately measured using ELISA (enzyme-linked immunosorbent assay) and RIA (radioimmunoassay), the samples can be obtained through a wide variety of harvesting procedures that minimizes stress response in fish (including anesthesia), the plasma cortisol level has a tendency to rise when the fish is exposed to different stressors.

Key words: *cortisol, fish, stress response*

INTRODUCTION

The stress response in fish

According to Branson E. J. (2008), in comparison with the stress research in domestic animals, the study of stress in fish has a relatively short history starting with the late 1960s and early 1970s.

Primary response: The hypothalamo-pituitary-interrenal axis

Neil Martin Ruane (2002) mentions in his thesis that fish response to stress is controlled by the hypothalamo-pituitary-interrenal axis (HPI). He also relates that after the stressor is detected, sensory neurons in the brain are activated and direct the information to the hypothalamus. According to van Enckevort et al. (2000) and van den Burg (2002) the main factors that trigger the release of the pituitary peptides are the corticotropin releasing hormone (CRH) and the thyrotropin releasing hormone (TRH), freed by the hypothalamus. The adrenocorticotropic hormone (ACTH), the α-melanocyte-stimulating hormone (α-MSH) and the endorphin are the main peptide hormones produced by the pituitary gland. Although the role of the ACTH hormone to stimulate the production and release of cortisol from the interrenal cells (24) is well known, the function of the α-MSH hormone and endorphin is still subject to discussion (3; 4; 28). The precursor of cortisol is represented by cholesterol. This sterol is transformed to pregnolone by the

action of the enzyme P450 (18). Afterwards pregnolone is further converted into 11-deoxycortisol by the action of steroidogenig enzymes and finally converted to cortisol by 11b-hydroxylase (18, 10)

The interrenal gland:

According to Ruane N.M. (2002), the teleost kidney is composed of a head and body kidney. The head kidney is represented by a wide haematopoietic, endocrine and lymphatic tissue, while the body kidney is composed of nephrons and interstitial lymphoid tissue (27). Milano et al. (1997) describes the anatomy of the head kidney as consisting of the interrenal gland (the correspondent of the andrenal cortex in mammals) and the chromaffin cells (adrenal medulla); these structures are presented as surrounding the postcardinal vein and it's collaterals. According to Wendelaar Bonga (1997), the main steroid hormone synthesized by the interrenal cells is represented by cortisol, whereas the cromaffin cells secrete catecholamines. The interrenal gland is represented in *Cyprinus carpio* by clearly defined glandular masses, sorrounding the postcardinal vein with branches that infiltrate the head kidney (24). Imagawa et al. (1995) describes the chromaffin cells as being located singly or in clusters in the postcardinal vein walls and delimited by the interrenal cells. The regulation of the interrenal and chromaffin cells by the endocrine system through the circulatory system, is enhanced by their location near the postcardinal vein (24).

Shortly, during the activation of the HPI axis, the corticotropin releasing factor (CRF) induces the pituitary corticotropes to secrete ACTH (2). Some autors (1, 14) suggest that a specific binding protein for CRF (CRF – BP), as well produced in the POA, may have a role in the CRF – mediated ACTH regulation during stress response. Bernier et al. (2009) report that blood-born ACTH in his turn stimulates synthesis and secretion of cortisol into the circulation.

The secondary response:

According to Ruane N. M. (2002), the secondary stress response appears consecutively to the neuroendocrin changes that follow the primary stress response; thus metabolism and immune capacity are being influenced. The same autor states that in order for an animal to respond to a stressor, a supplementary metabolic cost is required, the organism needing to dispatch energy substrates such as glucose and free fatty acids; the increase in plasma glucose concentration is due to elevated glycogenolysis (brakedown of liver glycogen) and gluconeogenesis (production of glucose), whereas

lipolysis (lipid breakdown) leads to the formation of free fatty acids from triglycerides in fat stores.

The tertiary response:

The tertiary response is represented by the adverse consequences of exposure to stressors (5). Branson E. J. (2008) states that the indicators of a tertiary stress response can be: reduction or even ceasing of growth, low body condition score, increased incidence of infection (bacterial, viral, fungal, parasitic), low reproductive status.

Cortisol measurement: in blood, bile, whole body homogenates or water.

Cortisol in blood

Edward J. Branson (2008) states that from a research point of view, the blood contains the most relevant concentration of cortisol. Also he recommends sampling the blood with a hypodermic needle and syringe from a lightly sedated fish. The same author mentions that the blood samples can be obtained by heart puncture, from the caudal vein or artery or from the cuvierian ducts (posterior cardinal veins). The problem of this approach, from the author's point of view, is that the time elapsed between the initial disturbance (catching the fish in nets) and blood harvesting should be less than five minutes. If this is not possible, the initial disturbance associated with the sampling maneuver will generate a stress response or possibly increase an already existing response (21). Authors like Marcel Martínez – Porchas et al. (2009) state the following plamatic cortisol values from the literature (table 1):

Table 1. Plasma cortisol values in carp and trout, before and after different stressors (18).

Species	Stressor	Cortisol nmoli/l		Exposure	References
		Prestress	Poststress		
Rainbow trout *Oncorhynchus mykiss*	Chemical exposure	49	110	Chronic	Benguira et al. 2002
Rainbow trout (diploid) *Oncorhynchus mykiss*	Handling and confinement	77	698	Acute	Benfey & Biron 2000
Rainbow trout male *Oncorhynchus mykiss*	Trapping	16	380	Acute	Clements et al 2002
Common carp *Cyprinus carpio*	Density	19	206	Acute	Ruane et al 2002

An other study by Mackenzie Macintyre C. (2008) states the following results:

Materials and methods: the author describes that samples were harvested from 3699 trouts removed from the pool by netting, immediately transfered into water with anesthetico (2-phenoxy ethanol, Sigma, Dorset, UK) and stunned. Immediately following death, blood samples were harvested using syringes and a heparinised 23 gauge hypodermic needle from the caudal vena cava. The samples were stored on crushed ice. The plasma cortisol values were determined using the radioimmunoassay method as described by Ellis et al. (2004), adapted by North et al. (2006a). Concentrations were reported in ng/ml.

Results: the author reports the following results: average concentration ± standard deviation of 8.29 ± 13.36 ng/ml, with differences between system type. The highest cortisol concentrations (15.4 ± 19.62 ng/ml) were reported in fish provided from cage systems in comparison with raceways where the concentration was 5.89 ± 7.81 ng/ml. For fish from ponds and tanks, the mean ± standard deviation for cortisol concentrations were 7.51 ±12.34 and 8.25 ±14.31 ng/ml.

Cortisol in the bile:

Branson E.J. (2008) states that cortisol is inactivated and cleared from the body through hepatic biotransformation processes. A study by Pottinger et al. (1992) on rainbow trout, *Oncorhynchus mykiss*, showed that the levels of cortisol metabolites and their conjugates in the bile are significanlty higher in fish exposed to a chronic stressor. Thus it is concluded by the author that the analysis of biliary steroid content may provide a useful tool for

identifying stressed fish under conditions where an supplemental sampling stress is inevitable.

Matherials and methods: the accumulation of corticosteroids and their metabolits in the bile was measured using radioimmunoassay (RIA) and gas cromatography – mass spectrometry (GC-MS). One of the experiments was represented by: „The effect of acute and chronic stress on plasma cortisol levels, respectively free and conjugated biliary steroid levels in rainbow trout *Oncorhynchus mykiss*". The author describes that the fish were held in 1500 L capacity circular outdoor tanks with a constant intake of lake water, (30 l/min, with water temperature of 6.5 ^{o}C). The fish received daily food except during the experiment. Three groups of 10 fish were netted from their holding tank and sampled after being subjected to 24 h of confinement (first group – chronic stress), 1 h of confinement (second group – acute stress) and immediately in the third group (control). The blood samples were harvested from the caudal region using a heparinized syringe and the bile samples from the gall bladder with a syringe and a needle. The following were determined: cortisol levels, biliary free and total steroid levels

Results: the unstressed fish had a low plasma cortisol concentration 1.8 ± 0.3 ng/ml, in comparison with the 1 h acutely stressed fish **(**83.1 ± 17.0 ng/ml), and the 24 h chronically stressed fish (129.1 ± 13.5 ng/ml).The acutely stressed fish had the following unconjugated steroid levels in the bile: 53.3 ±20.7 ng/ml not significantly higher in comparison with the control fish (51.5 ± 18.5 ng/ml) but much smaller than the chronically stressed fish (169.1 ± 24.4 ng/ml). In the same manner, total (free + conjugated) steroid levels in acutely stressed fish had no major differences

when compared to control fish (4.6 ± 1.2 µg/ml in comparison with 3.4 ± 1.0 µg/ml) but were significantly higher in the chronically stressed fish (13.4 ± 1.0 µg/ml).

Cortisol in whole body homogenates:
When the fish is to small to obtain a sufficient blood sample, cortisol can be measured in whole body homogenates. It is necessary to humanely kill the fish before homogenising it in a special apparatus (9). In his article, Pottinger et al. (2002) shows that this method is usefull in monitoring stress response in small fish:
Materials and methods:Pottinger et al. (2002) used in his study three-spined stickleback that were maintained 4 months until the start of the experiment in outdoor 1000 l flow-through tanks supplied with lake water (10 l/min and ambient temperature (4-17 ^{o}C – seasonal range) and light period. Commercial trout fry feed was given to the fish. Two of the experiments conducted were:

Experiment 1: "Effects of an acute stressor"
At time 0, eight fish were taken from an outdoor holding tank (controls). The author relates that, immediately after this, 160 fish sampled from a second holding tank were transferred to eight 2.0 l aerated beakers filled with 500 ml of lake water (20 fish per beaker) and at 0.5, 1, 1.5, 2, 3, 4, 6 and 24 h removed one fish from each beaker (sample size of eight at each time point representing the stressed group). Also at 1, 2, 4, 6 and 24 h after the first sample, groups of eight fish were sampled from the holding tank implying minimum stress (control group).

Experiment 2: "Effects of a chronic stressor with food withdrawal"
At time 0, eight fish were taken from an outdoor holding tank (controls). The author relates that, immediately after this, 105 fish sampled from a second holding tank were transferred to seven aerated beakers filled with 1500 ml of lake water (15 fish per beaker). After 1, 2, 4, 6, 8 and 10 days from transfer, eight fish were sampled from a single baker (sample size of eight at each time point representing the stressed group). Also after 4, 6, 8 and 10 days from the first sample, groups of eight fish were sampled from the holding tank, implying minimum stress (control group). In both experiments the fish were humanely killed and stored at -70 ^{o}C until

required. The immunoreactivity of cortisol was conducted in ethyl acetate extracts of whole body homogenates by radioimmunoassay (23).

RESULTS

The control group had the mean whole body levels of immunoreactive corticosteroids in the interval 2-8 ng/g. The acutely stressed fish had a high level o corticosteroids in the first 30 minutes of stress (reaching 35-40 ng/g within 1 h) and remaining significantly elevated in comparison with the control fish, during the entire acute trial (24 h). The mean corticosteroid value of the chronically stressed fish was similar to that of the acutely stressed fish (35 ng/g in the first 24h, respectively 50 ng/g within 4 days). Values decreased later in the study but remained significantly elevated in comparison with the control fish. Food restriction also increased the corticosteroid whole body levels (9.9, 14.1 ng/g in the fasted fish in comparison with 5.5, 8.1 ng/g in the fed fish).

Cortisol in water:

Branson E.J. states that a major part of the circulating cortisol is removed by bioconversion and excretion through the bile. Also according to other authors (30, 13), a major amount of free steroids are passively eliminated from the blood as it passes through the gills. Although this method can reveal important information about the endocrine status of the fish population, in a non-invasive manner (26, 12, 16), there are two major disadvantages of this method (9): first, an enclosed volume of water is required with known inputs and outputs, and second, individual variation is lost.

Ruane N. M. si Komen H. (2003) measured the cortisol in water in order to measure the stress in common carp (*Cyprinus carpio*), caused by increased loading density.

Materials and methods: the authors describe that on day 0 of the experiment fish were organised into two density groups composed of four low-density tanks with 25 fish and three high-density (HD) tanks with 100 fish. Both blood and water samples were harvested from the LD and HD tanks on days 1, 3, 8, 14 and 28. Plasma cortisol concentrations were measured by radioimmunoassay (RIA) and cortisol in the water was measured as described by Scott & Sorensen (1994) for free steroids and according to the instructions of the cartridge manufacturers for glucocorticoid measurements (31)

Results: in time, the plasma cortisol concentration was significantly higher in all the groups in comparison with the first day. The highest value of the plasmatic cortisol due to the increased loading was recorded in day 3. In the HD groups cortisol concentrations were significantly elevated during the experiment, excepting the 14th day. In comparison with the cortisol concentrations in day 1, the LD group had a higher cortisol value in day 8 and the HD group in day 8 and 28.

CONCLUSIONS

Cortisol appears to be an accurate and accessible stress witness in fish. A major advantage is that it can be determined through a wide variety of methods. Thus, it is concluded that cortisol corroborated with other fish health parameters can be of a real useful tool in evaluating the welfare status of fish in different aquaculture systems.

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A CASE OF A 2 YEAR ADOPTED DSH CAT WITH ACUTE ABDOMINAL TRAUMA, RICKETS SKELETAL ABNORMALITIES AND UNILATERAL RENAL HYPOPLASIA

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INTRODUCTION

This is the case of a 2 year old DSH presented on 25.09.2012 with acute abdominal trauma after a road accident. During investigations, abdominal ultrasound scan, whole body plane and contrast radiography, abdominal exploratory surgery, one can find different, unrelated pathology and stunning abnormalities which remained undetected and with no clinical presentation until now. All investigations follow a step-by-step protocol in order to further discover and assess hidden pathology. This case stands for a better medical care for our pets and for the gross pathology that one can find in stray animals, which is the "tip of the iceberg" pathology that these animals can harbor and still survive, as a wonder o life itself.
Keywords: abdominal trauma, renal hypoplasia, rickets
This is the case of a 2 year old DSH presented on 25.09.2012 with acute abdominal trauma after a road accident. The cat is in post-traumatic shock and after shock therapy is submitted for further investigations. From the owner questioning one can find out that the cat was castrated a year ago. There is no ascendency information as the cat was adopted from the street while a kitten.
The cat is in a poor shape, confused, in pain and the owner said that she saw the cat urinating blood-like color. Supportive care and pain management protocols are put in place and the cat is registered as in-patient(4). The cat is then prepared for abdominal ultrasound scan. After the ultrasound scan next step is the plane and contrast radiography. For confirmation of the preliminary findings an exploratory abdominal surgery is prepared.

MATERIALS AND METHODS

The case is admitted in Salvavet-Ilioara Animal Hospital. For the abdominal scan is used a Esaote Ixos Vet Doppler scanner with micro-convex and

linear, 3 frequencies probes. The frequency of choice for abdominal scan was 10 mH for the micro-convex probe. For x-rays I used a mobile unit Philips Practix 33 Plus. Exposing protocol was 48 mV/10 mAs. The contrast is Iopamiro solution used 0,5 ml/kg and it was injected IV(2, 5). The cat has 3 kgBW. During investigations, abdominal ultrasound scan, whole body plane and contrast radiography(8), abdominal exploratory surgery, one can find different, unrelated pathology and stunning abnormalities which remained undetected and with no clinical presentation until now. All investigations follow a step-by-step protocol in order to further discover and assess hidden pathology. There is described a parallel of the ultrasound, plane/contrast radiology and the macroscopic aspect of the renal abnormalities(7).

RESULTS

First abdominal scan reveals the reason for urinary blood-like color. One can detect a regular shaped urinary bladder that is filled with liquid and other floating structures which are considered to be blood clots. There is no integrity loss hint. Furthermore there is no free liquid inside the abdominal cavity or around the urinary bladder, nor into Douglas space. The left kidney becomes visible and is considered to be enlarged, with the longitudinal axis of 4 cm (fig.1) but with normal architecture.

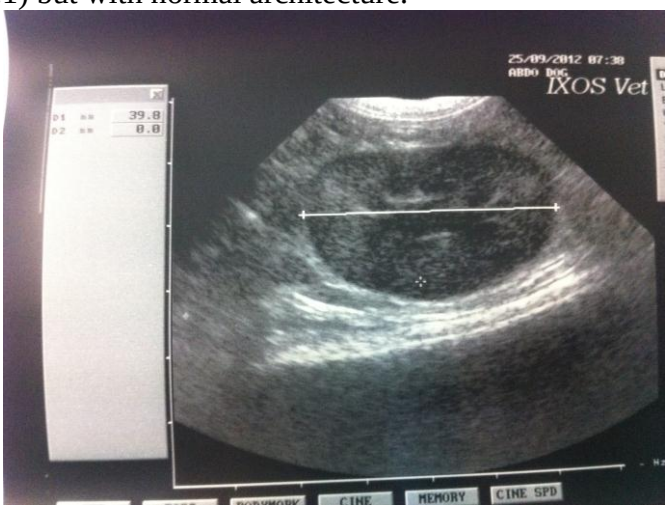

Fig.1 Left kidney of the traumatic cat. Long ax of 4 cm with normal architecture.

There are no other findings worth mentioning until right kidney is reached for examination. It appears to be somehow hard to find. Right kidney has no visible normal architecture and is very small, 2,4 cm/1,2 cm (fig.2.).

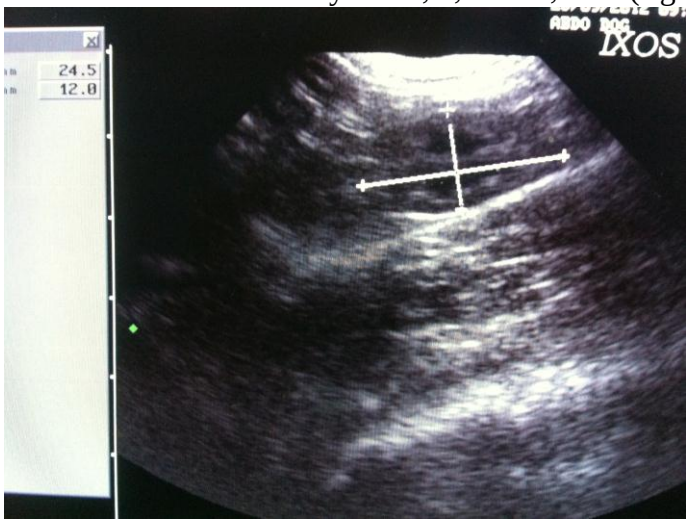

Fig.2 Right kidney of the traumatic cat. No visible renal architecture, 2,4 cm/1,2 cm.

After these findings the cat is submitted for further examinations for two reasons. First of two is to eliminate/confirm any large bone/vertebral injuries and thoracic trauma. Second reason is to confirm renal abnormalities. Two x-rays were taken, one VD and one in lateral recumbence. First lateral x-ray reveals a visible thoracic vertebrae abnormalities-lordosis (fig.3.)(1,6). On the VD aspect one can additionally find scoliosis. In contrast there is no visible thoracic trauma. The abdominal view (fig.4.) reveals also some new findings. Caudal to the left kidney there is an area overlooked at the abdominal scan which appears to be a large hematoma. Left kidney is easily found but the right one is very difficult to detect. Even after intravenous pielography one can struggle to find the right kidney. This kidney is considered to be a hypoplasic one. We can not know if this finding is doubled by genital abnormalities as by the time the cat is presented it was already castrated with no information in regards to any pathology findings.

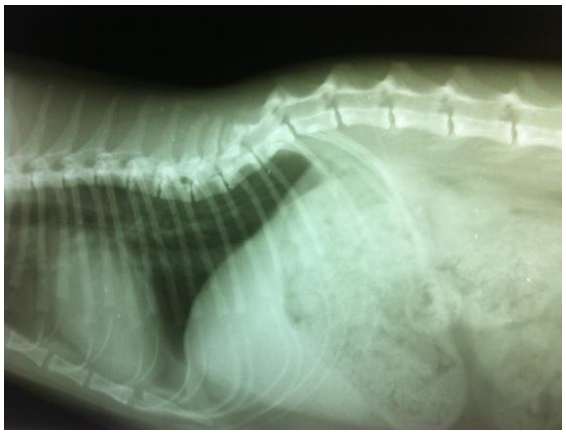

Fig.3 Lateral aspect plane x-ray of the cat with thoracic post-rickets skeletal abnormalities-lordosis.

After the patient is stabilized an exploratory abdominal surgery is put n place. One can easily no confirm all previous findings: urinary bladder injury, large hematoma caudal to the left kidney, enlarged left kidney and very small, hard to find right kidney (fig. 5/6.).

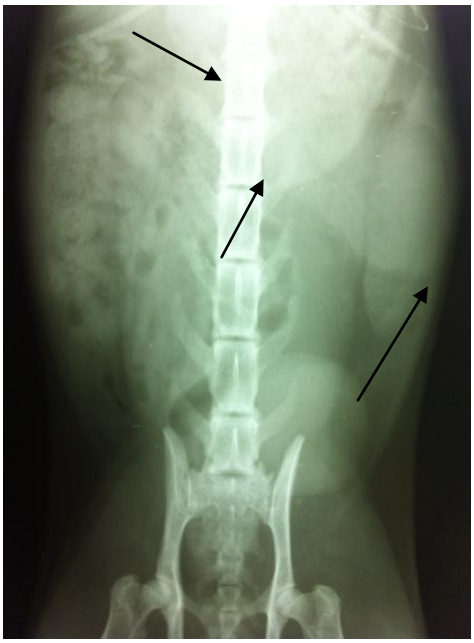

Fig.4 VD aspect, contrast x-ray. On the left, the two arrows point out the right hypoplasic kidney. On the right arrow point the left enlarged kidney with large caudal extracapsular hematoma. Urinary bladder filled with contrast.

In this case presentation, one will surprisingly find abdominal hemorrhage and hematoma, urinary bladder injury, skeletal abnormalities after rickets, unilateral renal hypoplasia. This is a surprise case and reveals the importance of emergency imaging scan means but at the same time it also reveals shocking discoveries about developmental abnormalities that can remain undetected even after usual abdominal procedures and regular clinical examinations.

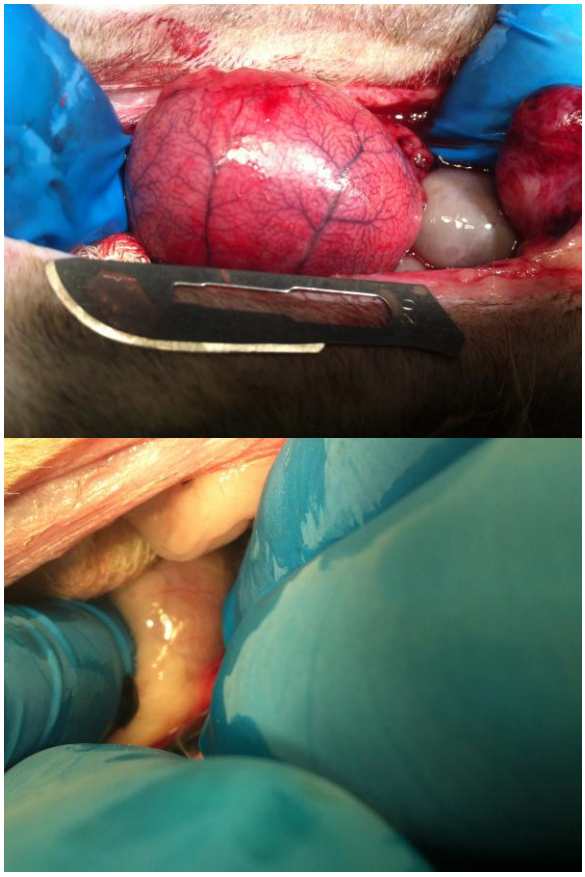

Fig.5 Left kidney after exploratory abdominal surgery. Fig.6 Right hypoplastic kidney

CONCLUSION

Step-by-step and complete abdominal scan and whole body radiography can reveal not only physical injuries in the post-traumatic emergency patient but also unrelated and previously undetected pathology.
Considering past discoveries I can suggest that renal hypoplasia is one condition that is not uncommon in cats.
All the imaging means produce a clear diagnostic confirmed by exploratory surgery.
It is worth mentioning that it is preferable to use rapid/non-invasive techniques as the ultrasound scan for emergency purposes and leave all other for further examinations after patient stabilization.
Exploratory surgery is the one procedure that produces eye-shocking results but is the most invasive procedure of all.
This case stands for a better medical care for our pets and for the gross pathology that one can find in stray animals, which is the "tip of the iceberg" pathology that these animals can harbor and still survive, as a wonder o life itself.

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STUDY CONCERNING THE URINARY TRACT DISEASES IN CATS IN THE DÂMBOVIŢA COUNTY

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Abstract

The results of the examination of a number of 198 of cats with urinary tract diseases in the Agervet-Târgovişte clinic during 2012 are presented. The sick animals were both mixed breeds (69.19%) as well as pure breeds (30.81%), aged between 1 and 15 years (with an average of 7.53 years). The urinary tract diseases had an acute evolution in 62 cats (35.96%), and in 136 cats (64.04%) they had a chronic evolution. Cystitis (58.08%), urolithiasis (56.06%), nephritis (30.80%), pyelonephritis (11.62%), renal cysts (1.5%) were diagnosed. The clinical manifestations were represented by: dysuria (63.13%), periuria (51,52%), pollakiuria (49.49%), stranguria (43.94%), hematuria (39.90%), anorexia (32.83%), vocal signs (31.31%), vomiting (16.16%), pyrexia (29.29%), hypothermia (23.23%).

Key words: cat, diseases, urinary tract

INTRODUCTION

Urinary diseases can record different clinical aspects depending on the cause producing the disease, the affected segment, the evolution level, and the patient. The anomalies of the urinary tract may be due to the damage of the lumen of its segments, to own structural changes or to changes in the organs of other systems, which together will cause functional alterations of the urinary tract (Buffington, 2011). The range of the urinary tract diseases found in cats is wide, including idiopathic cystitis, urolithiasis, bacterial infections, birth defects, neoplasia, behavioural and neurological disorders (eg, reflex dysnergia) (Hostutler et al., 2005). The signs and symptoms are not specific, and diseases with a different etiology have similar signs (Gerber, 2008). Stranguria, dysuria, hematuria, periuria, pollakiuria are typical signs of urinary tract disease, but these signs rarely indicate a specific etiology (Sævik et al., 2011).
This study presents the results concerning the diagnosis and evolution of the urinary tract diseases in cats in a private clinic in Dâmboviţa County.

MATERIALS AND METHODS

The study was carried out in the Agervet clinic -Târgovişte, during 2012, where 198 cats of various breeds and ages were recorded with urinary tract diseases. The physical examination, the X–ray and abdominal ultrasound, catheterization and urine examination were performed. The diagnosis was based on the clinical signs and on the results of complementary examinations.

RESULTS AND DISCUSSIONS

Following the epidemiological study conducted during 2012 we found out that out of the 1874 cats recorded, 198 were diagnosed with urinary disorders, representing a prevalence of 10.57%. Sick animals were represented by 61 (30.81%) pure breed cats and 137 (69.19%) mixed breed cats (86 domestic short-haired and 51 cases of domestic longhaired). Pure breeds were represented by: Burmese (19 cases), Persian (14 cases), Siamese (10 cases), Russian Blue (6 cases), Norwegian Forest (5 cases), British Short Hair (3 cases), Turkish Angora (3 cases), Sphynx (1 case). The age of the animals ranged from 1 to 15 years (with an average of 7.53 years), from which 156 (78.78%) males and 42 (21.22%) females.

Following the analysis of the clinical examination, we found that 62 (35.96%) cats had acute forms of the disease, coming for the first time at the veterinary office, and 136 (64.04%) cats had chronic forms of the disease, being known as having prior episodes of illness. Out of the total cases 73 (36.87%) cats had upper urinary tract diseases, affecting the kidneys and ureters, and 125 (63.13%) au had lower urinary tract disorders, the changes being located in the bladder and urethra.

The clinical signs were represented by: dysuria (125/198; 63.13%), periuria (102/198; 51.52%), pollakiuria (98/198; 49.49%), stranguria (87/198; 43.94%), hematuria (79/198; 39.90%), anorexia (65/198; 32.83%), vocal signs (62/189, 31.31%), vomiting (32/198, 16.16%), pyrexia (58/198, 29.29%), hypothermia (46/198, 23.23%).

The imaging examination revealed the presence of calculi in 111 (56.06%) cats, at the level of the kidneys (9/198; 4.55%), bladder (100/198, 50.51%), urethra (8/198; 4.04%; from these 7 also had bladder stones). Following the imaging examination we found: nephritis (61/198; 30.80%), cystitis (115/198; 58.08%), pyelonephritis (23/198; 11.62%), renal cysts (3/198; 1.5%).

Surgery was performed on 7 cats, and calculi of different shape, size and number were removed. For 91 cats urethral sounding was necessary to evacuate urine, due to urethral obstruction. The samples collected were centrifuged, aiming the microscopic examination of the urinary sediment. Crystalluria was found in all samples, and struvites (59/91; 64.84%) and calcium oxalate (32/91; 35.16%) were observed, but the presence of crystalluria is not necessarily a sign of disease (Gerber et al., 2005), as it can be present in healthy animals as well.

The urinary tract of cats has a various pathology, and there are diseases where in which pain is localized in one segment of the urinary tract or in several segments. Functional alterations of the urinary system are classified into upper urinary tract and lower urinary tract diseases. Induced changes may be focal (cysts, abscesses) or diffuse (inflammations, neoplasic, toxic, congenital).

Following the analysis of the results that mixed-breed cats (69.19%) were more affected than those of pure breeds (30.81%) (30.81%). Sævik et al. (2011), in Norway it was found that 86.60% of the patients with lower urinary tract diseases are mixed breeds, and 13.40% are pure breeds. Also Sævik et al. (2011) found that males are more affected (73.90%) than females (26.10%), the results being similar to those obtained by us, 78.78% males and 21.22% females respectively. Gender is one of the risk factors for developing urinary tract diseases in cats (Palm and Westropp, 2011). The anatomy of the male urinary system is characterized by the narrowing of the penile urethra, thus this gender is prone to the development of urethral obstruction and implicitly to dysfunctions of the urinary tract (Hostutler et al., 2005).

The results of this study indicate a high prevalence of lower urinary tract diseases (63.13%) compared with the upper urinary tract diseases (36.87%). Previous studies note the fact that lower urinary tract diseases in cats are the most frequent diagnosis in feline patients (Eggertsdóttir et al., 2007). The study carried out by Kruger et al. (1991), indicates a prevalence of 45% of the lower urinary tract diseases in cats. While Lund et al., (1999) estimate that the prevalence of lower urinary tract diseased in cats in America is 1.5%. The differences can be explained by geographical factors, the variation of the reported feline population, difference maintenance conditions.

The signs of the urinary tract disease in cats may have an acute or chronic evolution, being the result of various combinations of abnormalities of the urinary tract lumen (external local abnormalities), urinary parenchyma

(intrinsic defects) or of the organs of other systems, care which subsequently leads to the urinary tract dysfunction (Buffington, 2011). Our results indicate an increased prevalence of chronic diseases (63.13%) at the expense of acute (36.87%).

The clinical picture includes a wide range of signs, different from one case to another, depending on the evolution and the affected segment. The signs of functional disorders of the urinary tract (dysuria, periuria, pollakiuria, stanguria, hematuria) were predominant, but changes in the general condition were also present (anorexia, vomiting, hyperthermia, hypothermia, tenesmus, vocal noises consecutive to pains). These signs are not specific to a particular disease or to a disease in a certain segment of the urinary tract, they can be seen in cats who have bladder stones, urinary infections, urinary tract tumours, etc. (Westropp, 2007). Therefore, it is always necessary to perform such a physical examination very carefully, so as to include the urinary tract and the surrounding areas, and to supplement it with the results of additional examinations.

Cystitis had the highest prevalence (58.08%), followed by urolithiasis (56.06%) and nephritis (30.80%). Most cases of cystitis were a consequence of stones, but there were cases where the cause could not be determined, which were deemed as idiopathic cystitis, according to the terminology adopted in the literature (Westropp and Buffington, 2010). The diet, the overlapped infections, the stress, the breed, the age, the environment and the lifestyle are among the factors incriminated in the occurrence of urinary diseases. The idiopathic cystitis is inflammation of the bladder without a precise etiology. In a previous study, Gerber et al. (2005) reported that the main disease diagnosed in cats was idiopathic cystitis (58%), followed by urolithiasis (22%), urethral plugs (10%) and urinary infection (8 %). While Sævik et al. (2011), identified idiopathic cystitis at 55.50%, urethral plug to 21%, urolithiasis at 11.8% and bacterial infections at 11.8%.

The urethral obstruction is a medical emergency and the evacuation of urine must be done so that the survey should be carried out. In our study it was applied for 45.96% of the cats. The palpation revealed a dilated and tense bladder, and the inspection and the observations of the owner revealed that the animals were restless, they frequently licked the perianal area, showed tenesmus and vocalizations when trying to urinate. Sævik et al. (2011), in Norway, the urinary tract obstruction was identified in 28.60% of the cats examined, and the main cause was a urethral plug (21%). Recent studies note that the main causes of the urinary tract obstruction in cats are the urethral plug, urolithiasis or urethral spam (Walker, 2009). The

pathogenesis of the urethral plug is not clearly determined, it is assumed to be the consequence of urinary infections with crystalluria resulting in the aggregation of proteins, crystals, white blood cells, red blood cells, which in their turn are surrounded by amorphous material, forming plugs (Hostutler et al., 2005).
In relation to the cases observed in this study, the therapy was applied differently from case to case, with the purpose of obtaining the hydroelectrolytic balance, to stimulate diuresis, to combat and eliminate the causes of pain. Diet and nutrition supplements to maintain urinary pH were recommended, and an improvement in the overall condition of the respective animals was noticed. Diet is the most important part of long-term treatment of this disease.

CONCLUSIONS

The diseases of the urinary tract in cats have been predominantly chronic forms – 64.04%, recorded mostly in the lower urinary tract – 63.13%.
Mixed breed cats were more affected than pure breed cats, recording a prevalence of 69.19% and 30.81% respectively.
Cystitis – 58.08%, urolithiasis – 56.06% and nephritis – 30.80% were the main diseases diagnosed.

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CURRENT DATA ON PANCREATITIS IN DOGS

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Abstract
The current study presents current data on the evolution and prevalence of pancreatitis in dogs examined in the Agervet Târgovişte Clinic, Dâmboviţa County. Out of the 573 dogs with digestive disorders, 56 (9.77%) were diagnosed with pancreatitis. Twenty-one patients were male and 35 were female patients, aged 5 to 17 years. The clinical picture was polymorphic, and blood examination showed alterations in biochemical parameters. Based on the history of the clinical data, combined with additional examination data we determined that 17 (30.36%) had acute forms, and 39 (69.64%) had chronic forms. For the diagnosis rapid tests were used to assess the level of the specific pancreatic lipase. Also, histopathological examination was performed. Fibrosis, acinar atrophy and lymphocytic infiltration indicated the chronic form, while the pancreatic cell necrosis and neutrophilic infiltration indicated the acute form.

Key words: *acute form, chronic form, dogs, pancreatitis*

INTRODUCTION

Pancreatitis is an inflammatory condition of the pancreas and is the most common disease of the canine exocrine pancreas, but the accurate diagnosis and the reliable treatment of this disease remains a challenge for practitioners (Mix and Jones, 2006). From the clinical point of view pancreatitis can be acute or chronic, and the effects on the patient can be classified as mild or severe, nonfatal or fatal (Simpson, 2006). The signs and symptoms are polymorphic, being dominated by uncharacteristic signs. There are no pathognomonic signs for either of the two forms, the evolution of the signs and symptoms clinic and the clinical results depend on the severity and duration of the inflammatory process, and on the systemic changes caused (Spillmann, 2007). It can be seen in all dogs, but age is a risk factor, so middle-aged and elderly animals are more prone to pancreatitis (Washabau, 2009). The etiology is varied and often unclear, but knowing some risk in the emergence and evolution of pancreatitis in dogs is essential in determining the patient's examination plan.
The present study aimed to assess and determine the prevalence of pancreatitis in the dogs examined at the Agervet-Târgovişte Clinic.

MATERIALS AND METHODS

In the database of the Agervet-Târgoviște Clinic throughout 2012, 573 dogs with digestive diseases were recorded. For each case we obtained information from the owners concerning the origin, food and lifestyle. The age of the animals ranged between 6 months to 17 years. The animals were examined physically, by imaging exam and haematologically. Rapid tests were used to assess the level of the specific pancreatic lipase (SNAP cPL Test, IDEXX) following the procedure indicated by the manufacturer. For a number of 5 cases post-mortem samples of pancreas were collected, in order to obtain microscopic preparations subsequently stained by usual techniques (HE and van Gieson).

RESULTS AND DISCUSSIONS

Following the physical examination and the complementary examinations we determined that 56 (9.77%) dogs had diseases of the pancreas. The diagnosis was based on clinical signs, biochemical analyzes, enzymatic tests and histological examination (for 5 dogs). The clinical signs leading to the diagnosis of pancreatitis, supplemented with the results of the complementary tests, were similar to those described in the literature (Watson, 2004; Mix and Jones, 2006; Simpson, 2006; Spillmann, 2007; Xenoulis et al, 2008; Mansourian et al., 2009; Washabau, 2009). The animals diagnosed with pancreatitis were 21 males and 35 females, who ranged in age from 5 to 17 years. Although we did not aim to distribute them specifically on breeds, we determined, however, that the pancreatic disease were found in: mixed-breeds – 14 (25%), Cocker spaniels – 7 (12,5%), Yorkshire terrier – 5 (8.93%), Chow-chow – 5 (8.93%), German shepherd 5 (8.93%), Labrador 5 (8.93%), Pitt-bull –0 4 (7.14%), Bucovina shepherd – 3 (5.35%%), West-Highland Terrier – 2 (3.57%), Shar-Pei – 2 (3.57%), Shih-Tzu – 1 (1.78%), Rottweiler – 1 (1.78%), Caucasian shepherd – 1 (1.78%), Pekingese – 1 (1.78%).

Seventeen dogs (30.36%) had acute form and 39 dogs (69.64%) had chronic forms. The symptoms who pleaded for the inclusion in the acute form of the disease were anorexia (14/17, 82.35%), sudden vomiting (14/17, 82.35%) and abdominal pain (12/17, 70.59%), signs occurred in animals with a good maintenance condition. The animals included in the chronic form had a history, presenting: deviation (33/39; 84.61%), recurrent digestive disorders

(30/39, 76.92%), weakness (28/39, 71.79%), dull hair (19/39; 48.71%), vomiting (14/39, 35.90%), jaundice (9/39, 23.07%), abdominal pain (9/39, 23.07%), pruritus cutaneous (8/39; 20.51 %).

The results of the biochemical blood tests showed: hyperamylasemia (48/56; 85.71%), hyperlipasemia (44/56; 78.57%), azotemia (39/56; 69.64%), hyperglycaemia (37/56; 66.07%), hypercholesterolemia (25/36; 44.64%), increased liver enzymes (23/56; 41.07%), hyperproteinemia (21/56; 37.5%), and hyperproteinemia (15/56; 26.79%). Seventeen (30.57%) patients were registered with diabetes.

The evaluation of specific pancreatic lipase levels by rapid test performed on 10 dogs with acute gastrointestinal signs showed intense staining of the sample spot compared to the control sample spot, which is an inadequate level of specific pancreatic lipase in all patients evaluated by this method. The radiological examination was performed to exclude the presence of more foreign bodies that could trigger acute gastrointestinal signs, or vomiting or abdominal pain. The ultrasound examination showed an enlargement of the pancreas, with a hypoechogenic image, low abdominal collections, pancreatic duct dilation, of the bile ducts and the liver.

There were 5 deaths, two of them had acute forms of the disease, and 3 were recorded with relapses, being included in the chronic form. The hystopathologic examination a showed acute lesions indicated by pancreatic tissue containing areas marked by amorphous eozinophil material present both in the interlobular areas as well as in the intralobular ones, due to massive necrosis, with neutrophil infiltration and adipocytary necrosis. The chronic lesions were represented by lobular atrophy, fibrosis, lymphocytic inflammatory infiltrate, intra-and interlobular ducts contain amorphous plugs, some calcified, representing ductal protein plugs.

Pancreatitis is an inflammatory condition of the pancreas. Previous studies have found that pancreatitis is the more frequent disorder of exocrine pancreas in dogs (Newman et al., 2004; Mix and Jones, 2006; Xenoulis et al., 2008). However, the diagnosis of pancreatitis in dogs remains a challenge for practitioners, being difficult to determine due to the non-specific nature of its clinical signs and the lack of specific and sensitive diagnostic tests (Cordner et al., 2010; Van den Bossche et al., 2010). Obtaining a detailed history, performing a thorough physical examination and carrying out the available tests are essential steps in diagnosing pancreatitis (Xenoulis et al., 2008). The timely diagnosis of pancreatitis is essential because the disease can be associated with significant morbidity and mortality (Mix and Jones, 2006). Resorting to the example in human

medicine, dog pancreatitis were classified into acute and chronic, based on the potential reversibility of the pancreatic histopathological changes (Xenoulis et al., 2008). Other authors classify pancreatic diseases according to the effect on the patient, and there are mild forms and severe forms, non-fatal and fatal (Simpson, 2006). In acute forms the pancreatic changes are reversible after the elimination of the cause of the inflammatory process, while in the chronic form, the long action of the "spinal irritation" determines irreversible structural changes.

The etiology of the disease, whether in the acute or chronic form, is varied, and it often remains unknown, which is called idiopathic pancreatitis (Neiger, 2012). There are a number of potential risk factors leading to pancreatitis and that we must take into account in determining the diagnosis. In the case of acute pancreatitis the following are incriminated: obesity, fatty meals, hiperlipidemia, severe intestinal disease, endocrine disorders (diabetes), systemic infectious diseases (babesiosis), trauma, certain toxins or drugs (Spillmann, 2007; Neiger, 2012). The possible factors incriminated for causing chronic forms are: the genetic predisposition, the existence of immune-mediated processes or the poor healing of acute episodes of pancreatitis (Spillmann, 2007).

The results obtained in this study show the presence of pancreatic disease in adult animals, aged between 5 and 17 years, the age being considered a risk factor in the occurrence of pancreatopathies. Previous studies have reported that the most affected are the middle-aged and old animals (Hess et al., 1999; Washabau, 2009, Watson et al., 2010), our results are similar to these findings.

Most patients affected were half-breeds (25%), followed by Cocker spaniels (12.5%), Yorkshire terrier, Chow-chow, German shepherd and Labrador (8.93% each). Previous studies have found that Yorkshire terriers, Skye terriers and Miniature schnauzers an increased risk of developing pancreatitis, suggesting a hereditary component (Washabau, 2009). In another study, Cavalier King Charles Spaniels, Collies, boxers and cocker spaniels were the most affected species (Watson et al., 2007). The variations can be attributed to the differences in the canine population specific to various geographic areas.

Previous studies report a prevalence varying between 0.8% and 1.46% (Gal., 2011; Thompson et al., 2009). Variations may be due to the different number of cases studied or to the canine population for which the report was made. Our results indicate a high prevalence compared with the previous studies. The actual values concerning the prevalence of pancreatitis is

difficult to determine because of the difficulties faced in the ante-mortem diagnosis (Mix and Jones, 2006), especially in the case of subclinical forms which may remain undiagnosed.

Our results present a variety of clinical signs, being similar to the previous studies (Watson, 2004; Mix and Jones, 2006; Simpson, 2006; Spillmann, 2007; Xenoulis et al, 2008; Ludlow, 2009). Dehydration (97%), anorexia (91%), vomiting (90%), deviation (79%), abdominal pain (58%), diarrhoea (33%) and jaundice (32%) are the most common signs found in previous studies (Hess and al., 1998). And another study reported that vomiting (90%), abdominal pain (58%), dehydration (46%) and diarrhoea (33%) are the most common symptoms found (Ludlow, 2009). These aspects prove the polymorphic nature of the signs and symptoms of pancreatitis, the signs are non-specific and usually transient (Xenoulis et al., 2008). We can not talk about pathognomonic signs for the acute or chronic pancreatitis (Spillmann, 2007).

The blood changes are expressed by a wide variety of physical and biochemical parameters, as shown in the current literature (Simpson, 2006). Similarly to these data, our results prove the presence of such blood variations in the examined animals. According to previous studies, azotemia, hyperglycemias, hypercholesterolemia, an increase in the liver enzymes, are the parameters indicating the presence of pancreatitis (Mix and Jones, 2006; Van den Bossche, 2010). The presence of diabetes mellitus is a risk factor in triggering pancreatitis (Washabau, 2009). The similarity of our results to those in the literature eventually led to a diagnosis of pancreatitis. Moreover, the rapid test results helped confirm the diagnosis established initially in the respective cases. An increased level of lipase and amylase was associated with pancreatitis for a long time, but no predictive value of these parameters could be determined (Mix and Jones, 2006). On the other hand, normal levels of these enzymes can not rule out pancreatitis, previous studies demonstrating the existence of cases in which dogs with pancreatitis had normal values of the amylase and serum lipase (Xenoulis et al., 2008). However we should not neglect the increase in the value of these serum enzymes, some studies suggesting that the value three times bigger than the upper limit of the reference range may be suggestive for the evolution of pancreatitis.

The histopathological examination is considered “the gold standard” for diagnosing pancreatitis, but it is difficult to perform, especially due to reluctance and lack of cooperation of the owners. Our study presents the results obtained in 5 cases of deceases. The lesions obtained following the

histopathological confirmed the initial diagnosis. The veterinary terminology used for the histopathological description of acute or chronic pancreatitis is not standardized (Thompson, 2009), thus the lesional classification of dogs becomes more difficult. Previous studies deem that the presence of acinar atrophy and fibrosis (permanent changes) are the histological key for the classification of the chronic process, while the necrosis of pancreatic cells defines the acute form (Xenoulis et al., 2008). Moreover, previous studies performed in cats have established that the predominance of the neutrophil infiltrate, described as suppurative pancreatitis, pleads for the acute form, while the predominance of lymphocytic infiltrate indicates the chronic form (Hill et al., 1993; Ferreri et al., 2001).

The diagnosis of pancreatitis is difficult to determine, thus a carefully performed physical examination is necessary, which should be supplemented by a detailed history of the case and efficient complementary examinations.

The treatment aimed to restore the function of the pancreas and the alleviation of the abdominal pain. Anti-nausea medication, antispasmodics, fluid-therapy, anti-diarrhoea treatments were used, as well as antibiotics for the animals with fever. In addition to these, a dietary and hygienic treatment, very important in the treatment of pancreatitis, as well as diet food after alleviating the clinical signs are absolutely necessary.

CONCLUSIONS

Our study shows the presence of two evolutionary forms of pancreatitis, acute and chronic, with a higher prevalence of the chronic form (69.64%) compared to the acute form (30.36%).

The signs and symptoms were polymorphic, being dominated by anorexia, vomiting and abdominal pain.

The biochemical changes revealed alterations in the blood parameters, characterized by azotemia, hyperglycaemia, hypercholesterolemia, hyperamylasemia, hyperlipasemia and increase in the hepatic enzymes.

The evaluation of the specific pancreatic lipase levels by the rapid test and the histopathological examination of the pancreas to the final diagnosis.

The correlation of the symptoms with the results of the complementary examinations is necessary in determining the diagnosis of pancreatic diseases, and the early therapeutic intervention increases the chance of survival of the animals.

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STUDY CONCERNING ECTOPARASITES INFESTATION IN DOGS AND CATS IN THE TÂRGOVIŞTE-DÂMBOVIŢA AREA

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Abstract

The objective of this study was to determine the prevalence of ectoparasites infestation in dogs and cats, and their type in the Târgovişte-Dâmboviţa area. In the period September 2011 - September 2012, 685 dogs and 180 cats were examined. A prevalence of ectoparasites from 52.41% to 51.67% in dogs and cats was identified. The following species of ectoparasites were identified in dogs: Ctenocephalides canis – 33.71%, Ctenocefalides felis – 7.24%, Rhipicephalus sanguineus – 24.51%, Dermacentor reticulatus – 11.42%, Trichodectes canis – 16.99%, Demodex canis – 7.52%, Sarcoptes scabiei var canis – 4.18%. While the following species of ectoparasites were identified in cats: C. felis – 88.17 %, Otodectes cynotis – 12.90%, Rh. sanguineus – 6.45%, Felicola subrostratus - 2.15%, Microsporum canis – 2.15%. It was found that 5.57% of patients had multiple infestations in dogs, and in cats 12.90%. To our knowledge, this is the first report on infestation with Rh. sanguineus in cat in the Târgovişte-Dâmboviţa area.

Key words: *cats, dogs, ectoparasites, infestation, prevalence*

INTRODUCTION

Dogs and cats are important hosts for many species of ectoparasites that can produce a wide range of pathogenic effects. Ectoparasites are a common cause of skin diseases in domestic animals (Curtis, 2012). Some ectoparasites, after feeding by stinging, cause skin lesions accompanied by pruritus, erythema, excoriation, papules and crusts (Wall, 2007). Fleas are a common cause reported in the etiology of dermatitis, being responsible for producing allergic dermatitis (Sousa, 2012). Mange is incriminated in producing localized or generalized dermatitis, some being strongly infectious (canine sarcoptic mange). Secondly, some ectoparasites act as vectors, so when they feed, they can inoculate to the (animal or human) various bacterial, viral or parasitic agents (Cosoroabă, 2005). Ticks are responsible for the transmission of infectious (borreliosis, rickettsiosis, babesiosis) (Shaw, 2008) or parasitic (*Cercopithifilaria sp.*) diseases (Brianti et al., 2012).

Due to the low specificity and to the increased mobility they can easily go from one species to another, so that some parasites found in animals can pass to humans, causing serious diseases (Niculescu and Didă, 1998).
In this study we aimed to determine the prevalence of ectoparasites infestation in dogs and cats and their type, at a private clinic in the city of Târgoviste, Dâmboviţa County.

MATERIALS AND METHODS

During the period September 2011 - September 2012 in the Agervet Clinic - Târgovişte a total number of 685 dogs and 180 cats aged 4 weeks to 12 years were examined. Information on the breed, age, gender, diet and place of origin were obtained by interviewing the owners. Part of the animals were found in the streets and brought to examination, making it difficult to obtain such information. Each animal was examined systematically all the body areas, in order to detect and analyse ectoparasites, or skin lesions respectively. For the collection of fleas and lice the Scotch test was used, ticks were collected by hand, and when skin lesions were found scraping was used. For the ear mange cotton sticks were used. The samples obtained were displayed on blades, clarified with a lactofenol solution and examined under a microscope. The identification of ectoparasites was conducted based on the descriptions provided by Niculescu and Didă (1998).

RESULTS AND DISCUSSIONS

Following the clinical and microscopic examination it was found that 359 (52.41%) dogs and 93 (51.67%) cats were positive. We found two species of fleas in dogs, represented by *Ctenocephalides canis* and *Ctenocefalides felis* (Figure 1 and 2), two species of ticks - *Rhipicephalus sanguineus* (Figure 3) and *Dermacentor reticulatus* (Figure 4), a species of louse - *Trichodectes canis* (Figure 5), two species of scabies – *Demodex canis* and *Sarcoptes scabiei var canis*. The following species of ectoparasites were identified in cats: one species of fleas – *C. felis*, one species of scabies – *Otodectes cynotis*, one species of ticks – *Rh. sanguineus*, one species of lice – *Felicola subrostratus* (Figure 6), one species of fungus – *Microsporum canis*. The prevalence of the ectoparasitic infestation in dogs and cats is presented in Table 1.

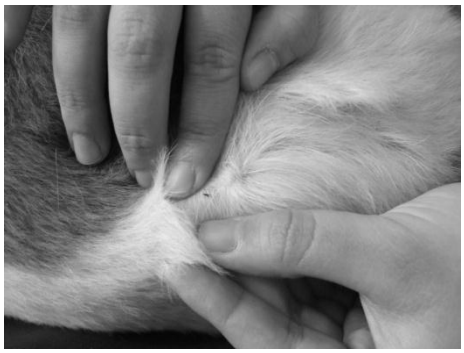

Figure 1 – Cat flea

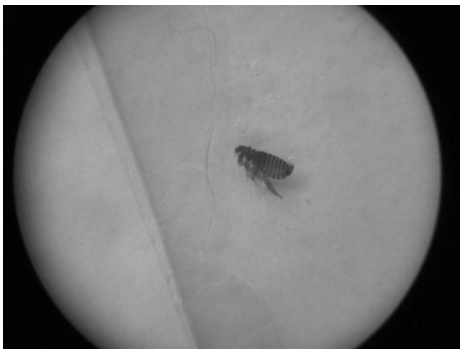

Figure 2 – *Ctenocephalides felis*

Figure 3 – *Rhipicephalus sanguineus* female after feeding

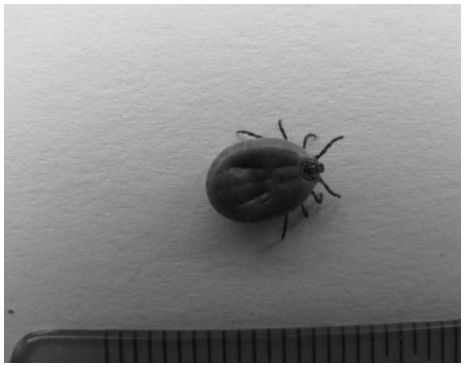

Figure 4 – *Dermacentor marginatus* female after feeding

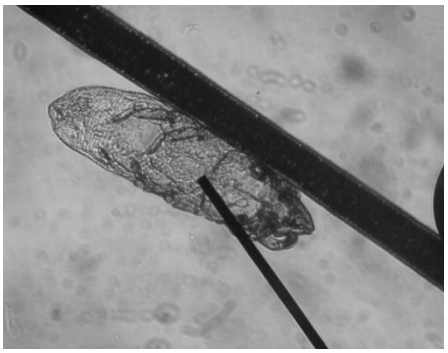

Figure 5 – *Trichodectes canis* larve *(x20)*

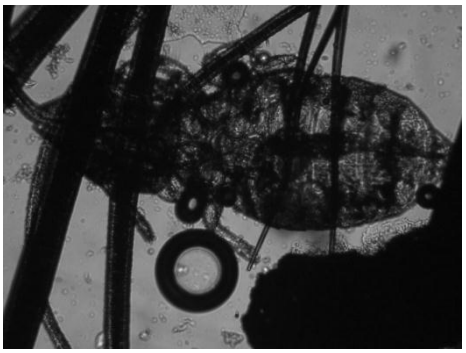

Figure 6 – *Felicola subrostratus (x20*)

Table 1

Prevalence of the ectoparasitic infestation in dogs and cats

	Dogs (n = 359)		Cats (n = 93)	
	Number	Prevalence %	Number	Prevalence %
C. canis	121	33.71	-	-
C. felis	26	7.24	82	88.17
Total fleas	147	40.95	82	88.17
Rh. sanguineus	88	24.51	6	6.45
D. reticulatus	41	11.42	-	-
Total ticks	129	35.93	6	6.45
T. canis	61	16,99	-	-
F. subrostratus	-	-	2	2.15
Total lice	61	16,99	2	2.15
D. canis	27	7.52	-	-
S. scabiei	15	4.18	-	-
O. cynotis	-	-	12	12.90
Total mange	42	11.70	12	12.90
M. canis	-	-	2	2.15

In dogs we have identified 20 (5.57%) cases of polyparasitism, out of which: 11 cases - fleas + ticks and 8 cases - fleas + scabies, 1 case - fleas + lice + scabies. In cats we have identified 12 (12.90%) cases of polyparasitism, out of which: 7 cases – fleas + mange, 4 cases - fleas + ticks, 1 case – fleas + lice.

The distribution according to the age of the animals found positive following the clinical and microscopic examinations are presented in Table 2.

Table 2

Prevalence of the ectoparasitic infestation according to the age of dogs and cats

	< 3 months	3 – 6 months	6–12 months	1- 3 years	3 - 6 years	> 6 years
	No. (Prevalence %)	No. (Prevalence %)	No. (Prevalence %)	No. (Prevalence %)	No. (Prevalence %)	No. (Prevalence %)
Dogs (n = 359)	154 (42.89)	82 (22.84)	63 (17.55)	27 (7.52)	15 (4.18)	18 (5.01)
Cats (n = 93)	23 (24.73)	18 (19.35)	19 (20.43)	13 (13.98)	15 (16.13)	5 (5.38)

In the present study, in the dogs and cats examined in the Agervet-Târgovişte Clinic, 9 species of ectoparasitic arthropods (7 species in dogs and 4 species in cats) and one species of fungi were identified, thus we determined a prevalence of the ectoparasitic infestation of 52.41% in dogs and 51.67% in cats respectively. The presence of ectoparasites in more than half of the number of animals examined in both species indicate the

existence of health problem for them and is a major risk of infestation for their owners and for other animals. Studies conducted in various parts of the world have shown the presence of a large variety of ectoparasitic species in dogs and cats, recording a different prevalence. This aspect may be due to differences in the geo-climatic and epidemiological factors. Thus, in Albania, Xhaxhiu et al. (2009) determined a prevalence of the ectoparasitic infestation of 79% in dogs, identifying 9 species of arthropods, respectively a prevalence of 100% in cats, identifying one species of ectoparasites (*C. felis*). In Ethiopia, Kumsa and Mekonnen (2011) identified a prevalence of 99.5% in dogs, identifying 6 species of parasitic arthropods, and respectively a prevalence of 91.5% in cats, identifying 3 species of ectoparasitic arthropods. In Iran, Bahrami et al. (2012) determined a prevalence of the ectoparasitic infestation of 44.26% in dogs, identifying 7 species of ectoparasites, respectively a prevalence of 58% in cats, identifying 3 species of ectoparasites.

Fleas have the highest prevalence in this study, both in dogs and in cats, 40.95% and 88.17% respectively. These results are similar to previous reports in Turkey (Aldemir, 2007), Thailand (Jittapalapong et al., 2008), Albania (Xhaxhiu et al., 2009), Iran (Bahrami et al., 2012). Farcas et al. (2009) in Hungary, obtained a 14.1% prevalence of fleas in dogs and 22.9% in cats. In Romania, previous studies have reported a prevalence of 45.52% of the infestation with *C. canis* in dogs, which is the main ectoparasites species identified (Tudor, 2009). In this study, two species were identified in dogs, *C. canis* and *C. felis*, and in cats only the latter species was identified. In another study, Borji et al. (2011) identified one species of cat fleas, represented by *C. felis*. In Pakistan, Arijo et al. (2007) identified the presence of the species *C felis* both in dogs and in cats, with a prevalence of 34%, respectively 28%. Beck et al., (2006) determines a prevalence of the infestation with fleas of 5.13% in dogs and 14.33% in cats, identifying 5 species out of which *C. felis* had the highest prevalence, 81.5% respectively. Flea infestation of animals and of their environment is frequently seen. The high prevalence of this ectoparasite is a serious problem for practitioners. Firstly, because they cause discomfort the hosts by stinging, causing allergic reactions and itching. Secondly, they are also a vector for numerous parasitic and microbial agents with medical veterinary and human importance, among which *D. caninum*, the cat scratch disease (*Bartonella sp.*) and the spotted fever rickettsial species (*Rickettsia felis*) (Shaw, 2008).

Ticks were ranked second in frequency of ectoparasites in both species of animals, with an overall prevalence of 35.93%, which is represented by two

species in dogs, i.e. *Rh. sanguineus* (24.15%) and *D. reticulatum* (11.42%). Only one species was identified in cats, i.e. *Rh sanguineus*, 6.45%. Similar results were also reported by Aldemir (2007) in Turkey and Xhaxhiu et al. (2009) in Albania, who nevertheless does not identify ticks on cats. On the other hand, in Nigeria, Adamu et al. (2012) identified ticks as the main species of ectoparasites in dogs, with a prevalence of 47%, and *Rh. sanguineus* was predominant (24.3%). In France, Zenner and Drevon (2003) reported the identification of three species of ticks in dogs (*Ixodes ricinus*, *D. reticulatus* and *Rh. sanguineus*) and 2 species in cats, i.e. *I. ricinus* (97.2%) and *Rh. sanguineus* (2.8%), due to the presence in that region of the three species of ticks, the species *I. ricinus* being predominant. To our knowledge, this is the first report on the infestation with *Rh. sanguineus* in cats in the Dâmboviţa County. Previous studies carried out on dogs in this area showed the prevalence of the species *Rh. sanguineus* compared with the species *D. reticulatus* (Mateescu et al., 2011). Ticks are spread across the continent and occur in large numbers, especially in areas with vegetation, such as forest edges but also in the urban environment, i.e. in parks and gardens. They are responsible for transmitting babesiosis to animals, which is why they require paying increased attention to this ectoparasite. Moreover, ticks are responsible for transmitting certain diseases to humans, which have recorded increased values lately.

Lice were the third ectoparasitic species in terms of frequency in dog and the fourth in cats, with values of 16.99% and 2.15% respectively. Low levels of lice infestations have been reported in previous other (Gonzalez et al., 2004; Jittapalapong et al., 2008; Chee et al., 2008). On the other hand, Mosallanejad et al. (2011) reported lice as the main species of ectoparasites in dogs, with a prevalence of 8.73%. These differences can be explained by the geographical differences, the animal population studied, the time dedicated to the study. Lice cause discomfort and dermatitis to the infested animals, but can also be a host for the tapeworm *D. caninum* (Niculescu and Didă, 1998). The low prevalence of these ectoparasites compared to the other species may be the result of applying preventive treatment against fleas.

Mange reported low values in both species. While two species were identified in dogs, *D. canis* (7.52%) and *S. scabiei var canis* (4.18%) respectively, while only one species was diagnosed in cats, i.e. *O. cynotis* (12.90%). Significantly lower values of scabies infestations have been reported in several previous studies (Aldemir, 2007; Xhaxhiu et al., 2009; Duarte et al., 2010; Bahrami et al., 2012). On the other hand, Chee et al.

(2008) that the most common species of scabies found in dogs is *O. cynotis* (22.3%), followed by *S. scabiei var canis* (19.4%) and *D. canis* (4.9%). Ali et al. (2011) determined a prevalence of scabies infestation of 62.5% in dogs, noting that the most frequent species was *S. scabiei var canis* with 50%, followed by *D. canis* with 35.4%. Jamshidi et al. (2010) au determined a prevalence of scabies infestation of 25.9%, the species *S. scabiei var canis* being the most frequently found, with 21%, followed by *O. cynotis* (2.8%) and *D. canis* (2.1%).

The fungal infestation showed low values, being found only in cats (2.15%). Unlike our results, Mancianti et al. (2002) diagnosed dermatophytes in 18.7% and 24.7% of the examined dogs, and cats respectively, and *M. canis* was the most frequent species, 83% and 97% respectively. Cafarchia et al. (2006) found the presence of dermatophytes in 20.5% dogs and 28.2% cats. Duarte et al. (2010) identified 4 species of dermatophytes in cats, determining a prevalence of 29.4%, while *M. canis* was the most frequent species (12.5%). Tel and Akan (2008) identified the presence of dermatophytes in 7.5% of the examined dogs, and in 42% of the cats. Previous studies have reported an increased occurrence of the infection with *M. canis* in European countries, especially in the Mediterranean ones (Lunder, 1992). Dermatophytosis is a frequent health problem in pets, and its contagious nature and the high cost of the treatment, as well as the implications for public health require increased attention to its causative agents.

The results obtained in this study showed that simple infestations were predominant, in both animal species studied. While polyparasitism, was recorded only in 5.57%, and 12.90% respectively of the examined cats and dogs. Unlike us, Gonzalez et al. (2004) determined that 56.9% of the examined dogs had triple infestation, while 39.6% had a double infestation. Xhaxhiu et al. (2009) also identified polyparasitism in 38.1% of the dogs, 29.8% with two, and respectively 8.3% with three species of ectoparasites.

This study shows that there is a high prevalence of ectoparasites in dogs and cats in the examined area. This aspect is significant both for veterinarians as well as for human doctors, due to the effects these ectoparasites cause on animals and humans. Informing pet owners about the role of ectoparasites in the transmission of zoonoses and educating them to observe the preventive and control measures against parasites is an important step in reducing the prevalence of parasitic infestation. Due to the well-known the role of vector some species of ectoparasites have in the transmission of infectious

diseases, we recommend the application of the preventive treatment against parasites as early as possible to pets.

CONCLUSIONS

The study concerning ectoparazitoses in dogs and cats in the Târgovişte-Dâmboviţa area, recorder a relatively high prevalence in both animal species, 52.41% in dogs, and 51.67% respectively in cats.
The main species of ectoparasites identified in dogs were fleas and ticks, 40.95% and 35.93 %, respectively, while fleas prevailed in cats – 88.17%.
For the first time, the tick infestation was reported in cats, with a prevalence of 6.45%.
Polyparasitism had relatively low values, i.e. 5.57% in dogs and 12.90% in cats.
The young individual of both species was found to have the highest rate of infestation with ectoparasites, compared to adult animals.

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RESEARCH AND OBSERVATION ON CLINICAL AND THERAPEUTIC ASPECTS REGARDING TRANSMISSIBLE VENEREAL TUMOR IN DOGS

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Abstract

Transmissible venereal tumor, also known as infectious sarcoma or Sticker tumor, has a different incidence from one race to another although the range is known as a cosmopolitan character disorder. In our study. In our cases the incidence was 23%. The initial lesions are superficial, small pink to red, 1 mm to 3 mm diameter nodules can be observed, then multiple nodules fuse together forming larger, red, hemorrhagic, cauliflower-like, friable masses, that draws the owners attention. Tumors bleed easily and while becoming larger, normally ulcerate and become contaminated (Hoque, 2002). For this study two groups were used, the first group included 11 dogs, the second group 12 dogs, different race and age, all males, raised in freedom, with little socialization with other dogs. For the first group the treatment was made with vincristine sulfate at a dose of 0.025 mg/kg, i.v, administered weekly, for a period of 7 weeks. The second group was treated with vinbleastina at a dose of 0,1 mg/hg i.v, administered weekly, for 6 weeks. The best results were with vincristine, 95%, while the results for the treatment with vinblastine were between 85-95%.

INTRODUCTION

Transmissible venereal tumor (TVT) , also known as Sticker sarcoma or infectious sarcoma, is a benign reticuloendothelial tumor of the dogs male or female (Smith, and Washbourn, 1998) but it can be observed in wild canide (Dominguez-Tejerina et al., 1996), sexually mature. Usually it is transmited during mating (Calvet, 1983), and it is more prevalent in temperate climates (Rogers, 1997).

It is commonly observed in dogs that are in close contact one with another, or in stray and wild dogs that exhibit unrestrained sexual activity (Cangul, 2003 citated by Purohit G.N.,2009).

This are the explanation for the high incidence (Jain, et al. 2002) of sarcoma in the dogs, or other studies performed on dogs in the same condition.

MATERIALS AND METHODS

The studies were conducted in the Surgery Clinic and Pathology of Veterinary Medicine Faculty Cluj-Napoca, on 100 dogs from different breeds and ages (2-6 years), male and female. 23 (23%) were treated by chemotherapy. The history reveled diferent maintenance systems: 25 of them were free (stray dogs), 26 raised in semi-freedom, the meaning of this refers that the dogs had periodic contact with other stray dogs, the rest of them had owners and they were raised in a restricted area, the contact with other dogs was rare and short. For diagnosis, clinical exam (an individual exam chart was made), cytological and histological exams by imprints of the tumors, fine needle biopsy.

Chemotherapy was made by using two products Vincristine sulphate and Vinblastine. For checking the efficacy of this two products we took in study a number of 23 dogs (15 males and 8 females) divided in two lots: the first one included 11 dogs (7 males and 4 females) and treated with Vincristine sulphate at a dose of 0,025 mg/kg i.v, once a week, for 7 weeks.

The second lot included 12 dogs (8 males and 4 females) and treated with Vinblastine at a dose of 0,1 mg/kg i.v, administered weekly, for 6 weeks. At the beginning of treatment animals were evaluated clinically by checking the temperature, heart rate and respiratory, all being in normal parameters.

RESULTS AND DISCUSSION

Clinical signs vary according to the localization of the tumors. On our cases lesions were localized on the external genital organs, cranially on the glans penis in males, 8 cases (34,78%), (Figure 1) followed by preputial mucosa on 5 cases (21,73%), and on the bulbus glandis 2 cases (8,69%) (Figure 2). On some subjects we noticed the lesions at the beginning, when they are superficial and pink to red color, multiple noduls shape, with 1-4 mm in diameter, then in time this develop, rise and fuse together forming larger, red, hemorrhagic, cauliflower-like, friable tumor (Figure 3).

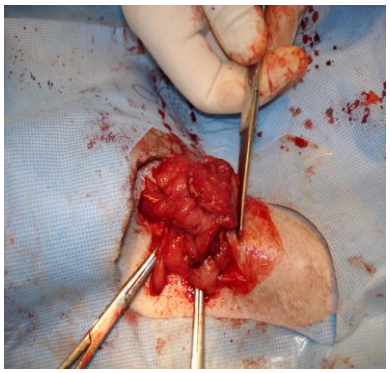

Figure 1. Tumor located on the glans penis

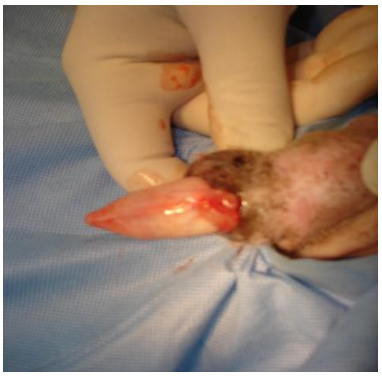

Figure 2. Bulbus glandis location aspect

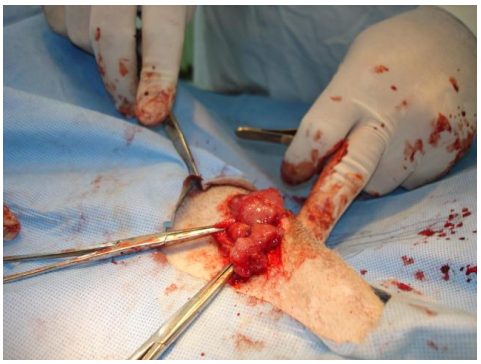

Figure 3. Cauliflower-like aspect

The tumoral mass can reach 5-8 cm in diameter which then progress deeper in the mucosa, and also outside it, often protruding from the prepuce, also observed by (Higgins, 1966) resulting the appearance of phimosis mentioned by authors (Mc Envoy, 1987), bleedings, ulcers and necrosis of the tumoral tissue. Tumors bleed easily, and when becoming larger, normally ulcerate and become contaminated (Hoque, 2002), hemorrhagic discharge at the prepuce, the blood has a dark color with a repulsing smell. The discharge can be confused at least at the beginning (before the tumoral growth) with urethritis, cystitis, or prostatitis (Rogers K.S., 1997). The involvement of regional lymph nodes is frequent in males with large tumors, in bitches this reaction may be absent. In our study the age of occurrence was between 2-6 years, which overlap with sexual maturity activity. In bitches the tumors have similar gross appearance as in male dogs and can be localized in the vagina (2 cases, 8,69%) or vulva (6 cases, 26,08%) deforming the region, protruding from the vulva and frequently causing a deformation of the perineal region. Because of repeted trauma, interference

with urine during micturition, the tissues suffer necrosis, they tear causingbleedings witch in time leads to anemia, this state has been encounter in 2 of the bitches from the study. During our study we did not find other locations besides genital area.
Histologically tumoral cells are round to oval in shape in different mitotic phases, with hyperchromatic nucleus and one or two prominent nucleoli, aspects observed by (Gonzalez, et al. 2000; Singh, et al. 1996) (Figure 4). Remarcable is the presence of multiple clear cytoplasmic vacuoles, observed since 2004 (Tella, et al., 2004). Another aspect that should be mentioned is that the tumor mass increases, the cells become tightly packed and irregular in shape and fibroblasts appear, resembling features are also mentioned by other authors (Calvet, 1983; . Kennedy, et al., 1977).

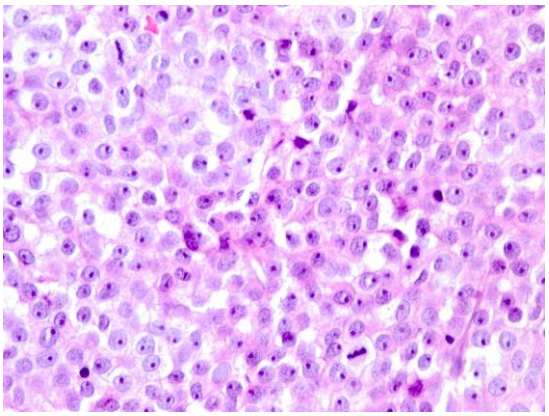

Figure 4. Tumoral cells with eosinophilic cytoplasm and hyperchromatic nucleus

Regarding the treatement, during time besides the surgical removal were used radiotherapy, immunotherapy, biotherapy and chemotherapy. In our study the animals treated with Vincristine sulphate had a 95% rate of succes after 7 weeks from the first administration. The second lot was treated with Vinblastine but the results was not as efficient as the first one, the succes rate was between 85-90% after 10-15 weeks from the first administration. During treatement the animals were kept under surveillance. At the end of the treatement blood samples were taken and haematological exam were made, to see if chemotherapy affects the blood values.

Table 1. Hematological aspects (average)

TEST	Reference values	Vincristine		Vinblastine	
		Result average	% average	Result average	% average
Hemoglobin (g%)	12-18	10,2	15	10,5	12,5
PCV (%)	37-55	32,38	12,5	32,93	11
RBC ($x10^6/\mu L$)	5.5-8.5	4,93	10,5	4,84	12
WBC ($x10^3/\mu L$)	6-17	5,22	13	5,07	15,5
Neutrophils (%)	60-70	47,7	20,5	50,7	15,5
Lymphocytes (%)	12-13	9,36	22	9,78	18,5
Platelet Count ($x10^3/\mu L$)	200-500	187	6,5	171	14,5
Monocytes (%)	3-10	2,62	12,5	2,58	14

Even if the registred values are at the lower end of the normal, this are not statistically significant. Three weeks after last administration the haematological values increased to normal values.
We mention that the administration was made in i.v solutions. Throughout treatment we monitored all the possible side effects especially those mentioned by authors as (Calvet, 1983; Withrow and McEwen 1996). For lots that we used Vincristine and Vinblastine we noticed that in the sixth and seventh week appetite decreased, the animals look tired, with muscle tonus decreasing. 10 days from last administration the animals recovered to normal status, without other side effects.

CONCLUSIONS

TVT is the most frequent neoplasic state of the external genital area in dogs, commonin males and females, with similar evolution.
Incidence of this disorder in our study was 23%, the high incidence is assigned to the fact that most of them were stray.

The most effective and practical treatment in TVT is using Vincristine sulphate weekly in a dose of 0.025mg/kg in i.v solutions.
In the last week of treatment, the animals from the study had muscle tonus decreased, lack of appetite, without any other side effects.

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PRIMARY CUTANEOUS ASPERGILLOSIS CAUSED BY *ASPERGILLUS FLAVUS* IN CAT - CASE REPORT

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Abstract

Aspergillosis is recognized as an opportunistic infection in human and animals often occuring in association with other chronic diseases (immunodeficiency, diabetes mellitus, long-term antibiotherapy, chemotherapy, surgery, etc.). Over 95% of human aspergillosis are produced by A.fumigatus, A.flavus and A.niger commonly found in the environment. Primary cutaneous aspergillosis with A.flavus has been rarely reported in human, mainly in immunocompromised and diabetic patients after surgery.
Aspergillus infections have been more less reported in cat than dog, with two clinical forms: nasal and systemic. Early detection and treatment are important factors in infection control.
This paper illustrated a primary cutaneous aspergillosis of the tail in a spayed mixed-breed female cat, 10 years old, with no general symptoms. The samples prelevated from tail lesions were submitted to bacteriological, mycological and cytological investigation. The results demonstrated the infection with a strain of A.flavus. The case is still under investigation and represent a real therapeutic challenge for us considering the chronic infection and the age of patient.

Key words: *aspergillosis, cat, tail.*

INTRODUCTION

Aspergillosis is recognized as an opportunistic infection both in human and animals, often occuring subsequently to other conditions (diabetes mellitus, immunosuppression, trauma/surgery, long-term antibio- cortico- or chemotherapy, etc.). In human, the most infections are caused by *A.fumigatus, A.flavus* and *A.niger*, species with a worldwide distribution in the environment (soil, plants, air, water, food, etc.). The primary mode of fungal transmission is by inhalation of *Aspergillus* conidia.
A.flavus is the common cause of human synusitis and superficial dermatitis and the second responsible agent for invasive aspergillosis after *A.fumigatus,* predominantely in arid dry regions: Middle East, Africa, Southeast Asia (Krishnan, 2009). Human cutaneous aspergillosis was classified as primary (following direct inoculation at sites of skin injury) and

secondary infection (by hematogenous spread from pulmonary sites or by contiguous extension from neighbouring sinus) - Hedayati, 2007.
Aspergillosis has been rarely reported in pets (more frequent in dog than cat) developping two clinical forms: nasal-sino-orbital infection with *A.fumigatus* and systemic infection with *A.terreus* (Kano, 2008; De Lorenzi, 2006; Barachetti, 2009).
Due to a high invazivity and allergic, immunosuppressive, toxic, teratogen and carcinogen potential of *A.flavus*, early fungal detection and treatment are very important to clear *Apergillus* infection. According to the latest data, *A.flavus* seems to be more virulent and more resistant to antifungal drugs than most other *Aspergillus* species (Hedayati, 2007).
This paper reported a case of primary cutaneous aspergillosis in a cat with chronic evolution and sarcoma transformation which in our opinion has been promoted by environmental conditions and repeated trauma of the tail.

MATERIALS AND METHODS

Patient history and clinical findings: A 10 years old spayed female cat from common breed was dermatologically examined at Faculty of Veterinary Medicine of Bucharest. Initially, we have received a tail fragment after surgery for microbiological evaluation, but subsequently to laboratory tests we decided the clinical examination of the cat to get a complete view of the case. So, patient history revealed repeated trauma of the tail consisting in an initial fracture resolved by surgery which was followed by a second intervention one year later due to persistent self-mutilation to the tail. Routine biochemical and hematological analysis demonstrated a hepatic insufficiency (high values of ALT and bilirubin) and a moderate polycitemia with leucopenia. Another key-element from patient history was constant exposure of the cat to an inadequate damp habitat.
Tail lesions were characterized by hair-loss, diffuse edema and induration, superficial brown crusting with the expression of petechiae and pus after crust removing (fig. 1 a,b,c). Moreover, a fatty-sarcomatous aspect was detected on the cut-section. No general symptoms have been associated with these cutaneous lesions.
Paraclinical evaluation included cytological, bacteriological and fungal examination. Cytology was performed on the smears obtained from scraping and aspiration of superficial and cut-section lesions which were stained by May-Grünwald Giemsa and Gram method. For bacteriological investigation, cutaneous samples were inoculated into brain-heart infusion broth (BHI

broth) and blood agar with incubation at 37˚C. Fungal cultures were prepared in Sabouraud dextrose broth, Sabouraud dextrose agar CAF CEX (with chloramphenicol and cycloheximide) and Czapek Dox agar which were incubated at 27˚C and 37˚C. The identification of *A. flavus* was made based on gross colony morphology and microscopic features (in lactophenol cotton blue-stained wet mounts).

RESULTS AND DISCUSSIONS

Clinical lesions were reproduced in figure 1 a,b,c.

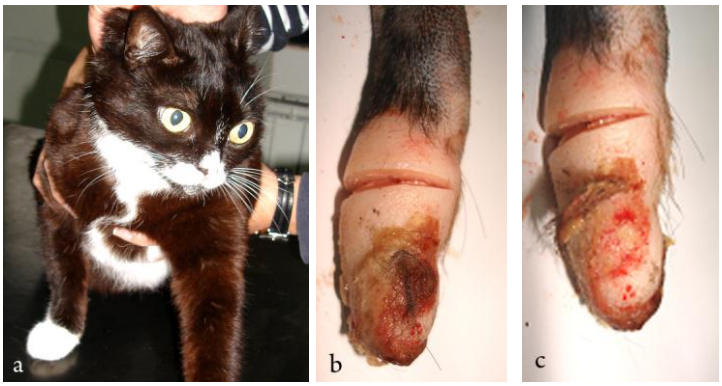

Figure 1 a, b, c. Tail lesions

Routine cytology supplied the first clue for diagnosis. In Gram stained smears, few septate hyphae intricated by numerous cellular remnants were observed (figure 2).

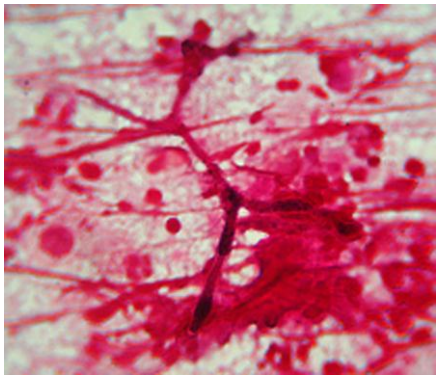

Figure 2. Branched septate hyphae in Gram stained smear (X100)

In May-Grünwald Giemsa staining, smears obtained from superficial scrapings revealed an inflammatory infiltrate with predominant degenerate neutrophils (nuclear pyknosis and karyorrhexis), red blood cells and fibrin filaments. Surprisingly, the aspirates from fatty cut-section lesions evidenced a moderate relatively uniform population of hystiocyte-like cells entrapped into an oxyphil matrix lacking other inflammatory cells (figure 3 a,b).

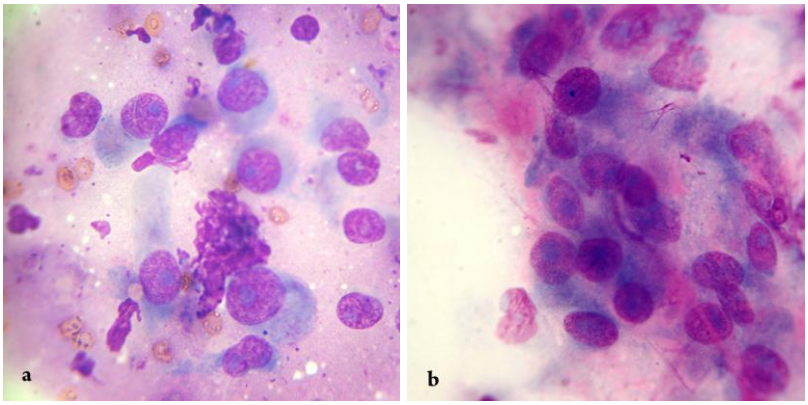

Figure 3 a,b. Hystiocyte-like cells entrapped into an oxyphil matrix from the aspirate of tail lesion (MGG stain, X100)

No granulomatous reaction typically found in fungal infection has been detected. Sarcomatous transformation in the deep tissue of the tail could be the result of combined action of repeated trauma and slow releasing of mycotoxins by *Aspergillus* isolated from these lesions.

Routine bacteriology was negative since no bacterial colony was identified in inoculated broth and agar. Instead of bacterial growth, fungal colonies have developped both in BHI broth (at 10 days of incubation) and blood agar (at 3 days of incubation) under 37˚C (figure 4 a,b).

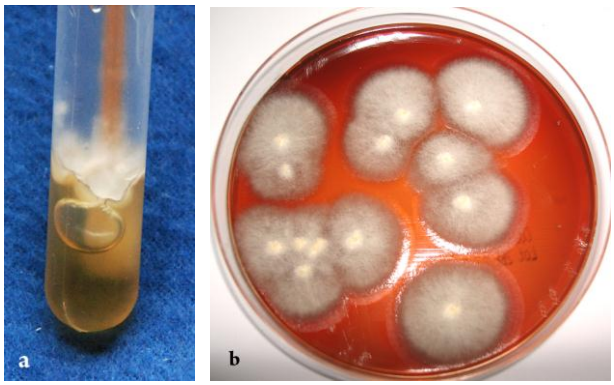

Figure 4 a,b. Cultures in BHI broth (10 days, 37˚C) and blood agar (3 days, 37˚C)

Fungal cultures proved the most relevant for diagnosis by isolation of *Aspergillus flavus* in pure culture. Typical flat powdery colonies ranging in colour from yellow-greenish to olive green-brown on averse side and cream to gold on reverse with radial grooves were observed in Sabouraud dextrose agar CAF CEX and Czapek Dox agar both at 27˚C and 37˚C at 10 days of incubation (figure 5 a,b).

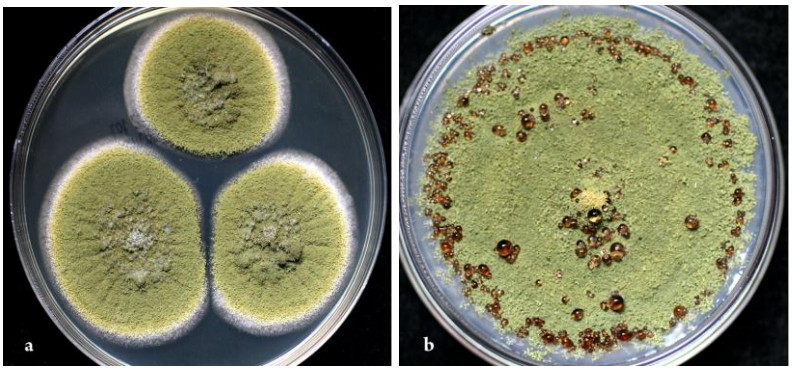

Figure 5 a,b. Colonies of *A.flavus* on SDA CAF CEX and Czapek Dox agar (10 days, 27˚C)

Sclerotia production was also observed in fungal colonies on Czapek Dox agar, at 27˚C and 37˚C at 10 days of incubation (figure 6 a,b).

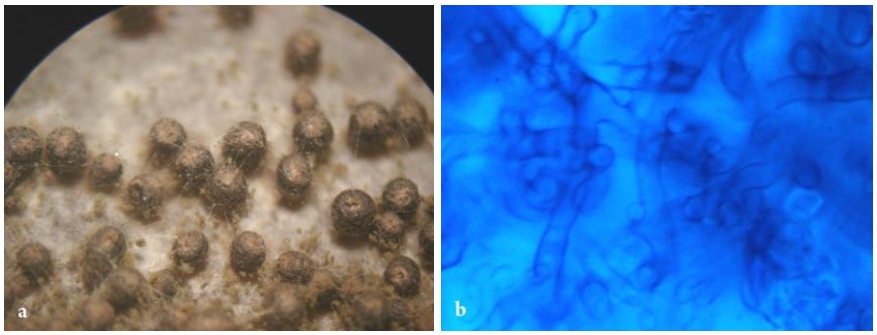

Figure 6 a,b. Macroscopic and microscopic features of sclerotia on Czapek Dox agar (10 days, 37˚C)

These gross findings of fungal cultures has been correlated with the typical microscopic features of *A.flavus* (figure 7 a,b).

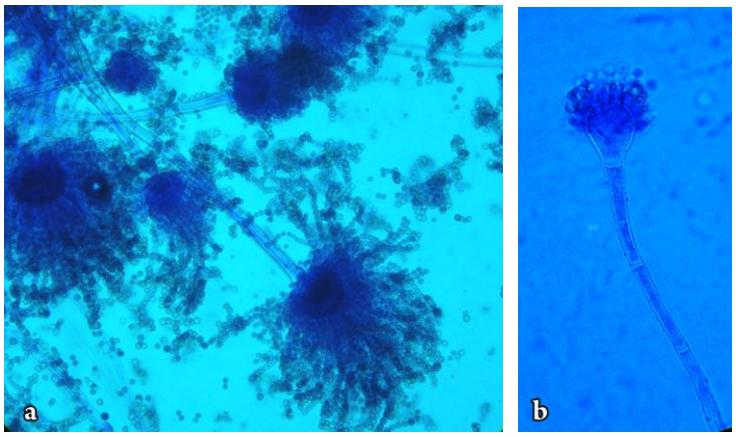

Figure 7 a,b. Conidiophores of *A.flavus*

A.flavus is known as a relative fast growing thermotolerant fungus able to grow at temperatures from 12 to 48˚C (Hedayati, 2007). Moreover, the growth of the fungus in the presence of cycloheximide indicated the isolation of a pathogenic strain of *A.flavus* from the tail lesions.
Sclerotia production is considered of key importance for identification of an *A.flavus* strain (Krishnan, 2009) and may be also a reliable marker of aflatoxins production (Leema, 2010; Hedayati, 2007). According to Leema's opinion (2010) the production of aflatoxins by a *A.flavus* may contribute to the severity of clinical lesions in human keratitis being

necessary the suppression or neutralization of deleterious effects of aflatoxins for a good response to usual antifungal therapy.The same author has found that aflatoxin production occured more frequently in isolates of *A.flavus* from patients with keratitis compared to *A.flavus* isolated from environment (Leema, 2010). Surprisingly, other studies on human aspergillosis demonstrated a reduced genetic diversity amongst isolates of *A.flavus* in comparison with *A.fumigatus* though the strains of *A.flavus* group (with 9 species and 2 varieties) are highly polymorphic in nature (Hedayati, 2007).

In our case, a strain of *A.flavus* was incriminated in a primary cutaneous infection in cat. The chronic infection (about 1 year) most likely was due to the inoculation of fungal spores into deep tissues of the tail secondarily to repeated trauma (surgery and persistent self-mutilation) and exposure to dampness. The isolated strain of *A.flavus* may be also toxigen inducing a local sarcomatous reaction, but not clasical granulomatous response (in cytology) with no general symptoms excepting a subclinical hepatic inssuficiency (in routine biochemistry).

The case is still under investigation and represent a real therapeutic challenge considering the chronic infection possibly combined with chronic toxicity beside the advanced age of patient.

CONCLUSIONS

In this paper, we reported a case of primary cutaneous aspergillosis in cat with an atypical localisation to the tail.

Detailed history, clinical and paraclinical investigations helped us in diagnosis. Repeated trauma of the tail by fracture, surgery and self-mutilation beside a persistent exposure of the animal to dampness were the most significant data from patient history. Routine bacteriology was negative, while the mycological examination (morphological and cultural evaluation) was definitive for diagnosis, confirming the infection with an *A.flavus* strain.

Moreover, sclerotia production on Czapek Dox agar beside a particular tissue response consisting in sarcomatous, but no granulomatous reaction are indicative for the toxic potential of the isolated strain of *A.flavus*. A role of aspergillar toxins from cutaneous site in subclinical hepatic insufficiency detected in this case cannot be excluded.

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EPIDEMIOLOGIC STUDY AND MORPHOLOGIC DIAGNOSIS ON LESIONS IDENTIFIED IN PSITTACINES

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Abstract

Tumoral lesions in psittacines are, nowadays, clinically diagnosed with increasing frequency. This study is aimed to evaluate clinic and epidemiologic characteristics together with the efficiency of the cytologic, histopathologic and necropsic diagnosis on lesions in parrots. A total of 23 cases were examined at the Department of Pathological Anatomy of Faculty of Veterinary Medicine, Bucharest during September 2011 - October 2012.
19 standard budgerigars (Melopsittacus undulatus), two cockatiels (Nymphicus hollandicus), one lovebird (Peach-Faced Agapornis) and one cockatoo (Cacatua sulphurea) were clinically examined. Sex of the birds was not significant in the tumor incidence. Regarding age, 17 cases were 1-5 years old and only 6 over 5. Regarding topography, 7 cases presented lesions in the pectoral area, 6 cases in the abdominal area and, same number, in the wing region and one case each for the uropygial region, legs, eye, cere and beak.
Microscopically, most of the cases were diagnosed as tumors and only one as inflamatory process. The majority were classified as malignant proliferations, from which five had mesenchymal origin (four fibrosarcomas and one hystiocitic cell sarcoma) and one, epithelial origin (a basal-cell carcinoma). The benign lesions had a mesenchymal origin (one hemangyoma and two lypomas). Malignant cases had a poor survival rate, under three weeks for mesenchymal neoplasms and one week for the epithelial one.
All in all, this study revealed that most cases of lesions in parrots were 1-4 year old, located either on trunk or wing and the majority confirmed a malignant proliferation.

Key words: *parrot, tumor, epidemiology, morphologic diagnosis*

INTRODUCTION

Psittacines, like all species, suffer from a range of cutaneous, subcutaneous and internal neoplasms. Approach to these should be similar to that used in other species. Fine-needle aspiration or biopsy is indicated prior to removal.

Species, site and age may be predictive in relation to tumor type (Samour, 2008).
Classification of tumors include pseudoneoplasies, benign lesions and malignant tumors, either solid or systemic, with epithelial or mesenchymal origin. Xanthomas are non-neoplastic, proliferative lesions, seen most commonly in budgerigars and cockatiels as a variable-sized, yellow mass. Benign lesions are most comonly diagnosed as lipomas, usually located on the sterno-pubic area, or hemagiomas, situated internal or external (Harrison 2007, Lightfoot, 2007 and 2009). Fibrosarcomas are neoplasies, most comonly seen in the oral cavity, associated with long bones, or in the abdominal cavity (Harrison and Lightfoot, 2007, Palmieri C et al., 2011). Squamous cell carcinoma is another neoplastic lesion, located anywhere on the body, ocasionally cited at the uropigyal gland (Paterson, 2006). Internal carcinomas are generally diagnosed at necropsy and include ovarian, renal, hepato-biliary and pancreatic neoplasies. Numerous reports of exophthalmos in psittacines, particularly young African greys, have been diagnosed as retrobulbar lymphoma.(Harrison and Lightfoot, 2007 and Samour, 2008)

MATERIALS AND METHODS

The present study involved a number of 23 psittacine cases, examined at the Department of Pathological Anatomy from Faculty of Veterinary Medicine, Bucharest, during september 2011-october 2012. They were submitted to a macroscopic examination, together with a general state evaluation. Clinical anamnesis was carefully carried to offer information about the age and sex of the bird, the time the owner observed the lesion, the growth rhythm and the eventual, previous feather or soft tissue trauma on the site of the lesion.
Citologic examination for the live cases was carried through fine-niddle aspiration technique, using 22G and 23G gauge needles. Of considerable importance is the physical contention of the parrot. In the case of the budgerigar (*Mellopsitacus undulatus),* this procedure means catching the bird and restraining the neck between index and middle finger and the feet supported by the thumb and the ring finger. This method ensures movement restriction, calming of the bird, and most important, proper breathing. As for the middle and large psittacine species (*Agapornis spp., Nymphicus hollandicus, Cacatua sulphurea*), a towel is used for restraint. For surgical excisions, the techniques used in citologic examination included scrape and imprint technique. The coloration method used was May-Grümwald-Giemsa and only for a limited number of cases, MGG-Quick.

Necropsies were performed in the Department's Necropsy Laboratory.
Histopathologic examinations were performed on lesions and organ samples obtained during necropsies. They were fixed in 10% neutral buffered formaldehid and tricloracetic acid, if they included bone structures. In the end, the samples were embedded in paraffin, sectioned at 4-6 microns and stained with Hematoxilin-Eosin (H&E).
The microscopic examination of citologic and histopathologic samples was carried at a Carl Zeiss Axio Imager A1 microscope with photographic system integrated.

RESULTS AND DISCUSIONS

A total of 23 parrots were examined, out of which 19 cases of budgerigars (*Melopsittacus undulatus*), two cockatiels (*Nymphicus hollandicus*) and one lovebird (*Agapornis roseicollis*) and one cacadu (*Cacatua sulphurea*).
Sex prevalence was not revealed, both were almost equally affected, 56,5% females and 43,5% males. As for the cases belonging to species without sexual dimorphism, as *Agapornis roseicollis* and *Cacatua sulphurea*, it was taken into consideration the existance of a mate, egg laying and behaviour.
As for the age of the birds, some important aspects were highlighted. A percentage of 73.9 % were aged between 1 and 5 years old, only 26.1% over 5 and none after the age of 8. (Table.1)

Table.1 Association between age and number of cases

Age of the parrot (years)	Number of cases
<1 year	1
1-2	2
2-3	3
3-4	7
4-5	4
5-6	1
6-7	4
7-8	1

Similar results were observed in 1983 by Neumann and Kummerfeld in a study regarding 74 cases of internal neoplasms in budgerigars. The highest incidence was registered between age 3 and 5 years old.
Regarding age of the parrots, lifespan is of great importance. *Melopsittacus undulatus* is considered to have a lifespan of 8-10 years (Jepson, 2009), *Nymphicus hollandicus* and *Genus Agapornis*, generally, live up to 15 years,

sometimes longer (Jepson, 2009). *Cacatua sulphurea* is the species with the highest longevity from the cases examined, up to 40 -50 years, with sexual maturity reached between 3 and 4 years of age. (Jepson, 2009) The case included in the study was two and half years old, which means the tumoral lesion developed at a young age, similar to ages before one year of small parrot species.

Regarding topography of the lesions, Table 2 reveals the anatomical regions of tumoral lesions identified in this study.

Table 2. Anatomic location frequency of lesions identified in psittacines

Topography of the lesion	Number of cases	Percentage
Pectoral region	7	30.6%
Coelomic region	6	26.1%
Wing region	6	26.1%
Uropigyal gland region	1	4.3%
Foot region	1	4.3%
Ceroma and beak region	1	4.3%
Eye region	1	4.3%

Survival rate proved, in most cases, to be correlated with the location of the lesion, meaning that lesions located at anatomical junctions, such as the carpal joint, tarso-metatarsal joint or maxilo-mandibular joint, are predisposed to rapid growth. These regions are intensely used, accesible to self mutilation, prone to septic or haemorhagic complications and, usually they were the site of repeatedly trauma, preliminary to the macroscopic lesion. On the other hand, pectoral or coelomic locations affect less the general state of the animal, due to the fact they infiltrate the pectoral muscle or the cavity with lateral deviation of the digestive organs, without self mutilation or septic complications. 7 cases with lesions located at jonctional site presented a survival rate under 30 days from the moment of diagnosis and only 2 cases overcame one year of survival. The cases with lesions on the torso showed a survival rate between 6 months and 3 years.

Another important epidemiologic aspect revealed in the study was the seasonal dynamics of the lesions. As a result, 10 cases (43.5%) were identified in spring, followed by 6 cases (26.1%) in automn, 5 cases (21.7%) in summer and in winter only 2 cases (8.7%). A high incidence in spring and automn is favored by thermal changes, photoperiod, atmospheric pressure changes, all of them reflected in metabolic and hormonal stress, to which parrots are vulnerable.

The cases submitted to anatomopathologic examination were represented by seven cases of *Melopsittacus undulatus* and one case, each, for *Nymphicus hollandicus, Agapornis roseicollis* and *Cacatua sulphurea*.
Microscopic examination revealed only one case of nontumoral lesion and the rest, tumoral lesions of different degrees and origins.
The nontumoral case was a *Nymphicus hollandicus* female with a lesion located at the uropigyal gland region. The area revealed a significant inflamation, with a diameter of aproximately 2 cm, coloured dark red, firm at palpation. The scientific data reveal cases of adenocarcinoma or squamous cell carcinoma, that evolve with swelling also and, as a result may be difficult cu differentiate from solely gland inflamation (Reavill D. R., 2004 and Samour J., 2008). In order to differentiate the two entities in the case studied, citologic examination was performed and, afterwards, coloration by May-Grümwald-Giemsa technique. Microscopic examination revealed the presence of a significant number of inflamatory cells, blood cells and cellular detritus. None atipic cells were identified, no atipic mitosis and nucleus-citoplasm ratio in normal limits. As a result, the citologic diagnosis was of cronic inflamation together with secretion impactation of the uropigyal gland. To support the diagnosis, at 10 days after the mechanical removal of the secretion, the inflamatory area retracted and the gland resumed normal function.
Regardind the tumoral lesions, 3 cases were identified as benign, all with mesenchymal origin. Pectoral lipomas were identified in two cases of Melopsittacus undulatus. Anamnesis recorded favoring factors as inappropriate feeding and limited or no daily physical training. Fine needle aspiration was performed at the edge of the pectoral deformation and a greasy material, hard to dry was noticed on the microscope slide. May-Grümwald-Giemsa was the stain used and citologic interpretation revealed a rare, uniform cellular population of adypocites, without inflamatory reaction. The cells appear as contour coloured vacuoles with a peripheric, small nucleus, pushed by the lipidic drop. Clinically, the size of these lesions remained constant or in little regression during 6 months of study, although the dietary and exercise deficiencies were corrected.
The third case of benign tumor was diagnosed at a specimen of *Cacatua sulphurea*. Anamnesis revealed two, parallel, round formations , with a diameter of 1 cm , located prepectorally, depicted by the owner in march 2011. In may, surgical excision of the left formation was performed with a rapid post-sugery recovery. During summer season, the right formation

presented daily size variations of up to 1-1.5 cm, enlarging at midday and regressing during night, sign of important vascular envolvement at this level. In september, a second surgery was performed for the right formation. Macroscopically, the tumor had a 2.5 cm diameter, well delimited from adjacent tissues due to a fibrous capsule and intense red colour. At sectioning, the formation expressed a quantity of 2 ml of intensely red liquid. Citology of this sample revealed a large cellular population composed of erythrocytes and groups of blood platelets which are of great significance in blood clotting. Among normal cellular forms, few bizzare erythrocytes were identified, such as schystocytes and acantocytes. These appear in local hemodynamic changes due to endothelial distruction. Another category, diagnosed as endothelial cells, were identified in the microscopic evaluation. These cells were characterized by poligonal shape, weak contour, ovalar nucleus, in groups of 2 or 3 cells, well differentiated without abnormal mitotic activity. After surgery, healing was conducted per primam, none relapse observed. The final diagnosis was of hemangyoma, based on anamnetic data, citologic examination and post-surgery recovery.

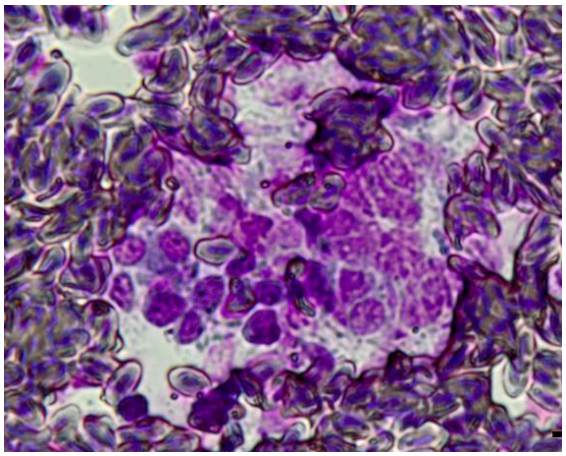
Of the 6 malignant lesions, 5 had mesenchymal origin and only one epithelial origin.

The epithelial neoplasm was diagnosed at a female of *Melopsittacus undulatus*, located on the left wing at the carpal joint. Initially, the lesion started as a plumage follicle inflamation and local irritation, evolved into a nodule with aproximately 5 mm diameter and, afterwards tripled in size in aproximately 2 weeks time. The ulceration area, presented a round shape, without any cicatrization tendancy. A citologic sample was obtained by fine-needle aspiration technique and stained M.G.G-Quick. The microscopic evaluation revealed cellular groups, scattered all around the sample, characterized by a very pale citoplasm, a high nucleus-citoplasm ratio, relative anisocariosis and nuclear hiperchromasia. The citologic aspects conducted to a suspicion diagnosis of basal cell carcinoma. Self mutilation lead to intense haemorrhage and death of the bird only a week after diagnosis.

Of mesenchymal origin neoplasms, one case presented unusual location of the lesion, the muscular region of the right leg of a budgerigar. The formation deformed roundly the whole region, was firm at palpation, dark red in colour with self mutilation crusts. Citologic examination of cellular aspirate revealed an important population of erythrocytes together with groups of large, ovoido-spherical cells, with large nucleus, multiple mitosis and cellular anisocytosis, as seen in Figure 1. The diagnosis for the case was

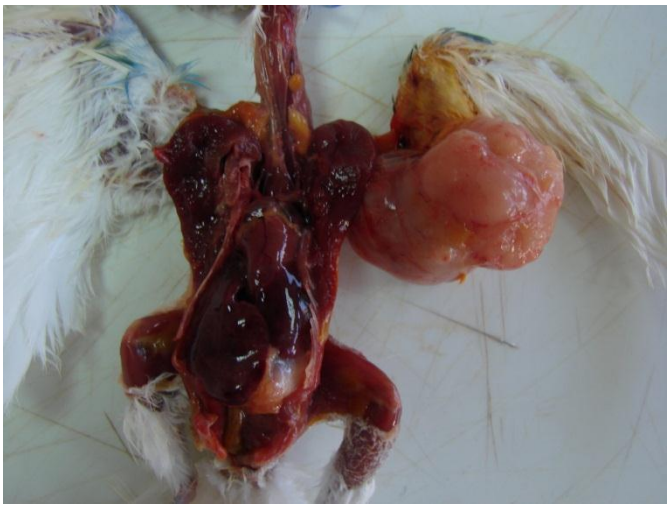
of histiocytic sarcoma.

Figure 1. *Melopsittacus undulatus*. Histiocytic sarcoma. Group of modified histyocites surrounded by a large population of erythrocytes (M.G.G. stain, 40x)



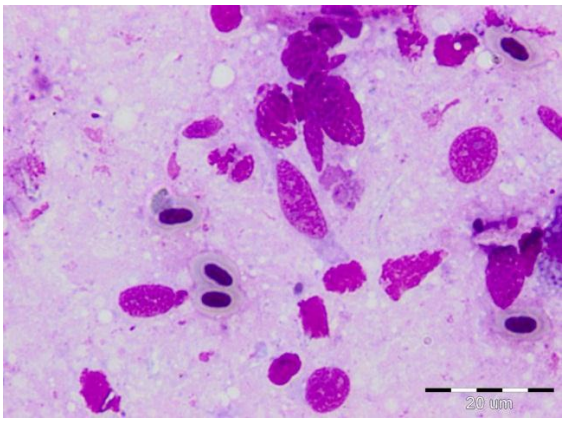
The survival rate, in this case, was of 1 month after examination.
The other 4 mesenchymal neoplasms examined in this study were represented by fibrosarcomas. Three cases were reported in *Melopsittacus undulatus,* out of which two in the wing region and one case in the caelomic cavity. One case, was diagnosed at a specimen of *Agapornis roseicollis,* located at the carpal joint. The lesions identified at the budgerigars were submitted to citologic, necropsic and histopathologic examination and for the Agapornis parrot a citologic examination was performed. The citologic examination revealed, in all four cases, a population of large, basophilic, spindle cells with large, round nuclei and obvious nucleoli. The mitotic activity was present in different degrees, same as the presence of bizzare cellular forms. The necropsies for the two cases of wing lesions revealed an obvious deformation of both muscular and bone structures at the site of the neoplasm as seen in Figure 2.

Figure 2. *Melopsittacus undulatus*. Macroscopic aspects of a fibrosarcoma located on the left wing



The emaciation of the pectoral muscles observed in these cases, together with the presence of seed content in the digestive tract was attributed to paraneoplasic syndrome of protein loss. Histopathologic examination of the tumor samples confirmed the diagnosis of fibrosarcoma, bizzare fibroblastic cells displayed in the internal derm layer, with a typical herringbone pattern. The examination of organ samples did not reveal any metastasis in those cases. The case of internal fibrosarcoma was submitted to necropsy after sudden death, in order to perform a differential diagnosis between egg binding, colibacilary granuloma and a neoplastic formation. After caelomic cavity opening, a spheroidal formation was depicted between the intestinal loops, logged to the mesentery, compressing regional organs. The tumor had a 2 cm diameter, external yellow colour and, after sectioning the internal structure, presented a compact aspect and whitish-grey colour. The citologic examination was performed as impression smears and the microscopic characteristics revealed atypical forms of fibroblastic cells, multiple mitosis, anysocytosis and anysocariosis.(Figure 3)

Figure 3. *Melopsittacus undulatus*. Abdominal fibrosarcoma. Pleomorphic spindle cells, rare erythrocytes (M.G.G. stain, 100x)



The cases of fibrosarcoma diagnosed at budgerigars had a survival rate of up to 2 months, while the lesion identified at the Agapornis presented a survival rate of over 6 months.

CONCLUSIONS

The most frequent location for tumoral lesions in common caged parrots are the ventral torso and the wing region.
Young age, up to 4 years old, involved most of the cases with tumors.
Seasonal change is envolved in tumoral development and clinical signs of disconfort.
Superficial neoplasies, located at anatomical junctions, are prone to bleeding or septic complications and a low survival rate.
Most cases diagnosed had mesenchymal origin out of which the most frequent type was the fibrosarcoma.

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CLINICAL AND MORPHOPATHOLOGICAL ASPECTS IN ANTI-FREEZE INTOXICATION OF DOGS

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Abstract

Anti-freeze intoxication is most frequently encountered in dogs and cats after accidental consumption of the liquid emptied from car radiators. In ruminants, the intoxication can appear as a consequence of erratic contamination of grazing fields with the liquid from tractor tires. Other cases have been reported, due to erronate treatments applied to silage, when ethilenglicole is mistaken taken for formic acid, or after contaminated water consumption. Ethylen glycol is oxidized by alcohol dehydrogenase in the liver to glycoaldehide, wich is in turn oxidized to glycolic acid, glyoxalate, and finally, oxalate. Calcium oxalates crystals may be found in tubular lamina, tubular cells and the interstitium; they are light yellow, arranged in rosettes or prisms, and are birefringent in polarized light. Tubular lesions range from fat degeneration to necrosis. Large numbers of crystals in tubules are pathognomonic for ethylene glycol poisoning.

Key words: *anti-freeze, dog, intoxication.*

INTRODUCTION

Antifreeze poisoning is most commonly observed in pet carnivores (dogs, cats). It is a toxicosis with nonspecific clinical symptoms, acute evolving as digestive disorders, cardio-respiratory and nervous and subacute form by nephrotoxic syndrome and renal failure.

Species frequently exposed are dog and cat, but intoxication was reported in cattle, dwarf goats and poultry (Solcan, 2001; Jubb et al., 2007).

Antifreeze is a syrupy, sweet liquid containing 95% ethylene glycol.

Poisonings are more common in autumn and spring, coinciding with the period of handling antifreeze for winter maintenance vehicles (Jubb et al, 2007).

Poisoning is more frequent in dogs than in cats, but the latter is more sensitive (Goicoa et al., 2003).

Following ingestion, the toxic is rapidly absorbed from the gastrointestinal tract and metabolized in the liver, where the action of alcohol

dehydrogenase and liver oxidase will turn it into oxalic acid (Paul, 2000). Intermediate compounds of metabolism: aldehyde glycol, glycolic acid and oxalic acid have neurotoxic and nephrotoxic action (Solcan, 2001).
Clinically, intoxication develops two forms: acute and subacute.
Acute form onset 30 'to 12h, manifested by nervous disorders, digestive and cardiovascular (5).
Acute form begins to 2^{nd}-7^{th} days with nephrotoxic syndrome and renal failure.
The accurate diagnosis consist in corroboration of toxicological, clinical and histopathological dates, the latter giving the most important data for diagnosis(Jubb et al, 2007).
Specific antidote is ethanol, which competes with ethylene glycol in using alcohol dehydrogenase. The enzyme has a higher affinity for ethanol than for ethylene, the latter being eliminated unchanged (Popescu and Enache, 1996; Solcan, 2001).

MATERIALS AND METHODS

Clinical and pathological investigations were performed on 6 dogs brought to the Faculty of Veterinary Medecine Iasi. The dogs were treated in the Internal Medecine and Toxicology Units; morphopathological investigations were performed in the Pathology Unit.
After necropsic examination, organ samples for histopathological investigations. Each case was prelevated kidney fragments, as well as fragments of different organs, physiologically closely related (heart, brain, liver, lung, stomach, intestine, spleen, etc.) were prelevated.
Organ samples were fixed in formaldehyde 10%, then paraffin - imbeded. The histological sections of 5 μm were stained Haematoxilin - Eosin - Methyl Blue (Tricromic - Masson) and Haematoxilin - Eosin.

RESULTS AND DISSCUTIONS

On clinical examination, the patients developed progressive nervous disorders, consisting in agitation, walking drunk, then progressive cortical depression, which occur periodically due to seizures or epileptiform manifestations type, digestive disorders (vomiting and diarrhea), signs of toxic shock (trend to hypothermia, tachycardia, cardiac arrhythmias or rhythmical heart, weak pulse, tachypnea, cyanosis mucosal and acute pulmonary congestion).

Subsequently, acute renal failure was installed with oliguria and anuria, and at biochemical examination of the blood was found hypercreatininemia (above 8 mg / dl) and increased uremia (above 300, reaching even 800 mg / dl). In this phase signs of uremic gastroenteritis (bloody vomiting, diarrhea) and secondary nervous disorders (muscle tremors, seizures and coma) were observed.
Ultrasound examination of the kidneys showed a diffuse hyperechogenic cortical with small shadow cones, suggestive for nephrocalcinosis (Figure 1). Ultrasound examination of the stomach revealed a secondary uremic gastritis (Figure 2).

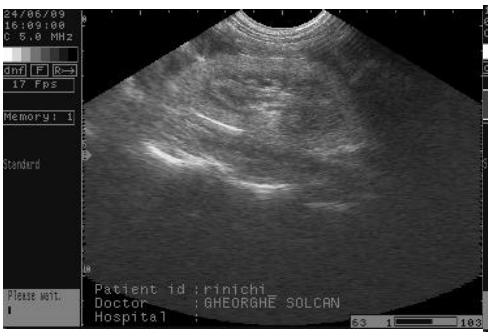

Figure 1. Diffuse renal calcinosis. Hyperechogenic cortical and medullar.

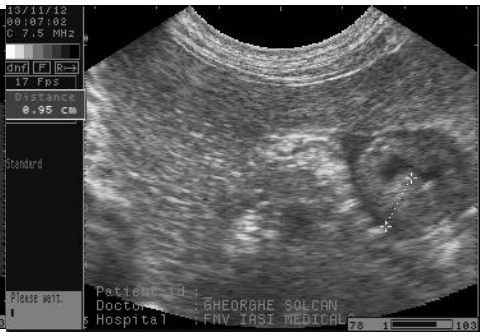

Figure 2. Secondary uremic gastritis. Thick pylorus.

Death occurred within the first 12-36 hours in most cases, due to nervous depression or convulsions, (2 from 8 were euthanized), and 2 cases 4-5 days later due to acute renal failure.
Necropsy. After the death of patients, necropsy was performed, stating the gross lesions observed.
The kidneys were pale, globular, wrinkled surface and showed discrete cortical petechiae (Figure 3).
Heart was distended with a discolored and soft myocardium and the left ventricle was very dilated (Figure 4, Figure 5).

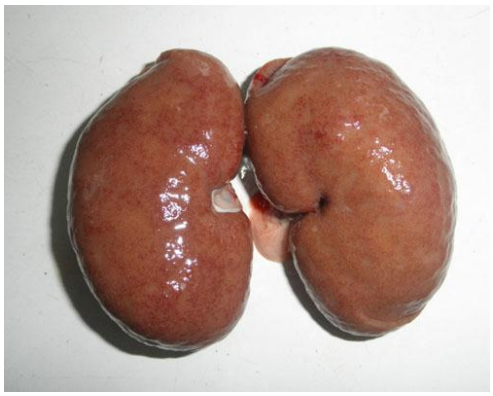

Figure 3. Dog. Discolored and wavy kidney.
Antifreeze poisoning.

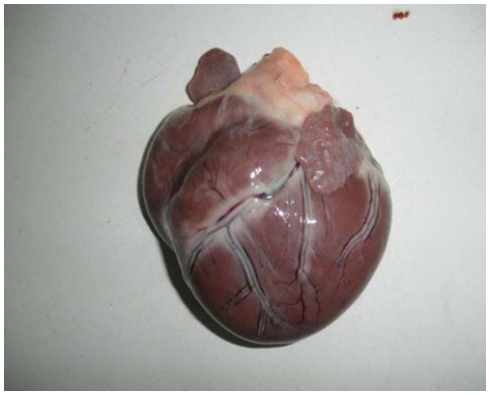

Figure 4. Dog. Discolored and soft heart.
Antifreeze poisoning.

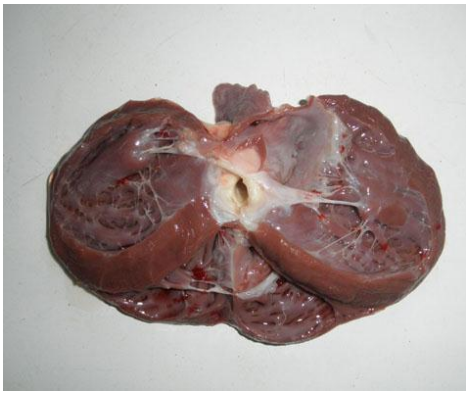

Figure 5. Dog. Left ventricle distension.
Antifreeze poisoning.

Lungs were expanded, pale or slightly reddish. They expressed on the section surface an aerated sparkling reddish liquid, also observed in the lumen of the trachea and the main bronchi (Figure 6, Figure 7).
Constantly in our cases, the spleen was enlarged in volume and weight, red and blackish, asphyxic blood being observed on the surface of section (Figure 8).
The gastric wall was much thickened with accented pleats, a lot of mucus and small hemorrhages on the mucosa. Stomach content was fluid, looking like "coffee grounds" (Figure 9).
In the duodenum were observed macroscopic changes which corresponded to a severe diffuse hemorrhagic inflammation (Figure 10).

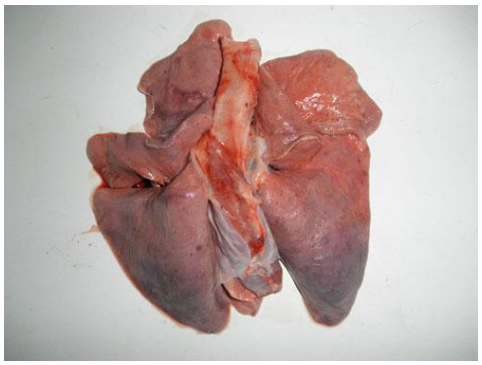

Figure 6. Dog. Pulmonary edema. Antifreeze poisoning.

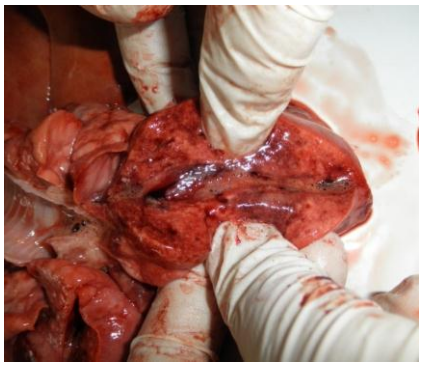

Figure 7. Dog. Pulmonary congestion and edema. Congestie și edem pulmonar. Antifreeze poisoning.

Figure 8. Dog. Spleen stasis. Antifreeze poisoning.

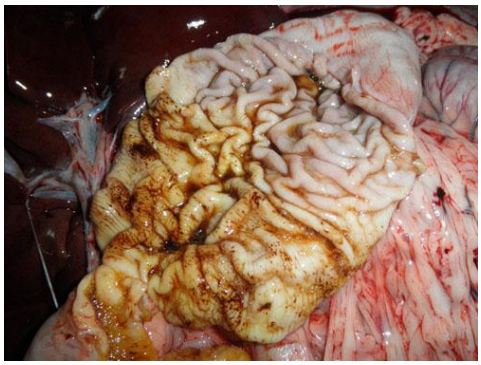

Figure 9. Dog. Focalised hemorrhagic gastritis. Antifreeze poisoning.

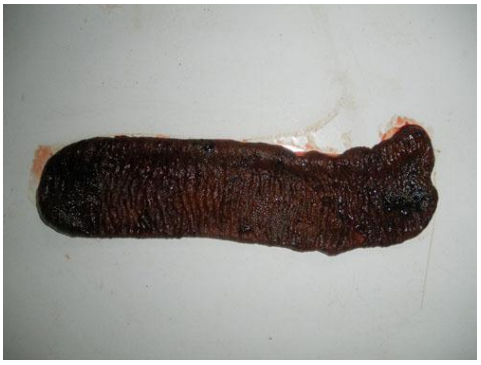

Figure 10. Dog. Hemorrhagic duodenitis. Antifreeze poisoning.

On histopathology, the lesions observed was located kidney, heart, lung, digestive tract and nervous, were characteristic for antifreeze poisoning.
Histopathological examination of the kidneys established the cause of dogs death.
In all cases were noted severe tubular degenerative lesions. Lipid and granular dystrophies, as well as hyaline cylinders were observed in tubular epithelium.
The presence of calcium oxalate crystals induced necrosis of tubular epithelium and its detachment from the basal membrane and also lymphohystiocytare and fibrous proliferation.
In convoluted renal tubules were identified radiar, yellowish calcium oxalate crystal deposits (Figure 11, Figure 12, Figure 13). The nephrocytes showed cloudy/granular cytoplasm and pyknotic nuclei.
On histological exam, the hearts showed a severe granular dystrophy, and in 2 cases subepicardic edema was observed(Figure 14, Figure 15).
Histologically, circulatory lungs disorders were represented by congestion and edema. The interstitial capillary were dilatated and filled with blood. Also, transsudat and hemosiderocytes in alveolar spaces were observed(Figure 16, Figure 17). In subacute form of posoning pulmonary emphysema was observed.
Hemorrhagic and catarrhal-haemorrhagic inflammation was observed in stomach and duodenum (Figure 18, Figure 19).
Cerebral edema was observed consistently in the cases, pointing out also the presence of calcium oxalate crystals in meningeal vessels (Figure 20, Figure 21).
Microscopically, the splenic sinuses were represented by uniformly colored lakes filled with red blood cells, rare lymphocytes and the capsule and trabecules were thickened. Hemosiderocytes were seen in large numbers (Figure 22).
In the liver, the overload of centrolobular vein and sinusoid capillaries was observed (Figure 23).

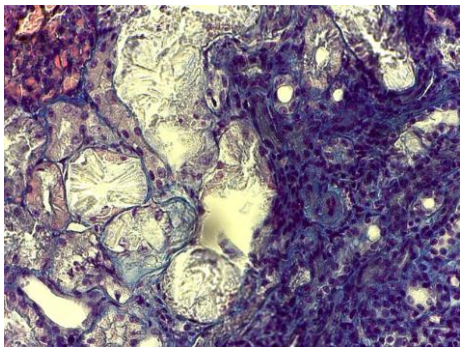

Figure 11. Dog. Oxalic nephrosis. Antifreeze poisoning. Col. HEA, x1000;

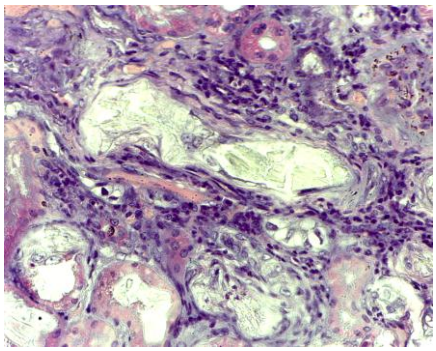

Figure 12. Dog. Tubular epithelium degeneration. Lymphohistiocytic interstitial inflammation. Col. HEA, x1000;

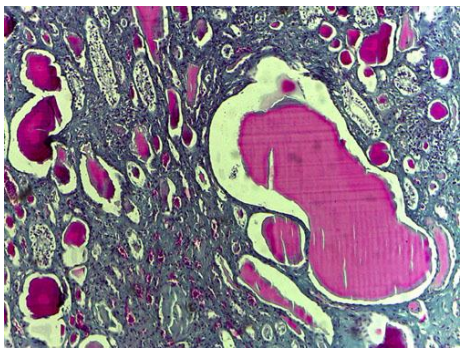

Figure 13. Dog. Hyaline cylinders. Kidney. Col. HEA, x200;

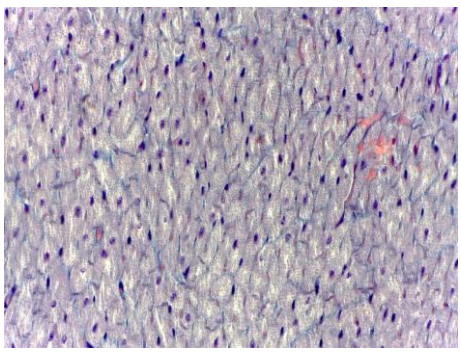

Figure 14. Dog. Granular myocardosis.Col. HEA, x200;

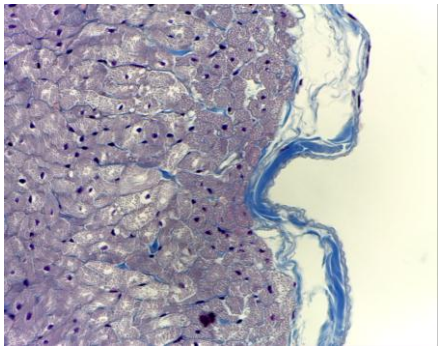

Figure 15. Dog. Subepicardic edema. Antifreeze poisoning. Col. HEA, x200;

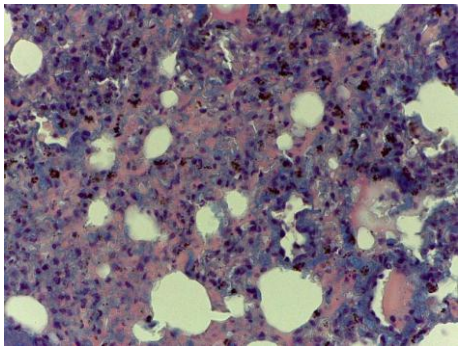

Figure 16. Dog. Pulmonary congestion and edema. Antifreeze poisoning. Col. HEA, x400;

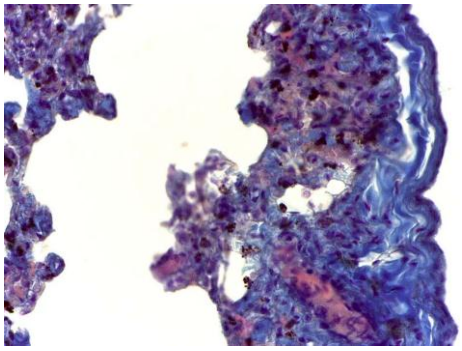

Figure 17. Dog. Pulmonary emphysema. Antifreeze poisoning.

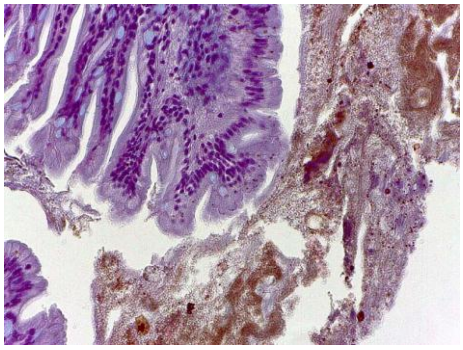

Figure 18. Dog. Haemorrhagic gastritis. Antifreeze poisoning. Col. HEA, x400;

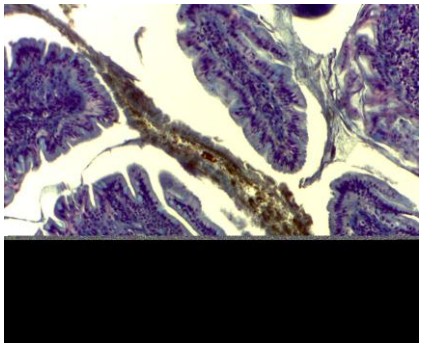

Figure 19. Dog. Catarrhal-haemorrhagic duodenitis. Antifreeze poisoning. Col. HEA, x200;

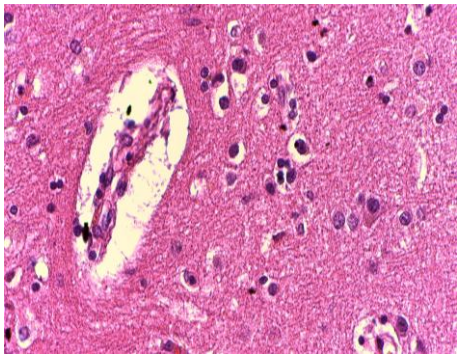

Figure 20. Dog. Cerebral acute edema. Virchow-Robin spaces dilatated. Antifreeze poisoning. Col. HE, x1000

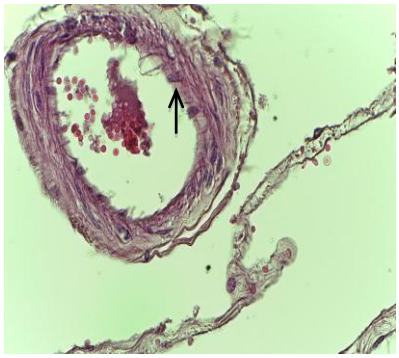

Figure 21. Dog. Cerebral acute edema. Oxalate cristals in meningeal artery. Antifreeze poisoning. Col. HE, x1000

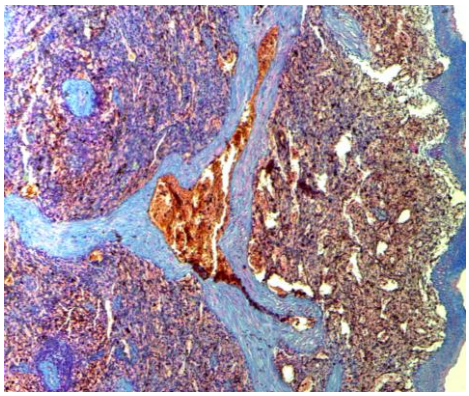

Figure 22. Dog. Spleen stasis. Antifreeze poisoning. Col. HEA, x200;

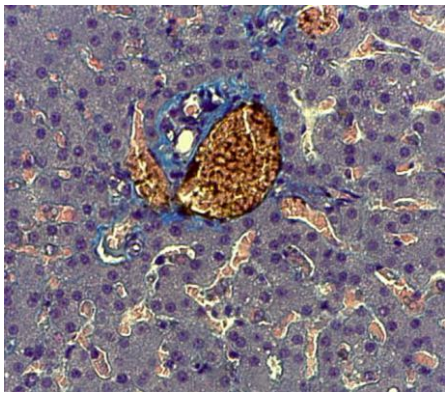

Figure 23. Dog. Liver stasis. Antifreeze poisoning. Col. HEA, x400;

CONCLUSIONS

Clinical signs in all investigated cases consisted in nervous signs (restlessness, walking drunk, progressive cortical depression, seizures or epileptiform), digestive disorders (vomiting and diarrhea), toxic shock signs (hypothermia, tachycardia, cardiac arrhythmias, weak pulse, tachypnea, cyanosis mucosal and acute pulmonary congestion) and acute renal failure.
Necropsy revealed pulmonary and splenic congestion lesions and severe dystrophic and inflammatory lesions of the digestive tract and kidneys.
Microscopic examination revealed hemorrhagic gastritis specific in uraemic poisoning outbreaks and severe degenerative kidney damage induced by the presence of calcium oxalate crystals.
The presence of a large number of calcium oxalate crystals in uriniferous tubules confirmed ethylene glycol poisoning.

AKNOWLEDGEMENTS

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TROMBICULIDAE HARVEST MITES (*NEOTROMBICULA AUTUMNALIS*) INFESTATION IN DOG IN WINTER SEASON – A CASE REPORT

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Abstract

Neotrombicula autumnalis *is a mite belonging to* Trombiculidae *family, which lives free on soil and vegetation, and in larval stages becomes an ectoparasite, attacking wild and domestic animals and humans. The harvest mites are responsible for producing cutaneous pruriginous lesions in infested hosts. This study presents a trombidiosis case during winter season, in a 5 year old mongrel dog from the Eastern part of Romania. The dog presented a papulo-erythematous dermatitis, pruriginous, localized in interdigital spaces of posterior limbs area. Diagnosis has been established by microscopic identification of* N. autumnalis *parasitic larval stage from skin scraped material. To our knowledge this is the first report of trombidiosis in dog from the Eastern part of Romania, with evolution during winter season.*

Key words: *dog, harvest mites, Romania, winter*

INTRODUCTION

Trombidiosis represents a parasitic cutaneous affection produced by acarian larvae which belong to *Trombiculidae* Family, *Acari* Order, with over 1500 known species worldwide, of which 50 are known to attack domestic animals and humans (Wall and Shearer, 2001).
In Europe, trombiculidae presence has been spotted along the continent (Kampen, 2000), (*Neo-)Trombicula autumnalis* (Shaw 1790) being considered the widest spread species (Schöler et al., 2005). In Romania, trombiculiasis larvae infestation in animals is little known. Nesterov (1984) quoted by Olteanu (2001), reported *Trombicula* infestation in rabbits (*Lepus europaeus* and *Oryctolagus cuniculus*), and Mircean et al. (2008) described a trombidiosis outbreak produced by *N. autumnalis* harvest mites in a household from Cluj district.
The acarian prefers warm biotopes, with damp soils, but well drained, where animals that could be hosts for the larva are found (Cosoroabă, 2005). Adults and nymphs live free in soil and plants, feeding on plant fluids, eggs

and other arthropods larvae. Only larval stages parasite the animals. Usual hosts are represented by small wild vertebrates (rodents), domestic animals, pets and humans being accidental hosts. In Europe, active life of *N. autumnalis* manifests at the end of summer and in autumn, with an intense activity in dry and sunny days (Wall and Shearer, 2001). This report presents a case of *N. autumnalis* harvest mites infestation in a dog during winter season.

MATERIALS AND METHODS

On the 18th of December 2012 inside the Clinic of Veterinary Medicine Faculty Bucharest, a 5 year old mongrel dog has been presented for evaluation, with multiple and extended old cutaneous lesions, in lumbar and thoracic area. Afterwards, a neoplastic cutaneous process was found. This modification wasn't the purpose of this study, but the lesions found 4-5 days ago, with pruritic character, papulo-erythematous, from posterior limbs extremities. Despite the fact that the animal presented those old cutaneous lesions, its general status was good. From patient's history, it was revealed that the owners lived in a household, in Constanţa district (44°10'24"N, 28°38'18"E), being the only animal there.
Skin scrapings were taken from limbs and interdigital level, and spread on a slide. Lactophenol was added for clarification and then examined on a microscope. Microscopic examination revealed the presence of parasites. Morphologic identification was carried out based on Cosoroabă (1994) descriptions.

RESULTS AND DISCUSSIONS

Microscopic examination revealed mite larvae presence, orange coloured, with smooth hairs across the body (Fig. 1), with 3 pairs of long legs, segmented, which end with claws (Fig. 2). The oral apparatus presented a long hypostome, two chelicerae which end with a reap shaped claw and two pedipalps, segmented. The palpal claw is three-pronged, morphological character which separates the genus (Wall and Shearer, 2001). Based on these characters, *N. autumnalis* larvae were identified. To our knowledge this is the first report of trombidiosis in dog from the Eastern part of Romania, with evolution during winter season.

Cutaneous modifications identified in our case, represented by papulo-erythematous areas, accompanied by pruritus, fit in the lesion scheme described in previous studies (Small et al., 2004; Kavitha et al., 2011).

Fig 1 –*Neotrombicula autumnalis* harvest mites, (x10)

Fig. 2 – *N. autumnalis* - the long legs, segmented, covered with smooth hair and ending with claws (x20)

Regarding lesion distribution, our results are similar to those obtained by Nuttal et al. (1998) that found lesions on interdigital skin level in two dogs (out of 18), but also in ear level, ventral side of the body an ventral side of the tail. Also, Mircean et al. (2008) signaled frontal-parietal lesion presence and auricular pavilions edge. Distribution of identified lesions identified by us corresponded with vegetation contact areas. Generally, larvae attach to hosts at ventral side body level, where skin is smoother and easier to penetrate (Jones, 1950). Limbs and especially interdigital spaces represent such preferred areas by mite's larvae.

While attaching to the host, larvae stick their chelicerae in superficial layers of the skin (mechanical action) and inoculate saliva rich in proteolythic enzymes (irritative action), which explains pruriginous character of lesions. Saliva enzymes digest host tissues, and the result is absorbed by the larvae through formed stylostome (feeding tube) (Jones, 1950). Stylostome feeding process, common to trombiculidae larvae, was surprised in histological sections reported in previous studies (Cunningham et al., 2001).

N. autumnalis harvest mites infestation is less known in our country, compared with other geographic regions worldwide, where it is found frequently, affecting both domestic and wild animals (Nuttall et al., 1998; Cunningham et al., 2001). In warm countries, trombiculides activity is manifested throughout the year, while in tempered areas it becomes active in warm seasons, with larvae appearing at the end of the summer and autumn (Cosoroabă, 2005). In Europe, previous studies have identified *N. autumnalis* harvest mites infestation in animals in autumn (Nutall et al., 1998; Cornegliani and Cavazzini, 1999; Mircean et al., 2008; Martiolle et al., 2011), from which the acarian's name is derived. In England, White (2001) reported an unusual *N. autumnalis* harvest mites infestation case, in a cat in January. Unlike previous signaled cases, our case was diagnosed in the second half of December, winter season. Larvae presence in December can be determined by high temperature situated above the climate averages of the season, from the respective area. It is possible for climate changes to influence dynamics and seasonal activity of the mites appearing also in winter months. In a second hand, evolution of biologic cycle and the fact that harvest mites stick to the host for only 3-7 days for feeding (Cosoroabă, 1994) and then falling on vegetation in order to continue their development, sustains the idea that the infestation was carried out in December.

In the past, trombiculiasis infection was attributed to rural areas, which were considered preferred biotopes for the mites due to rodent's presence, main

hosts of mites. Lately, their presence was signaled in urban areas also, this aspect being attributed to small vertebrate's presence (rodents) (Schöler et al., 2006). Our identified case came from a household area inside Constanţa city, where rodent's presence was signaled by owners, being in consistence with previous alerts.

CONCLUSIONS

Microscopic examination of scraped material obtained from a dog with pruriginous dermatitis allowed us to identify *N. autumnalis* harvest mites.
Papulo-erythematous lesions, accompanied by pruritus, had a localized distribution, including limb extremities and, especially, interdigital skin.
The study shown here has revealed *N. autumnalis* harvest mites infestation presence in winter season.

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GENERAL CONSIDERATIONS ACCORDING TO PITUITARY VERSUS PLACENTAL GONADOTROPHINS ACTIVITIES IN BITCH

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Abstract

The reproductive cycle of the domestic bitch, a mono-estrous species, is characterized by a follicular phase with spontaneous ovulations, followed by a luteal phase of about 75 days, and a non-seasonal anestrous of 2-10 months. The reproductive cycle is under control of the hypothalamic-pituitary-ovarian axis.

The ovarian hormones exert a feedback at the hypothalamic-pituitary axis, thereby also influencing, in a differential way, the secretion of LH and FSH.

Key words: bitch, pituitary gonadotrophins, placental gonadotrophins

MATERIALS AND METHODS

The literature reported that each FSH pulse occurs concurrently with a LH pulse, differential regulation of FSH and LH. The frequency and amplitude of the pulses of the hypothalamic peptide gonadotrophin-releasing hormone (GnRH) can only partly explain this differential regulation.

The specific hypo-thalamic FSH-releasing factor may also play a role of our investigations.

RESULTS AND DISSCUTION

The ovarian hormones are inducing a feedback at the hypothalamic-pituitary axis, thereby also influencing, in a differential way, the secretion of LH and FSH (Patrick, 2011; De Gier, 2006).

In addition to its role in transporting molecules between mother and fetus, the placenta is a major endocrine organ. It turns out that the placenta synthesizes a huge and diverse number of hormones and cytokines that have major influences on ovarian, uterine, mammary and fetal physiology, not to mention other endocrine systems of the mother (Schaefers-Okkens et al, 2005).

Several protein and peptide hormones are synthesized in placentae of various species. They have effects on the mother's endocrine system, fetal

metabolism and preparation of the mother for postpartum support of her offspring.

In addition to exogenous pituitary gonadotrophins, pregnant mare serum gonadotrophin (PMSG) and human menopausal gonadotrophin (HMG) have been used for estrus induction in bitches.

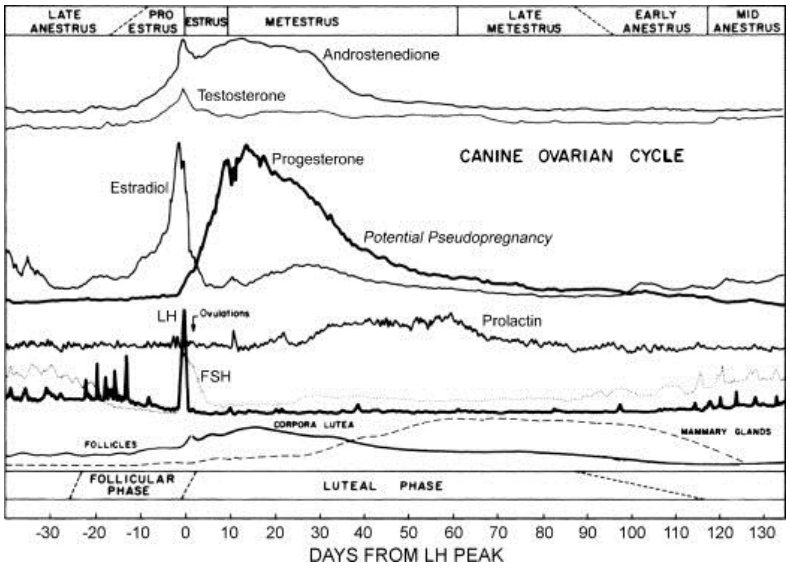
The most widely studied gonadotrophin for estrus induction in the dog is PMSG, with protocols ranging from daily to weekly injections using either subcutaneous or intramuscular routes of administration

As the name implies, chorionic gonadotrophins have the effect of stimulating the gonads, similar to the pituitary gonadotrophins. The only species known to produce a placental gonadotrophin are primates and equids.

The human hormone is called human chorionic gonadotrophin or simply HCG. This hormone is produced by fetal trophoblast cells. It binds to the luteinizing hormone receptor on cells of the corpus luteum, which prevents luteal regression.

Thus, HCG serves as the signal for maternal recognition of pregnancy. Equine chorionic gonadotrophin is also produced by fetal trophoblast cells. It is actually the same molecule as equine luteinizing hormone.

Chart 1. Schematic of typical changes in concentrations of reproductive hormones in the estrus cycle of the domestic dog



The most widely studied gonadotrophin for estrus induction in the dog is PMSG, with protocols ranging from daily to weekly injections using either subcutaneous or intramuscular routes of administration. Studies using PMSG (PG600®) have generally been more successful for estrus induction in bitches than those using FSH.

This product contains 80 IU PMSG and 40 IU HCG per ml. It was demonstrated that a single 5-ml injection of PG600® was highly effective at inducing proestrus in bitches. Unfortunately, the ovulation rate was poor (8 of 19), superovulation may have occurred and pregnancy rates were not reported. However, others have reported 50-84% whelping rates when PMSG and HCG are given in combination to induce estrus in bitches (Kooistra, 1999).

Histologically, luteal cells from *corpora lutea* formed in bitches following PMSG treatment have reticulated and vacuolated cytoplasm compared to luteal cells from corpora lutea of normal, non-fertile estrous cycles that have compact and granulated cytoplasm.

Administration of an ovulation induction agent in bitches as part of an estrus induction protocol is controversial since bitches are spontaneous ovulators and such a treatment would be unnecessary (Senovilla et al, 2005).

Administration of HCG has no positive effects on ovulation rates, pregnancy rates or number of offspring per pregnancy when administered at the onset of or during estrus.

In fact, treatment with HCG on the 1st and 3rd days of estrus significantly prolongs behavioral estrus and lowers serum progesterone concentration of day 5 of estrus.

(Shacham et al, 2001) found similar results when HCG was administered to bitches after day 40 of gestation; in that following an initial increase in serum progesterone concentrations, HCG dramatically suppressed progesterone secretion

CONCLUSIONS

Placental gonadotrophin administrations have been used for estrus induction in bitches.

Gonadotrophin administrations have varied in source, dosage, and bio-potency, as well as in pattern and frequency of administration.

PMSG administration at doses of 20 I.U.kg/day for 10 days, often causes hypersecretion of estrogen, with potential inducing uterine altered-function and/or uterine disease.

Improved pregnancy rates occurred when PMSG was administered for only 5 days and immediately followed by HCG administration (as a proestrus-enhancing) that apparently further stimulates ovarian follicle development such that the induced proestrus progresses and spontaneously culminates in an estrus in which ovulation occurs spontaneously.

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STUDIES ON THE THERAPY WITH GONADOTROPIN-RELEASING HORMONE (GnRH) AND HUMAN CHORIONIC GONADOTROPIN (hCG) IN GENITAL DISORDERS IN BITCHES

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Abstract

Infertility in bitch is characterized by a variety of clinical manifestations depending on the disorders of reproductive system. The purpose of this study was to asses the efficacy of gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) in the treatment of prolonged estrus caused by follicular cysts in bitch.
In this study, there were diagnosed 10 bitches with prolonged estrus having as etiology the presence of follicular cysts. The intensity of clinical signs was studied and vaginal smears were performed, resulting in high percentages of keratinized cells in bitches with follicular cysts. Determinations of estrogen and progesterone hormones were made, the presence of follicular cysts being suspected based on the high values of estrogen and duration of estrus. Duration of estrus signs ranged between 32-76 days, progesterone concentration was between 1.3-3.4 ng/ml, while the concentration of estrogen hormones ranged between 141.5-379.5 pg/ml. Based on the high values of estrogen hormones, the presence of keratinized cells, the duration of estrus and the age of bitch it was suspected the existence of follicular cysts. The efficacy of hormonal treatment (GnRH and/or hCG) was monitored by determining the values of estrogen and progesterone hormones, the results being correlated with the interpretation of vaginal smears and clinical manifestations. Following hormonal treatments, in 7 bitches clinical signs of estrus disappeared in 4-6 days, while 3 bitches still presented the clinical manifestations characteristic to estrus phase after the treatment.

Key words: *bitches, follicular cysts, gonadotropin-releasing hormone, human chorionic gonadotropin*.

INTRODUCTION

Perpetuation of the species and breed, even perpetuation of valuable qualities of certain individuals within the breed involves, beyond genetic selection, healthy females, especially from the reproductive point of view (Cernescu, 1995; Bîrţoiu and Seiciu, 2004).
With the increase in both number and importance of pet carnivores, multiple reproductive problems began to appear. The occurrence of infertility states in bitches can be determined by many complex etiological factors (Feldman and Nelson, 1996; Davol, 2002).

In addition to lesional changes, an important aspect of the incidence of reproductive disorders is the functional modifications, affecting the hormonal status, with implication in the reproductive sphere (Guerin et al., 1996).
By the researches conducted in this area, we intended to improve reproduction management by identifying and solving certain problems through treatments with gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) in prolonged estrus caused by follicular cysts in bitch (Vanderlip, 1987).

MATERIALS AND METHODS

In this study were diagnosed 10 bitches with prolonged estrus determined by the presence of follicular cysts. During the study, it was observed the intensity of clinical manifestations and vaginal smears were performed to determine the percentage of keratinized cells; at the same time, there were made determinations of the estrogens and progesterone hormones, and based on their values and duration of estrus the presence of follicular cysts was suspected.
Anamnesis was the first method used, supplemented by gynecological sheet. Based on history, data were obtained on previous estrous cycles and previous genital disorders.
Clinical examination included a general examination aimed to assess body temperature, condition of the skin and mucosa, circulatory, respiratory and digestive functions, after which a thorough genital examination was conducted.
Cyto-vaginal smear allowed the global assessment of cells number, form, presence or absence of leukocytes and erythrocytes, the grouping of cells (scattered, isolated or grouped in colonies), presence or absence of mucus and tinctorial affinity of the cell cytoplasm.
Hormonal determinations consisted in the determination of serum progesterone and estrogens, specific kits being used.
Based on ultrasound investigations in correlation with the results of anamnesis, clinical examination, vaginal smears and hormonal determinations, it was suspected the presence of ovarian cysts.

RESULTS AND DISCUSSIONS

In the present study, the causes of prolonged estrus were represented by follicular cysts. In bitches with follicular cysts, clinical signs had a high intensity, especially exacerbated libido.

Duration of estrous signs ranged between 32 and 76 days. Although females age was taken into account, it wasn't established a significant prevalence of cases depending on this feature.

Vaginal smears were performed in all bitches diagnosed with prolonged estrus; the percentage of keratinized cells was very high, ranging from 82-92%.

Bitches with prolonged estrus showed a blood concentration of progesterone ranging between 1.3 and 3.4 ng / ml, while estrogens concentrations ranged between 141.5 and 379.5 pg / ml (Table 1).

Table 1. Intensity of clinical manifestations and results of hormonal determinations in bitches diagnosed with prolonged estrus

No	Breed	Age (years	Clinical signs			Level of estro-gens (pg/ml)	Level of progeste-rone (ng/ml)	Diagnosis
			Vaginal bleeding	Exacerbation of libido	Acceptance of mating			
1	Bichon	4	+++++	++	++++	243.7	2.8	Follicular cysts
2	Irish setter	4	+++	+++	++++	156.2	3.4	Follicular cysts
3	Dalmatian	7.5	++++	++	++++	379.5	2.1	Follicular cysts
4	German Shepherd	4	++++	+++++	+++	291.3	3.0	Follicular cysts
5	Boxer	2	++++	+++++	+++	233.5	2.6	Follicular cysts
6	Collie	3.5	+++	+++	+++++	141.5	2.1	Follicular cysts
7	Doberman	6	+++	+++++	++++	205.2	2.6	Follicular cysts
8	Caniche	5	++++	++++	+++++	188.2	2.1	Follicular cysts
9	German Shepherd	3	+++	+++	++++	208.9	2.7	Follicular cysts
10	Cocker Spaniel	3	++++	+++	++++	167.3	1.3	Follicular cysts

For the treatment of follicular cysts it was administered gonadotropin-releasing hormone (GnRH) (Fertagyl – vials of 5 ml, 100 μg/ml) or human chorionic gonadotropin (hCG) (Chorulon – vials of 500 I.U.), in order to stimulate the release of pituitary hormones, respectively to induce luteinization or involution of follicular cysts, following physiological model.

In case of Chorulon (hCG), the administered dose was 22 I.U. / kg, while for Fertagyl (Gn-RH), the dose was 50 μg / animal. The number of administrations varied between 2 and 5, every 2 days.

The efficacy of hormonal preparations was monitored by determining the values of estrogens and progesterone hormones. Hormonal determinations were performed at 2 and 6 days after the last administration. The results were correlated with the interpretation of vaginal smears and clinical manifestations (Table 2).

Table 2. Therapeutic protocols and the results of hormonal determinations

No.	**Breed**	**Age (years)**	**Therapeutic protocol**	**Number of adm.**	**Estrogens after the treatment (pg/ml)**		**Progesterone after the treatment (ng/ml)**	
					2nd day	**6th day**	**2nd day**	**6th day**
1	Bichon	4	GnRH, 50 μg/animal (Fertagyl)	4	142.3	32.3	1.1	0.7
2	Irish setter	4	hCG, 22 UI/kg (Chorulon)	3	121.2	12.2	1.4	11.9
3	Dalmatian	7.5	hCG, 22 UI/kg (Chorulon)	4	376.2	365.2	2.0	2.1
4	German Shepherd	4	hCG, 22 UI/kg (Chorulon)	2	200.2	22.2	1.9	10.5
5	Boxer	2	GnRH 50 μg/animal (Fertagyl)	5	232.2	235.4	2.3	2.2
6	Collie	3.5	GnRH 50 μg/animal (Fertagyl)	4	106.2	6.3	2.9	24.6
7	Doberman	6	hCG, 22 UI/kg (Chorulon)	2	172.3	17.6	2.7	14.3
8	Caniche	5	GnRH 50 μg/animal (Fertagyl)	4	123.7	26.5	1.7	1.3
9	German Shepherd	3	GnRH 50 μg/animal (Fertagyl)	3	210.5	206.3	2.1	2.0
10	Cocker Spaniel	3	hCG, 22 UI/kg (Chorulon)	3	105.9	19.4	1.8	1.5

Thus, in four cases has been observed the significant decrease of estrogens hormones levels, 6 days after the last administration of medication. These changes were correlated with the disappearance of keratinized cells in vaginal smears and the appearance of cells specific to metestrus. Progesterone values registered significant increases in the 6th day after the last administration, and this was interpreted as a sign of cysts luteinization (cases no. 2, 4, 6, 7).
In three bitches, reduced estrogen levels were associated with basal values of progesterone (< 2 ng / ml), changes that can be interpreted by the regression of follicular cysts and anoestrus (cases no. 1, 8, 10). Vaginal smears were characterized by the presence of large numbers of parabasal cells and intermediate epithelial cells.
Following performed treatments, three bitches still presented elevated levels of estrogens, accompanied by characteristic clinical manifestations of estrous phase (cases no. 3, 5, 9); in these cases, ovariohysterectomy was applied.

CONCLUSIONS

One of the main genital disorders in bitch is the presence of prolonged estrus due to follicular cysts.
In the studied cases of bitches with follicular cysts, progesterone values ranged between 1.3 and 3.4 ng / ml, while estrogen concentration ranged between 141.5 and 379.5 pg / ml.
The treatment of follicular cysts consisted in the administration of gonadotropin-releasing hormone (GnRH) in five cases and human chorionic gonadotropin (hCG) in five cases.
Consecutive the performed treatments, clinical healing of seven bitches was registered.
Following the treatments, three bitches still presented characteristic clinical manifestations of estrous phase; in these cases, the treatment consisted of ovariohysterectomy.

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THE PRESENCE OF MYCOTOXINS (OTA AND ZEA) IN FEED FOR PIGS AND THEIR INFLUENCE ON REPRODUCTION

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Abstract

Today FAO estimates that about quater of world cereal crops are contaminated with mycotoxins. Contamination of agricultural products ca occur both befor and after harvest, especially during storage, being conditioned by the humidity and high temperatures. These factors favor the growth of mold and increase the resk of mycotoxins.Species of fungi: Aspergillus, Penicillium and Fusarium can produce and release secondary metabolites in feed type: Ochratoxin A (OTA), Zearalenone (ZEA), impact on reproductive performance. Toxicity of mycotoxin depens on the source and their dose, duration of exposure and composition. Damaging effects of moldy feed management are felt especially in youth and female reproduction subject. If consumption of moldy feed, the effects recorded in breeding disorders manifested by abortions, complications of parturition, uterine involutions delayed, prolonged estrus after calving, followed by infecunditate endometritis and sterility. In this case the percentage of pregnant females is very low, even after the repeated treatements. This paper aims to address the presence of mycotoxins (OTA and ZEA) in feed intended for pigs, with their direct influence on reproduction. To minimize the impact of the presence of mycotoxins in pig feed, control measures are carried out to establish the quality of feed used. This mycotoxin has been evidenced by laboratory tests. The working method used was ELISA. Values obtained from determinations were performed according to the legislation.

Key words*: mycotoxins, Ochratoxin, Zearalenone, feed, breeding swine.*

INTRODUCTION

A recent definition posed mycotoxins as "fungal metabolites after inhalation ingestion or absorption through the skin, altering the responsiveness of the

body and cause illness or even death in animals (JI Pitt,1996). Species of fungi: Aspergillus, Penicillium and Fusarium can produce and release secondary metabolites in feed type: Ochratoxin A (OTA), zearalenone, impact on reproductive performance. (Table 1)

Table 1.Species of fungi that can generate OTA and ZEA in cereals

Fungi	**Cereale/ matrice**	**Mycotoxins**
Aspergillus ochraceus; *Penicillium viridicatum* *Penicillium cyclopium*	Cereals	Ochratoxin A (OTA)
Fuzarium culmorum; *Fuzarium graminearum;* *Fuzarium sporotrichioides*	Cereals	Zearalenone

Regardless of their type, mycotoxins (aflatoxins, ochratoxin, zearalenone, fumonizine) cause serious damage to livestock. Food and Agriculture Organization (FAO) estimates that up to 25% of cereals crops are significantly contaminated with mycotoxins worldwide. Contamination of agricultural products can occur both before and after harvest, especially during storage, being conditioned by the humidity and high temperatures.

These factors favor the growth of mold and increase the risk of mycotoxins (Savu C. et al., 2004).

Following recent investigations, one can release a warning of the danger which poses for the health of animals.The occurrence of frequent and various forms of barrenness is caused largely by feed quality assurance.

Pigs are the most sensitive to the two mycotoxins: OTA and ZEA. High incidence of barrenness, profound disturbance of the sexual cycle (false estrus and repeated absence or irregularity of oestrus and vulvo vaginal swelling, vaginal and uterine prolapse are the main symptoms of moldy feed management. Reproduction disorders manifested by abortion complications at parturition, delayed uterine involutions, prolonged estrus after calving,

endometritis followed by infecunditate and sterility. in this case the percentage of pregnant females is very low, even after repeated treatments. Spores cross the gastrointestinal barrier through blood-and lymph, reach the gynecological organe, crossing the placenta (in de case of pregnant females). Roof covering the fetus and fetal multiply, causing abortion

Ochratoxin A (OTA) is considered a natural contaminant in cereal grains with affinity for plasma proteins that are fixed in 90%.(II' Icev YV, Perry,Rucher F et al,2002). OTA can delay the body's immune response,reducing cell-mediated immunity. As aflatoxins, it has carcinogenic action. In boars, OTA can reduce sperm motility and longevity.

Zearalenone is a non-steroidal estrogen mycotoxin produced by Fusarium spp it has been reported in many micotoxicoze at farm animals, especially in pigs.

Zearaleona is resistant to high temperatures and can be found in many cultures across the world contaminating grain, such as corn, oats, barley, wheat, rice and sorghum (Kuiper-Goodman et al., 1987, Tanaka et al., 1988).

Zearalenone is a known phytoestrogen that causes hormonal disorders in animals that ingest contaminated fodder, with the worst effects in pigs. Pigs are very sensitive to zearalenone, which produces a syndrome manifested by changes in estrogen and breast tissue of the vulva (congestion, increase in volume), abnormal lactation, infertility,abortion, birth of dead or viable products, vaginal and rectal prolapse .Absorbtion in pigs after a single oral dose of 10 mg / kg body weight was estimated at 80-85%. (Biehl et al., 1993) zearalenone and its metabolites were found in plasma of pigs less than 30 min after feeding began. (Kuiper-Goodman et al., 1987, Olsen et al.,1991, Biehl et al., 1993).

MATERIAL AND METHODS

To minimize the impact of the presence of mycotoxins in feed breeding pigs with direct influence on their determinations were carried out to establish the quality of feed used. This mycotoxins (OTA and ZEA) was evidenced by using ELISA method of working, a rapid quantitative method of screening. The determination is made based on working kit protocol used is based on the reaction of antigen - antibody.

ELISA kit (Enzyme-linked immunosorbent assay-enzyme immunoassay, or EIA) contains:

- Microtiter plate consisting of 12 strips with 8 wells each, coated with antigen;
- Standards of different concentrations of mycotoxins (5 or 6) with standard Cuba Trace
- All reagents and buffers required (Anti-body - specifically of mycotoxin, Conjugate (with enzyme), Substrate Solution, Stop Solution , Washing buffer)

To avoid contamination of samples was taken into account the observance of rules, namely:

- when entering the laboratory, samples were pureed
- it was a laboratory sample is stored in the freezer representative until determination;

To obtain valid results has been considered subject to the following precautions:

- All reagents were brought to temperature 20-25 ° C and were mixed before use
- these steps were imposed by the kit work in compliance with time forced
- to work in the solvent extract preparation - 70% methanol (OTA, ZEA)
- were observed using working volumes: 50, 100, 500 and 1000 µl-micropipets

Upon completion of the determination and use of equipment contributed: centrifuge, shaker, stirrer ELISA, ELISA plate reader at 450nm.

All kits must be certified according: detection limit (LOD), recovery rate, sample preparation and specificity (Table 2).

Table 2. Performance criteria and standard solutions for same ELISA kit´s

Mycotoxin	**Recovery %**	**LOD**	**Matrices**	**Standards**
Ochratoxin A ***RidaScreen***	85	625 ppt	Cereals, feed	0.0; 25; 75; 225; 675; 2025 ppt
ZEA ***BiooScientific***	-	1,0 ppb	Cereals, feed	0.1; 0.25; 0.5; 1.5; 4.5 ppb

*(ppb= ng/mL= µg/Kg ; ppt = ng/Kg)

RESULTS AND DISCUSSION

This paper has proposed to address the presence of mycotoxins (OTA and ZEA) in feed intended for pigs in 2010-2011. Samples were representative sample for each lot and have to comply with harvesting. If consumption of moldy feed containing secondary metabolites such as: Ochratoxin A (OTA), zearalenone (ZEA) swine, especially youth and females may be affected, the impact on reproductive performance. Toxicity of mycotoxins depends on the source and their dose, duration of exposure and composition. Samples analyzed samples were represented by the following matrix: combined fodder for pigs, corn beans, bran, ground grain.

The results of determinations made are shown in the table below (Table 3).

Table 3.Determinative mycotoxins in swine feeds: 2010-2011

Matrices	**Nr. Samples**		**OTA, (µg/ Kg)**		**ZEA, (µg/ Kg)**	
	2010	**2011**	**2010**	**2011**	**2010**	**2011**
Mixed fodder for pigs	3	3	Ned…0,478	Ned.	Ned…16,82	Ned….28,5
corn beans	6	4	Ned.	Ned.	5,12…51,64	3,44…30,09
bran	3	4	Ned.	Ned.	Ned. ...35,10	1,12…28,14
ground grain	6	5	0,36..0,74	0,12..0,24	5,34...35,18	Ned. .. 27,41

Ned.- nedetectabil

Values obtained from determinations were performed according to the legislation. Because toxic effects of mycotoxins, their highest level in feed for pigs is subject to COMMISSION RECOMMENDATION EC N0 567/2006 (Table 4)

Table 4. Maximum levels for ZEA and OTA in cereal for pigs feeding

Mycotoxin	**Products intended for animal feed value**	**Value in mg/kg (ppm)**
Zearalenone	Feed materials (*) — Cereals and cereal products with the	2

	exception of maize by-products — Maize by-products — Complementary and complete feegingstuffs for pigs	 3 0,250
OTA	Feed materials (*) — Cereals and cereal products; Complementary and complete feegingstuffs for pigs	 0,250 0,050

the previous year were much lower. This leads us to conclude that were rigorously respected veterinary rules on handling, transport and storage.
Considering the results, it can be said that the fodder for feeding pigs were not any danger to their health and therefore did not affect reproductive performance default. Analyzing the results of determinations made, it can be seen that no sample (matrix) was not contaminated with OTA or ZEA values over the maximum allowed.
More than corn grain and bran samples analyzed both in 2010 and in 2011 did not contain OTA. The remaining samples were found only traces of OTA, obtained values were located below 1 µg/kg.(ppb)
In terms of values found in ZEA, the highest value obtained was recorded in 2010 maize grain (51.64 ppb) value less than about 60 times the maximum permitted.
All the same matrix to registering and in 2011 the highest value determined (30.09 ppb) approximately 100 times lower than the maximum allowed.It also notes that ZEA determinations made in 2011, compared to

CONCLUSIONS

Type mycotoxins: Ochratoxin A (OTA), Zearalenone (ZEA), can contaminate food agricultural products used in swine, reproductive performance impact;
Mycotoxins toxicity depends on the source and their dose, duration of exposure and composition;

The quality of feed used is determined by extensive laboratory occupying it an important quantitative determination of mycotoxin –working method ELISA, a quantitative screening method is based on the reaction antigen – antibody can contribute to obtaining valid results, because performance criteria of the kit used;

Due to the toxic effect of mycotoxins the maximum level in food for their pigs in subject to European legislation;

The results of determinations made on the matrices analyzed in 2010 and 2011 did not exceed the maximum allowed neither OTA and ZEA;

So it can be said that these fodder for feeding pigs were not any danger to their health and therefore did not affect reproductive performance default.

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**** Test kit, OTA - ELISA method, RIDASCREEN,Catalog # R1311

.**** Test kit, ZEA - ELISA method, BiooScientific,Catalog # 1035

THE PRESENCE OF *OCHRATOXINS* IN FOODERS AND FOOD PRODUCTS AN THER IMPACT ON ANIMALS AND HUMAN HEALTH

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Abstract

Species of fungi: Aspergillus and Penicillium can produce and release, in certain circumstances of temperature, pH and humidity, secondary metabolites in fooders and food products type: Ochratoxins .
Toxicity of Ochratoxin depens on the type (A,B,C), source and their dose, duration of exposure . Up to the present time has been demonstrated nephrotoxic compounds, hepatotoxic and teratogenic effect. of OchratoxinA (OTA)
Due to the toxic effect of OTA the maximum level in fooder and food is subject to European legislation (Reg. CE N0 576/2006, Reg. CE 1881/2006)
The aim of the paper is to highlight the value of mycotoxins type OTA in feed and foods, as an possible risk on animals and human health. The working method used for teting was ELISA. Values obtained from determinations were performed according to the legislation.

Key words: *mycotoxins, OTA, animals health, human health*

INTRODUCTION

Mycotoxins, defined as "metabolites of fungi which, after ingestion inhalation or absorption through the skin, alter the ability of the body's reaction and cause illnesses or even death in animals" (Pitt JI,1996).
Once ingested they may lead to reduced performance and to change the metabolism of animals. Diseases that occur as a result of consumption of feed contaminated with mycotoxins are called micotoxicoze. They are negative: ingestion of feed; performance and changing metabolism of animals. The appearance of frequent and various forms of infecunditate is caused largely by the quality assurance of.

Contamination of agricultural products occur mainly during storage, being conditioned by the humidity and high temperatures. These factors favour the reproduction of fungi, leading to increased risk of mycotoxins. (Savu et al., 2004).

Due to the effects on human health and animal productivity and higher economic losses that occur inreaga world pay special attention to these mycotoxins.

Increasing control these mycotoxins contamination is due to numerous alerts on food: cereals, coffee, dried fruit(Bayman P, Baker J –2006) Species of fungi: *Aspergillus, Penicillium* generally develop after harvest and are called "mycotoxins of deposit". They can develop and release in feedingstuffs in certain circumstances secondary metabolites: Ochratoxin A (OTA)(Larsen et al.,2001; Pfohl-Leszkowicz et al., 2007).

These are:

In the genus *Penicillium:*

P. verrucosum

P. nordicum

In the genus *Aspergillus* :

- *A. ochraceus*
- *A.melleus*
- *A. auricomus*
- *A.ostianus*
- *A. petrakii,*
- *A.sclerotiorum*
- *A. sulfuroase*

In recent years the analyses of some food products and fodder demonstrated that, P. *viridicatum*, P.*griseofulvum* and possibly P. *solitum* also produced ochratoxins. From genus *Aspergillus*: *A niger* and A.*carbonarius* have been reported as ochratoxigenic fungi(Pitt, J.I.,1987; Abarca ML et al., 2001; National Library of Medicine, 2002).

Affects both animal health Ochratoxin and productive activity and can be match win in animal products such as meat, eggs, milk, presenting a potential risk for human health(Won-Bo Shim et al., 2004).

status of immune system, leading to reduced

In recent years due to its special form on the body human, animal and ochratoxinele are intensely studied. Depending on the degree and impact of health they may be divided into:

- Nephrotoxic
- Immunotoxic
- Neurotoxic
- Mielotoxice
- Carcinogens (IARC, 1993).

As a result of research carried out recently, you can pull up a stark signal of alarm that fungi pose for animal health and human health.

OTA- Toxicity in animals and in humans

OTA has cancerigena, genotoxica and mutagena to several species of animals and humans. Ochratoxin is primarily a toxin that affects the kidneys, but, if the concentration is high enough and injuries can occur in the liver(Rutqvist L. Et al. 1978; O' Brien E, Dietrich DR, 2005) .
By inhibiting the metabolism of glucose and insulin, OTA may cause the accumulation of glycogen in the liver.Neurotoxic effect was demonstrated in all mammalian species.
Main mechanisms by which manifests its toxicity mycotoxins are: stimulation of lipid peroxidation, apoptosis and inhibition of protein synthesis of DNA and RNA. In this respect immunotoxicity is the most important consequence of serious micotoxicozei (Bondy and Pestka, 2000).

Nefrotoxicitatea in pigs fed with feedingstuffs, at which the level of OTA has been comprised between 200-4000 g/kg, is manifested in the kidney, proximal tubular atrophy, fibrosis and glomerular sclerosis(Stefanovic, V.et al.,1991). Intoxicati pigs appear biochemical lesions: proteinuria, sugar, enzimurie, reducing the concentration of the urine. Later onset kidney failure (Petrova- BacharovaT et al., 1991; Pfohl-Leszkowicz et al.,2002)
Human epidemiological studies have shown that OTA can cause a higher incident of renal tumors, described for the first time in Bulgaria in the year 1956 (TanchevY,Dorossiev D -1956).
That is way the European Scientific Committee on Food indicates a lower tolerable intake, below 5 ng/kg /per day (Walker, R.; Larsen, J.C, 2005).
Carcinogeneza
In rodents, after ingesting food contaminated with OTA were detected in liver, kidney tumors, Mammary and testicular (IARC, 1993;Castegnaro, M. ,et al.,1998; Mantle, P.,et al.,2005)
Following administration of OTA in growing gilts over a period of 35 days found decreased phagocytic macrophages. Also the production of interleukin (IL) is compromised. (Harvey et al., 1992; Petzinger, E., Weidenbach, A. Mycotoxins,2002).
In the studies done have shown the effects of immunomodulation induced by ochratoxin, and the fact that OTA affects humoral immunity (antibody synthesis) in chickens, rats and mice (Surai, 2004).
Purified human lymphocytes after exposure to OTA has been a decrease in cellular capacity aspunde activation stimuli in vitro, is impaired production of IL-2 and IL-2 receptor by T cells activated. (Lea et al., 1989)The conclusion was that the toxin

led to immunosuppression by interfering with essential processes of cellular metabolism.
Inhibition of interferon production base has Locle after suppression of NK cell activity by OTA (Lea et al., 1989).
The man behind the studies conducted have found that kidney tumors often appear when food intake is greater than 70 g/kg per day of OTA(Pfohl-Leszkowicz, A et al. ,1993; Pfohl-Leszkowicz, A et al. 2007; Pfohl-Leszkowicz, A, 2009).

MATERIAL AND METHODS

We used the test kit with competitive enzyme immunoassay for the quantitative analysis of *Ochratoxin A (OTA)* in fodder and foods.
The determination is made based on working kit protocol used is based on the reaction of antigen - antibody. ELISA kit (Enzyme-linked immunosorbent assay-enzyme immunoassay, or EIA). After the sample preparation the test procedure, the measurement is made photometrical at 450 nm.
Reagents: - 1n HCl , 5 n HCl; CH_2Cl_2 ; 0,13M buffer ($NaHCO_3$) with pH=8,1
Equipment:
- microtiter plate spectrophotometer (450nm)
- centrifuge
- magnetic stirrer
- paper filter
- gradual pipettes
- micropipettes
- purification columns OTA

All reagents required for determinations had adequate quality according and the determinations were made using modern equipment from Sanitary- Veterinary and Food Safety Direction-laboratory of Brasov. This laboratory applies a GPL system and a quality system.
To avoid contamination of samples was taken into account the observance of rules, namely:
-when entering the laboratory, samples were pureed ;
-it was a laboratory sample is stored in the freezer representative until determination;
To obtain valid results has been considered subject to the following precautions:
-all reagents were brought to temperature 20-25 ° C and were mixed before use ;
-these steps were imposed by the kit work in compliance with time forced ;
-to work in the solvent extract preparation - 70% methanol (OTA) ;
-were observed using working volumes: 50, 100, 500 and 1000 µl-micropipets;
All kits must be certified according: detection limit (LOD), recovery rate, sample preparation and specificity (Table 1).

Table 1. Performance criteria for ELISA kit

Mycotoxin	Recovery %	LOD	Matrices
Ochratoxin A ***RidaScreen***	85	625 ppt*	Cereals, feed, food

*(ppb= ng/mL= µg/Kg ; ppt = ng/Kg)

RESULTS AND DISCUSSION

The present report refers to the determinations made in 2010-2012 on samples from farms, processing units, markets designated booth for animal feed and human food (cereals, wine, peanuts, etc).
The sample were representative of each batch separately and have respected the rules of collection.
The maximum level of *Ochratoxin A* in different types of products are indicate in following table 2:

Table 2. The maximum levels for OTA according to Europeans legislature

Mycotoxin type	Level	Products	Directive EU
Ochratoxin A	3,0 – 10,0 (ppb)	Cereals, peanuts , dry fruits, coffee	Regulation (EC) No 1881/2006
	2,0 (ppb)	Wine, juice of wine	
	0,50 (ppb)	Baby foods	
	0,05 – 0,25 (ppm)	Types of fodders	Regulation (EC) No 576/2006

Regarding the values of Ochratoxin A in the sample which we analysed are presented in the table below (Table nr.3).

Table 3. The values of OTA in same samples

Analysed sample	The variation of values for OTA (ppb)		
	2010	2011	2012
Cereals	0,00 – 3,200	0,00 - 2,84	0,22 – 1,64

Foods	0,00 – 0,120	0,00 - 0,300	0,00 – 4,28
Fodder	0,00 - 8,760	0,00- 0,54	0,12 – 0,24

Values obtained from determinations were performed according to the European legislation: Regulation (EC) No 576/2006 and Regulation (EC) No 1881/2006

CONCLUSIONS

The Ochratoxin A is on of the highly dangerous mycotoxins for human and animal health

The obtained results were reported to be tantamount to the laws regarding the levels of Ochratoxin A.

Since the values obtained from analyses did not exceed the maximum level permited by low , these samples are not harmful to humans and animals.

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REGULATION (EC) NO. 1881/2006 COMMISSION of December 19, 2006
setting maximum levels for certain contaminants in foodstuffs.
Test kit,OTA - ELISA method, RIDASCREEN, Catalog # R1311.

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RESEARCH ON IDIOPATHIC STOMATITIS IN CATS

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Abstract

Introduction.
In the last decade there have been great advances in the study of chronic oral inflammation impacts on the health of the whole animal body.
Materials and methods.
Clinical investigations were performed in the Surgical Clinic of FMV Iaşi, on 32 patients presenting characteristic symptoms.
Results and conclusions.
Secondary clinical outcomes of the research are those that revolutionizes diagnosis and treatment plan, or need treatment more than the technology itself. Gingivostomatitis have long been known, studied and systematized, but some of them have not yet been fully described and investigated as cat idiopathic gingivostomatitis. Failure diagnosis and treatment of this disease and others that are localized specifically in the oral cavity is due to the fact that dentistry is basically a new branch, implemented shortly in veterinary medicine and began to grow in treatment of pets. Treatment of this disease should be seen as a product of comparative analysis of the advantages and disadvantages involved in each case. In addition to restoring the functions of the stomatognathic apparatus, treatment raises a number of shortcomings related to threatening the integrity of various organs of the animal, to facilitate the emergence of other diseases, the sometimes high cost of treatment and, not least, the risk of its failure.

Keyword: cat idiopathic gingivostomatitis,cat stomatitis.

INTRODUCTION

Our research objectives were to diagnose and treat idiopathic stomatitis in cats called limfocytic plasmocytic stomatitis and its complications by clinical and laboratory examinations. We also aimed to implement a clear diagnostic techniques.
A key objective was to make differential diagnosis between: gingivostomatitis feline eosinophilic granuloma and some viral diseases such as feline leukemia virus (FeLV) and Feline immunodeficiency virus (FIV), feline calicivirus (FCV), which interference with the disease and often produce lesions in the oral cavity.

We seek to restore the integrity of the oral mucosa with effective treatment regimens of patients diagnosed.

MATERIALS AND METHODS

The research was carried out during 2008 - May 2012, a total of 184 cases examined cases.
Subjects included in the study were presented by their owners at clinics of the Faculty of Veterinary Medicine of Iasi, dental clinic.
Subjects included in the study were presented by their owners at clinics of the Faculty of Veterinary Medicine of Iasi, dental clinic.
The diagnosis was established by clinical and laboratory examinations.
The study was conducted on 32 patients of the total cases of which 17 cases were diagnosed with gingivostomatitis lymphocytes plasmocytes syndrome, 7 cases of ulcerative and necrotic gingivostomatitis**,** 4 cases with hyperplasia stomatitis and other 4 cases with other reasons.
Pet owners have been properly informed and help in decision making for a feature aimed at prolonging life and patient comfort habitat creation owners.
Clinical diagnostic elements and laboratory
Dental interventions were preceded by clinical examination of the oral cavity examination respecting semiological methods.
History, reason for the request, the conditions of feeding, maintenance, previous diseases, physiological status, and hereditary predisposition to previously used medications are important in diagnosis.
Opening the oral cavity (manual or appropriate speculum) and was one of its inspection examinations helpful in the diagnosis.
If necessary we performed a radiological examination. In case tissue proliferation is performed histopathological examinations, hematological and biochemical tests for feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and Feline calicivirus (FCV).
All these steps were necessary elements to develop a diagnostic certainty which allowed us to implement a correct therapeutic behavior.

Inflammatory lesions, stomatitis

Stomatitis is inflammation of the mouth, regardless of origin, location and evolution, in which the lesion extends over large portions of the mucosa.
If the inflammatory process is limited, we distinguish gingivitis, glossitis, oropharynx and palate.
Stomatitis involved in the etiology of a number of local and general factors.

Among the general factors that favor the development of stomatitis include: metabolic disorders, vitamin deficiencies, endogenous intoxications (uraemia, hepatic failure, diabetes) or exogenous (medicinal) endocrinopathies, infectious, allergic or immune-mediated diseases.

Local factors are the most frequent saprophytic germs of mouth, which become pathogenic under certain conditions trophic private. Depending on the evolution of the inflammatory process, the multitude of causes that generate them and pathological changes, stomatitis can be acute and chronic. Both forms of evolution, depending on the etiology, may be nonspecific and specific.

I met the nonspecific stomatitis inflammation of the oral mucosa, which we grouped by morphological changes in exudative stomatitis, stomatitis hyperplastic, necrotic stomatitis.

Hyperplastic proliferative stomatitis are inflammatory lesions that occur predominantly in the gums. Based on a hormonal disorder, drug or combination of other factors.

I found a histopathologic epithelial cell proliferation, as proving the presence of mitoses hyperplasia. In chorion found intraepithelial haemorrhage, infiltration lymphocytic and hstiocytic**,** histiocytic differentiation of and synthesis of collagen fibers (Figure 1).

Ulcerative and necrotic gingivostomatitis are the predominant inflammatory type alterative necrotic changes in gingival and buccal mucosa.

This is where a variety of biotic agents, whose aggression is favored by general and local factors.

Exacerbation of virulence factors that favor local saprophytic flora are chronic inflammatory processes: shallow marginal periodontitis and factors that maintain chronic irritation. These factors influence microbial germs multiplying - streptococci, aerobic, followed by increasing metabolism with high consumption of oxygen, which creates anaerobes development.

Macroscopic inflammatory process starts with congestion, mucosal edema, which appears purplish-red, swollen and bleed easily. Subsequently appear initially localized ulcers on the gums, the top inter-dental papilla, extending the oral mucosa, tongue, pharynx. Ulcers have irregular borders surrounded by hyperemia, have a tendency to confluence and ashes covered by false membrane. Ulcers can be shallow or deep. Evolving disease with enlarged jaw.

Microscopically, the absence of squamous epithelium limited to the presence of necrotic debris, fibrin, microbial germs and polymorphonuclear

neutrophil. In chorion appear hyperemia, edema and diffuse polymorphonuclear infiltration (Figure 2).
Evolution is generally good. By necrosis of interdental papillae amputation creates job retention, however, favors the formation of tartar.

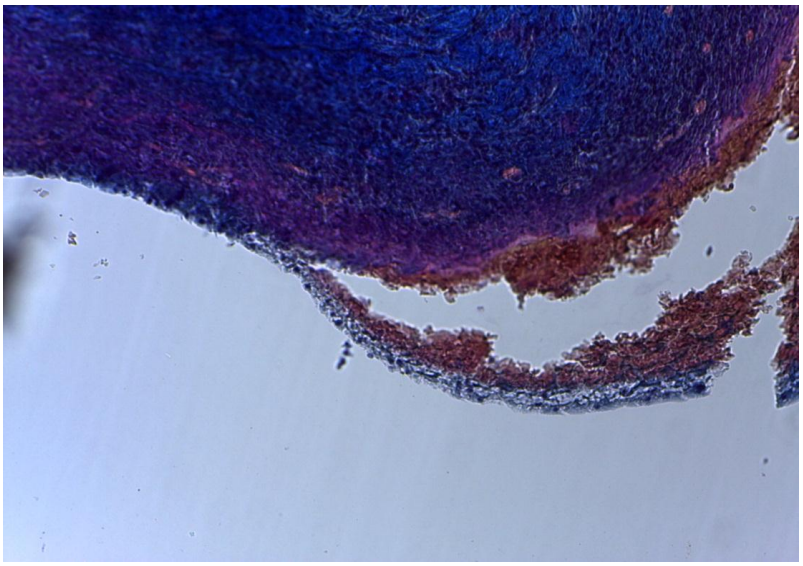

Figure 1. Gingivostomatitis hyperplasia. Col. HEA x 200.

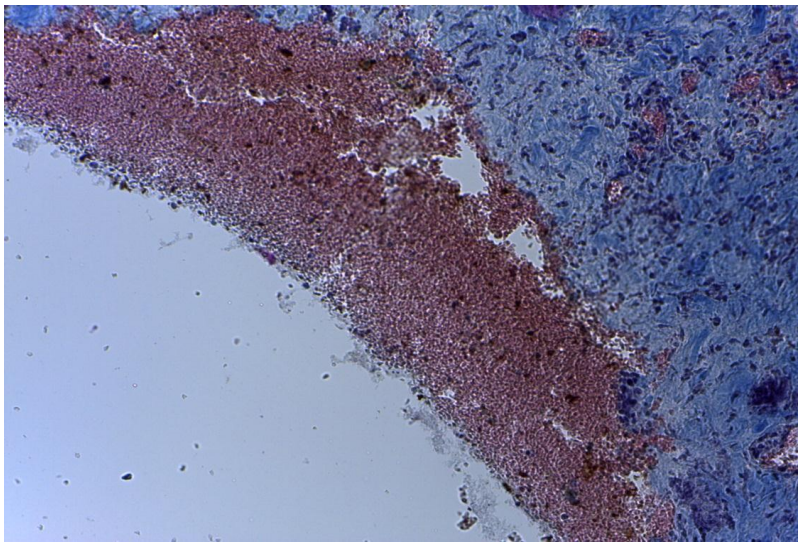

Figure 2. Active ulcer in the lining of the gingival Col. HEA x 200

Inflammatory hyperplastic lesions (pseudo lesions). Inflammatory hyperplasia is the term used to describe numerous nodular neoformaţii the mouth, which histologically resemble inflammatory granulation tissue.
This similarity is based on the degree of development of inflammatory action and reaction components in cicatricial.
Thus, sometimes the look is predominantly epithelial hyperplasia with reduced conjunctive reaction.
In other cases the lesion is fibroma, including angiomatosis changes or collagen sclerosis, with minimal epithelial component.
Depending on the degree of maturation of granulation tissue may be young, exuberant, rich neoformation vessels. Sometimes, reducing cell population and building a neovasculature sclerosis causes collagen scar.
This variation is reflected in the variability of histological aspects of clinical issues that may have inflammatory hyperplasia.
Terms like "fibroids" or "papilloma" are used to describe these lesions, even if there are no signs pointing to a neoplastic etiology.
The main etiologic factor of these injuries is chronic trauma (dental fractures etc). In many cases, chronic irritants can be clearly demonstrated (eg palatal hyperplasia).
Most lesions are localized on the surface of the mouth, irritation exposed location. Are classified as inflammatory hyperplasia two deeper lesions such as granuloma with giant cells of bone repair, thanks histological and clinical course.
Eliminating chronic irritant and inflammatory hyperplasia lesion, they will relapse, confirming their benign nature and etiology of chronic irritant.
Inflammatory fibrous hyperplasia may locate at any point in the mouth as a neoformation pedicle and sessile. Hyperplasia is often identified with papillomas, if the lesion is pedunculated and keratinized, and fibroids, if the lesion is sessile, tough and covered by a thin squamous epithelium. The gum, an injury of this type is often confused with epulides. Histopathological examination found neoformation vessels and fibroblast hyperplasia (Figure 3).
Most inflammatory fibrous hyperplasia remain small. Lesions with a diameter greater than 1 cm are rare on the tongue and floor of mouth, probably because it limits the size of masticatory trauma.
Biting pressure usually causes lesions keratinization.
In the differential diagnosis of inflammatory hyperplasia should be considered that the lesion may be true or injury papilomatoasă papilloma viral origin. Nodular formations on the surface of the tongue can be

neurofibromas, lymphoid nodules or cystic dilatation of mucous gland ducts.
Recurrences after removing inflammatory fibrous hyperplasia, can be interpreted as a persistence of chronic irritation factor action.
Inflammatory fibrous hyperplasia have malignant potential, however they must be removed surgically. Their appearance brittle, and often ulcerated bleeding arising from their histological structure.
Microscope is composed of a richly vascularized granulation tissue with minimal collagen support. Are present abundant polymorphonuclear cells and chronic inflammatory cells (Figure 4, Figure 5).
Tissue is swollen and the presence of microabscesses. The existence of these lesions indicates the need for periodontal inspections, appropriate treatment should eliminate the irritating factors scaling and gum pockets.
All these pathological conditions described above are irritants to the lining of the oral cavity, the premise is easily prone to a chronic inflammatory reaction that ultimately interested in the free edge of the gums, sublingual mucosa receses, soft palate and glosopalatal folds.
From this moment the actual installation of the syndrome of chronic stomatitis, things evolve in a poor outcome until it reaches the visible expression of clinical signs. They consist of difficulty in mastication and prehension, reaching a stage when sick cat can not chew or dealing with a high difficulty. In many cases animals unable to reach could feed, but usually refuse liquids, such stomatognathic apparatus is unable to work. Disease can be identified in the early stages of the veterinarian advised that the examination of the oral cavity will notify the changes. Early detection of disease or predisposing factors that are precursors worthy of attention for establishing a diagnosis and proper treatment to prevent disease progress will bring a sick animal disease control stage.
From research conducted both by us and by other practitioners symptoms do not resolve itself only in a very small percentage, which is considerably increased by the application of appropriate treatment regimens.
Association with immunodeficiency, feline leukemia feline coronavirosis is demonstrated in many cases of gingivostomatitis syndrome. For confirmation usually use standardized tests.
In terms outlined histopathological infiltration by lymphocytes, monocytes and plasma cells. These can generate advanced chronic stage appearance of plasmacytoma.

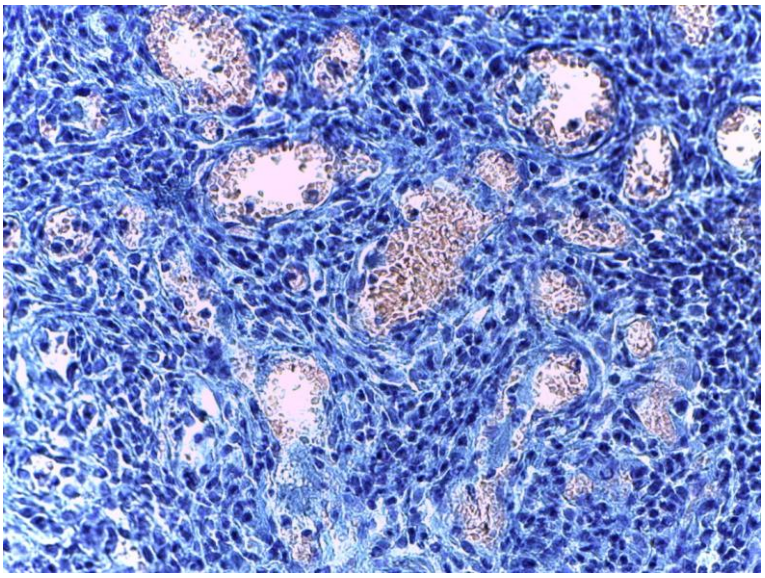

Figure 3. Fibroblast hyperplasia. Col.HEA, x 200.

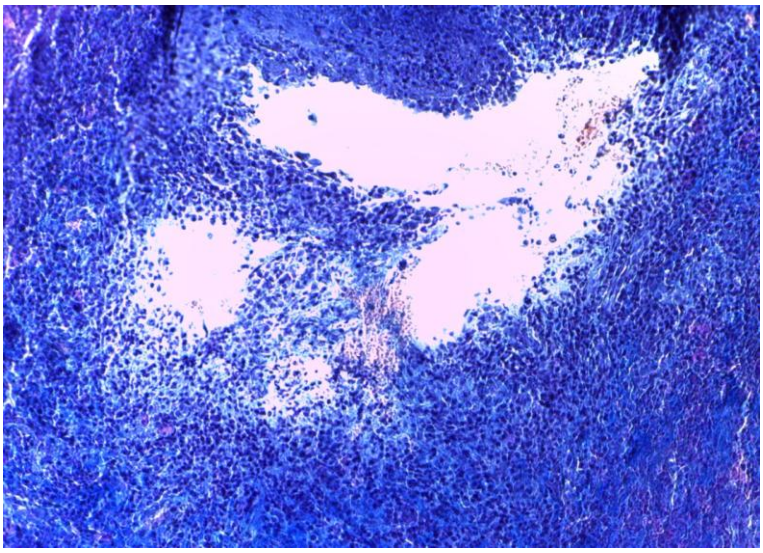

Figure 4. Focal inflammation. Col. HEA, x 200.

Following a strategy established treatment planning increases the likelihood of obtaining a positive result with satisfaction both parties. Treatment plan designed to ensure a net improvement in health status, a benefit for the patient and owner. They must understand and accept that treatment success

depends largely on their efforts sequentially adequate maintenance of oral health through the scheme imposed. Treatment planning is not an exact science, but an art supported by clinical experience, scientific knowledge filtered.

Treatment in controlling the inflammatory response consisted in prednisone administration at a dose of 2-4 mg/kg/day and if the patient responds well to treatment, the dose will be reduced gradually. Simultaneously applied vitaminotherapy A, and zinc mineral supplements based on soft tissue maintenance and insisted on local professional prophylactic treatment consisting of scaling and brushing and administration of chlorhexidine for oral hygiene maintenance.

Inflammation of the gums and mouth can come from a variety of local and systemic causes. The most common cause of gingivitis is dental plaque and tartar (Figure 6, Figure 7, Figure 8, Figure 9).

Calicivirus and herpes virus can cause ulcerative stomatitis. Cats affected by this virus presents as clinical signs: sneezing, runny purulent ocular and nasal, oral mucosal ulceration apathy and especially language and palate.

Chronic gingivitis, tartar unless it is associated with infection with feline immunodeficiency virus (FIV).

Success in the treatment of gingival stomatitis attention involved a primary cause of the disease if it can be identified.

Clinical studies have suggested a possible involvement of different viral agents, particularly herpes virus and species of Gram-positive bacteria anaerbobe. However, attempts to reproduce the disease using these presumed infectious etiological agents were unsuccessful.

Cats with chronic stomatitis require a thorough investigation before any treatment. Its aim is to reach a diagnosis in itself, but mostly an attempt to identify root causes. Such analysis includes testing for FIV and FeLV, haematological and biochemical blood tests and sometimes routine biopsy and microscopic examination of tissues affected. A thorough oral examination and dental, including radiographs of the entire oral cavity for the presence of periodontitis, resorptive lesions, root debris and other injuries is mandatory. Systemic diseases such as chronic renal failure and diabetes, which may predispose to the development of severe gingival inflammation in the presence of the plate, should also be excluded before starting any treatment.

Chronic gingivostomatitis (CGS) describes a clinical syndrome characterized by focal or diffuse inflammation of the gums and oral mucosa (Figure 10). The most common laboratory results described in cats with

CGS include serum globulins, predominantly hypergammaglobulinemia and submucosa inflammatory infiltrate consisting of plasma cells, lymphocytes, macrophages and neutrophils. Serum globulins in cats affected and inflammatory nature of the submucosa led some authors to suggest that there could be an immunological basis for disease. So far, no intrinsic immunological abnormality in cats affected with CGS was not identified, however, is immune mediated disease.

Another treatment consisted of up to 10mg Triamcinolone acetate/administration in combination with metronidazole 30-60mg/kg/day in two divided doses for 10-15 days. Vitaminotherapy complex A, B, C and E, and for FeLV and FIV viral disease was used specific treatment. In this case a possible cause was found essential for inflammation in the oral mucous membranes namely FIV infection.

Another general treatment consisted of administration of immunomodulatory effect of levamisole for dose 2-5mg/kg/day or 30mg/ animal three times a week plus clindamycin 5mg/kg corp/12 hours for 3-4 weeks. There were 3 doses of interferon within 7 days and vitamins therapy complex, A, B, C and E.

In this case the cat was re-examined at 6, 10, 14 weeks and oral samples were taken for histo-pathological examination. Notice a significant improvement in oral inflammation.

This disease is considered to be an inappropriate response to oral antigens, ie plaque present on the surface of teeth.

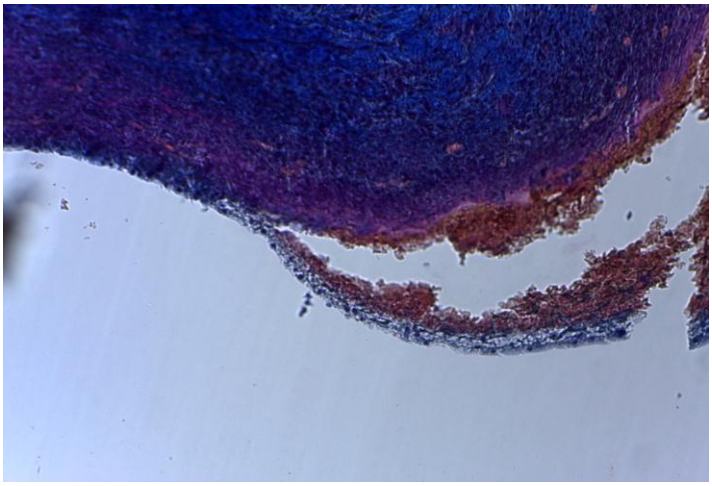

Figure 5. Gingivostomatitis hyperplasia. Col. HEA x 200.

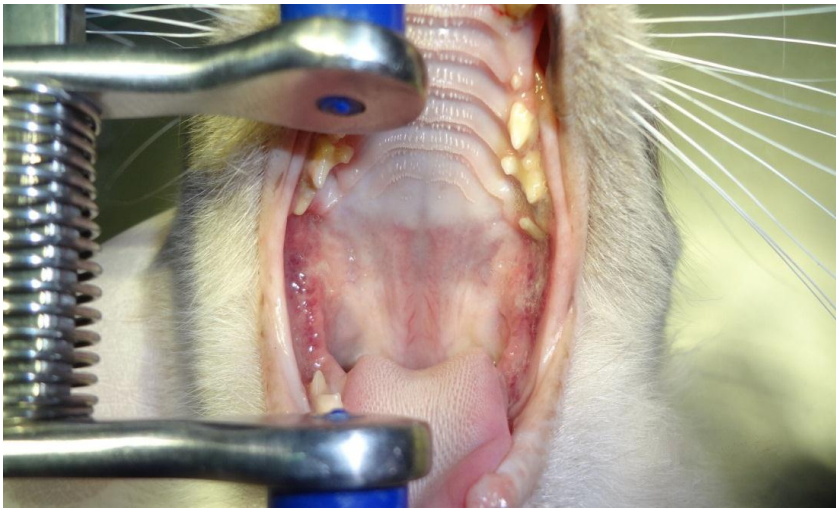

Figure 6. Gingivostomatitis on the soft palate and its folds.

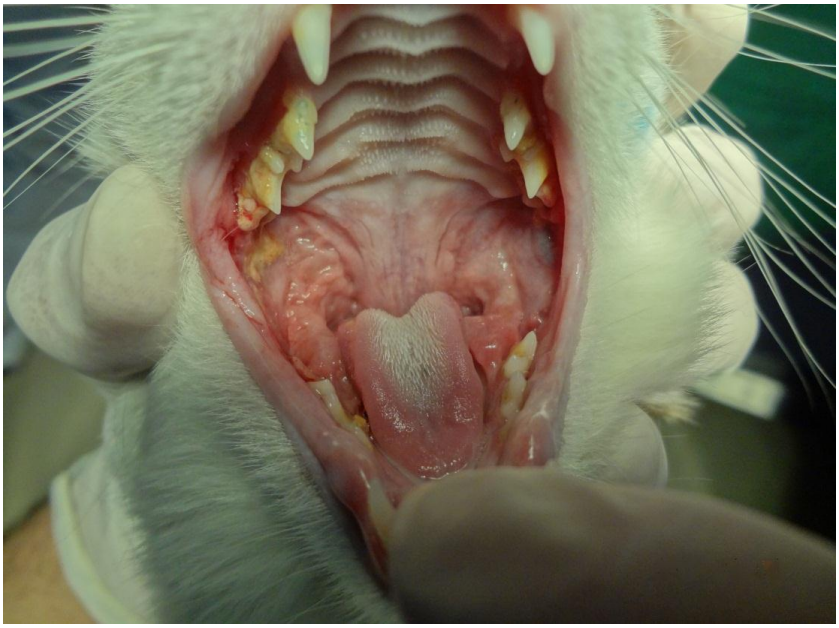

Figure 7. Glosopalatine mucosal hyperplasia

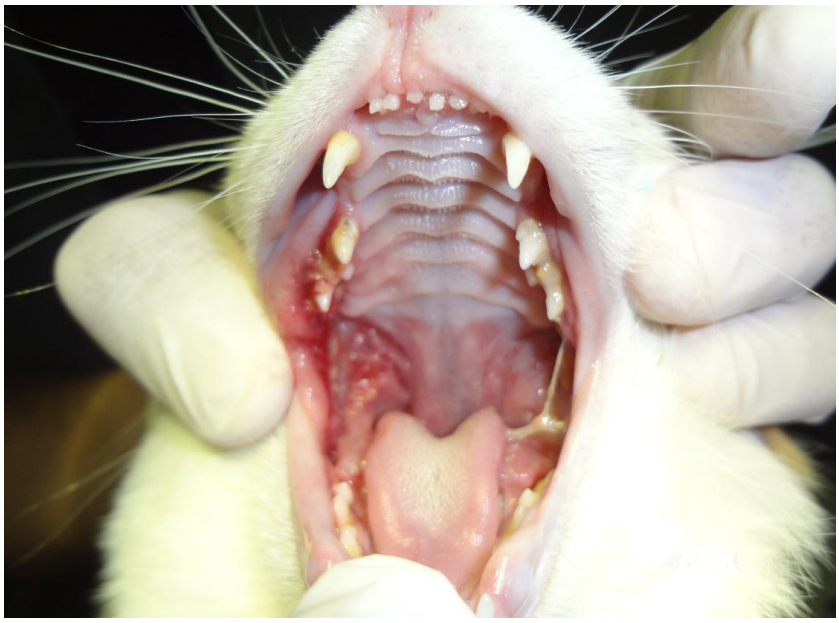

Figure 8. Location on molar gingival mucosa.

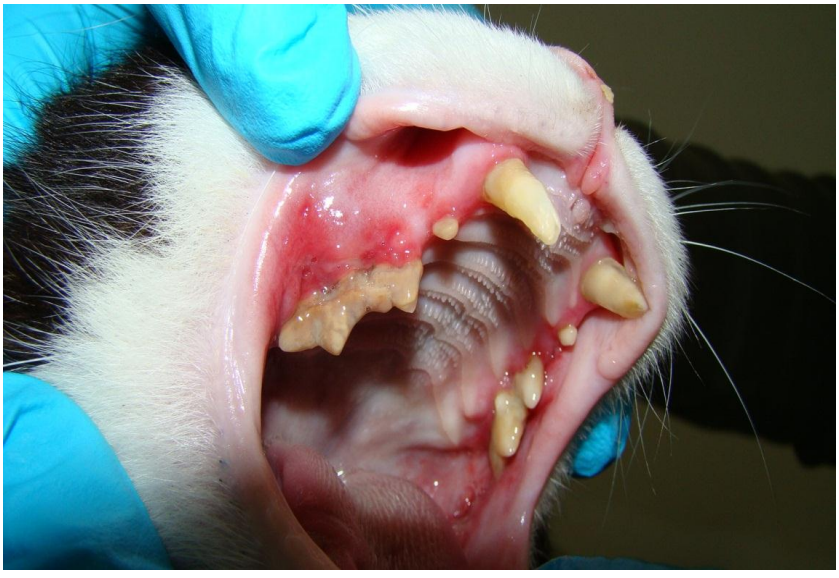

Figure 9. Lateral aspect of gingival inflammation and oral mucosa.

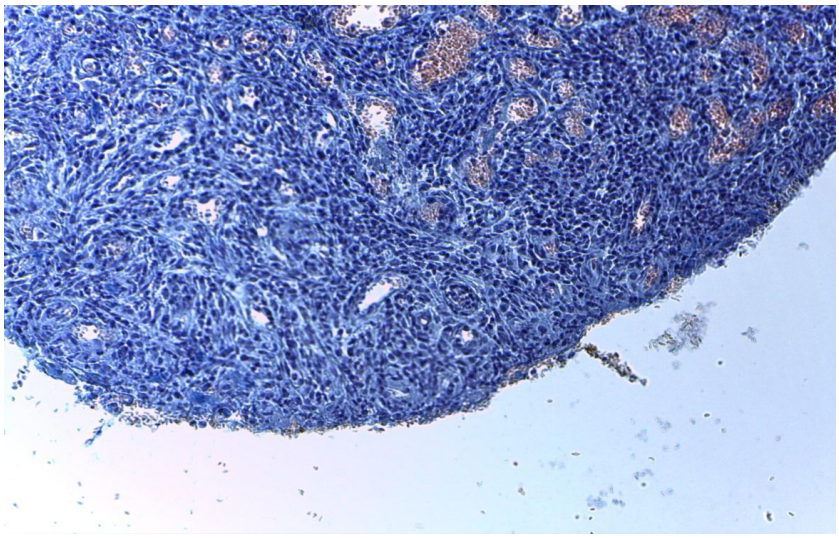

Figure 10. Fibrous chronic gingivostomatitis. Col. HEA, x 200.

CONCLUSIONS

Lymphocytic plasmocytic chronic stomatitis is a disease whose cause is somewhat unknown. Has several names including: chronic feline gingivo-stostomatitis, gingivo-pharyngitis plasma cell and idiopathic gingivostomatitis.

Cats of any age and breed can be affected, but young cats are prone to disease. Signs included anorexia, salivation, dysphagia and halitosis. Intermittent exacerbations and relapses are common.

Chronic gingivostomatitis describes a clinical syndrome characterized by focal or diffuse inflammation of the gums and oral mucosa. The most common clinical results described in cats with CGS include serum globulins, predominantly hypergammaglobulinemia and submucosa inflammatory infiltrate consisting of plasma cells, lymphocytes, macrophages and neutrophils. Serum globulins in cats affected and inflammatory nature of the submucosa led some authors to suggest that there could be an immunological basis for disease. So far, no intrinsic immunological abnormality in cats affected with CGS was not identified, however, may be immune mediated disease.

Cats with chronic stomatitis require a thorough investigation before any treatment. Its aim is to reach a diagnosis in itself, but mostly an attempt to identify root causes. Such analysis includes testing for FIV, FeLV and FCV,

haematological and biochemical blood tests and sometimes routine biopsy and microscopic examination of tissues affected.
Satisfactory results were recorded in general about treatment with prednisolone at a dose of 2-4 mg/kg/day. If the patient responds well to treatment, the dose will be reduced gradually. Simultaneously applied vitamin therapy with vitamin A, C and zinc mineral supplements based on soft tissue maintenance and insisted on local professional prophylactic treatment consisting of scaling and brushing and administration of chlorhexidine for oral hygiene maintenance.
Another regimen with equally good results consisted of Levamisole for immunomodulating effect dose 2-5 mg/kg/day or 30 mg/animal three times a week plus clindamycin 5 mg/kg corp/12 hours 3-4 weeks. There were 3 doses of interferon within 7 days and vitamin therapy complex, A, B, C and E.
A thorough oral examination and dental, including radiographs of the entire oral cavity for the presence of periodontitis, odontoclastic resorption lesions, root debris and other injuries is mandatory. Systemic diseases such as chronic renal failure and diabetes, which may predispose to the development of severe gingival inflammation in the presence of the plate, should also be excluded before starting any treatment.

Guidance

Lymphocytic plasmocytic stomatitis require aggressive therapy may include teeth cleaning, debridement of necrotic tissue and extracting any teeth close. Extraction of all premolars and molars may be necessary and results in even diminish distant lesions of teeth.
Cats with chronic stomatitis require a thorough investigation before any treatment. Its aim is to reach a diagnosis in itself, but mostly an attempt to identify root causes. Such analysis includes testing for FIV, FeLV and haematological and biochemical blood tests and sometimes routine biopsy and microscopic examination of tissues affected.
A complete blood count is needed to verify cytopenias every 2 weeks during the incubation period and every month during improvement. A urine analysis should be done to verify proteinuria.
Local application of chlorhexidine oral cavity is required. Accumulation of plaque can be reduced by special diets and using a dry toothbrush.
Recommended as using only interferon therapy in cats diagnosed positive tests for FeLV, FIV and FCV, which was an improved. It also assesses intralesional injection of interferon as a possible protocol.

Current treatment recommendations for cats with CGS include a combination of periodontal treatment and home care regimen that plaque buildup is kept to a minimum. In some cats can lead to a reduction in inflammation. Unfortunately, most cats do not cooperate adequately in the provision of care at home and board passes the critical level. These cats require extraction of premolars and molars. In some cats is necessary to remove all teeth. Extraction of all premolars and molars gave the most reliable results, up to 80% of cats were clinically cured. The other 20% that are not susceptible to extraction can be treated using the schemes described above.

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LIVER METASTASES IN MAMMARY CARCINOMA IN FEMALE DOGS: CASE STUDY

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ABSTRACT

At female dogs, the malign tumors have metastases potential, both on lymphatic way at the regional lymphatic nodules, as well as on sanguine way, on the lungs or in distant places of the body, including liver, spleen, heart and boned system.
This case study presents a half-blood female dog, 11 years old, with mammary tumors at the level of the right mammary chain (M_5) and partial mastectomy (M_3, M_4, M_5), on the left mammary chain. The clinical examination also underlined ascites, anorexia, diarrhea and severe dyspnoea. The female was undertaking several complementary examinations: ultrasound, radiology, necropsy and histopathology.
The ultrasound examination revealed the presence of liver hypo- and hyperechogenicity. The radiological examination revealed the presence of a large sized radio-opaque area (1.5-2 cm) on the right pulmonary diaphragmatic lobe and on the left cardiac lobe an area of smaller sizes (0.5 cm).
From the necropsy perspective, besides the pulmonary and hepatic lesions revealed following the complementary imagistic examinations, it was revealed the presence of both pancreatic and renal metastases.
The histopathological examination from the mammary lesion revealed the presence of a malignant epithelial tumor, the diagnosis being of complex type mammary carcinoma. The histopathological examination of the liver confirms the fact that the liver metastases have the same origin as the one in the mammary chain.

Key words: *female dogs, liver metastasis, mammary tumors*.

INTRODUCTION

Nowadays, mammary tumours account for 50% of the total neoplasms affecting lady dogs (Daleck et al., 1998, Oliveira et al., 2003). Between 40% and 50% of the mammary gland tunours are malign, and 50% of these may disseminate, following the tract of the lymphatic vessels adjacent to regional lymph nodes and blood vessels (Harvey, 1998; Robbins, 2003; Hedlund, 2007).
Mammary tumoral lesions are easy to identify, and matastases generally occur from a few months to a few years after the discovery of the primary

tumour. The average age for the detection of mammary tumoral lesions is 10 – 11 years old. (Ginn et al., 2007).
The literature in the filed indicates the fact that the lung is the main metastasation spot of mammary tumours (for 60-80% of the cases), but they may disseminate into other organs, as well: lymph nodes, suprarenal glands, kidneys, heart, bones, liver, brain, eyes, nose, spleen, uterus, serous parts (Lagadic and Cohn-Bendit, 1995; Sorenmo, 2003; Muller and Guaguère, 2006; Fontbonne et al., 2007). Occasionally, the skin may also be a metastasation spot of canine mammary tumours, determining cutaneous carcinomatosis (Muller and Guaguère, 2006).
The mammary carcinoma represents a tumour with a high tendency of haematogenous and/or lymphatic dissemination. The probability for such dissemination increases if there have been previously detected metastases at the level of the lymph nodes. Some cases recorded the occurrence of remote metastases, without being preceded by metastases at the level of the lymph nodes (Jassema, 1998).

MATERIALS AND METHODS

The present study is focused on an 11-year old, mixed-breed, lady dog, weighing 27 kg, with mammary tumoral lesions.
The investigations were performed at the Faculty of Veterinary Medicine in Bucharest, the female being subjected to the following protocol:

- The collection of anamnestic data
- Clinical examination: the morphoclinical examination of the primary tumour (identification of the number of affected mammae, the mortification/ulceration degree, the detection of affected lymph nodes).
- Complementary exams:
  - Abdominal ultrasound scan.
  - Lateral and ventro-dorsal pulmonary radiography, using the digitalised radiological technique.
  - During necropsy there have been biological samples collected in view of determining a definitive diagnostic.
  - Anatomopathological examination: macroscopic and histologic tests performed on the samples collected after the necropsy. The tumoral tissue fragments collected in view of performing the histological exam were placed in formaldehyde 10% solution, processed by means of the

classic histopathological method, with an inclusion in paraffin and cutting by section cutter, whereas the colouring methods were the bichromic hematoxilin-eosine (HE) method and the Masson trichromic method.

RESULTS AND DISCUSSIONS

Following the clinical examination, it was ascertained that the female displayed a mammary tumoral lesion located on the right mammary chain (M_5 – inguinal mamma), plus a partial ablation of the left mammary chain (M_3, M_4 si M_5), batrachian abdomen (ascites) and a state of inappetence. In the meantime, the female was subjected to the radiological exam in view of detecting any possible pulmonary metastases.
The *anamnesis* revealed that the primary tumoral lesion had been detected on the left mammary chain – M_5, three years before (2009), measuring approximately one cm. In November 2011, the female underwent a surgical procedure which comprised: sterilisation (OHT - ovariohysterectomy) and partial mammectomy with a tumoral lesion on M_5 (6 cm, hard, immobile).
In January 2012, it also developed on the right mammary chain - M_5 (2 cm, hard and adherent to the neighbouring tissue). At the time of the clinical examination, it measured 5 cm and was hard, adherent and with a bosselated surface (Figure 1).

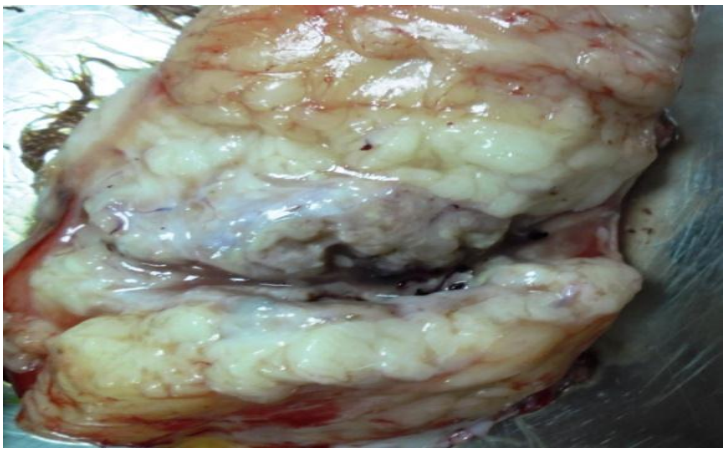

Figure 1. Mammary tumoral lesion M_5, right mammary chain (appearance in section).

Starting from March (2012), the lady dog began breathing heavily (dyspnea), and in April it was subjected to an ultrasound scan, due to the clinical, ascites-like aspect. Following the ultrasound procedure, the following were ascertained: hepatic modifications in the vicinity of the hydroperitoneum (irregular surface, with an alternation of hypoecogenic and hyperecogenic areas, suspicion of hepatic metastases), renal modifications (right kidney – 5,6 cm, left kidney – 5,8 cm, with the corticomedullary limit poorly delimited).

A therapy followed, consisting in the administration of diuretics (furosemide, manitol), hepatic protectors (aspatofort), antibiotherapy (amoxicilin), anti-haemorrhagic medication (etamsilat, fitomenadion, vitamine C), analgesic medication (algocalmin) and theramnekron (with some antitumoral effects).

During its lifetime the lady dog was not administered hormonal medication, it never gave birth, however, there have been multiple false lactations recorded (the ovariohysterectomy took place at a later date, when it was 10-years old). The literature data confirms that both the false lactations and the lack sterilisation led to the risk mammary tumour occurrence (Donnay et al., 1994; Schneider et al., 1969).

Age is one of the predisposing factors in the occurrence of mammary tumoral lesions, the incidence increasing with aging, as was the case in our study (11-years old). Out of the various studies conducted on this subject, the conclusion states that the maximum frequency applies to the 9 – 11-years old interval (Perez Alenza et al., 2000).

The radiological exam confirms the presence of two radiopaque areas at a pulmonary level (on the left diaphragmatic lobe, ⌀ ≈ 1.5 cm and on the right cardiac lobe, ⌀ ≈ 0,5 cm), which may belong to the category of multiple pulmonary metastases. Other conditions recorded were sternal lymphadenopathy, incipient mediastinal reactivity, hypertrophy of the aortic crutch, secondary to the reactivity of the pericardic madiastinal lymph nodes (Figure 2). Vincent (2010) recommends thoracic radiography in order to assess any possible pulmonary metastases and the sternal lymphadenopathy, including them within: single metastases, multiple metastases and interstitial ± peribronchial infiltrate (Vincent, 2010).

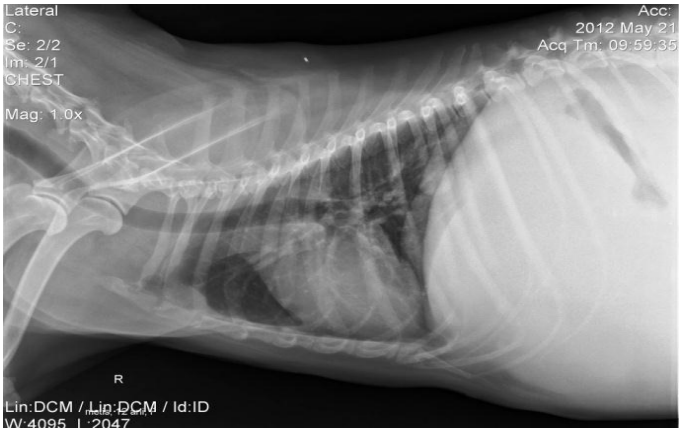

Figure 2. Pulmonary radiography, lateral incidence, confirming the presence of two radiopaque areas (multiple pulmonary metastases), sternal lymphadenopathy, hepatomegaly and hydroperitoneum.

With regard to the T.N.M. (tumour, lymph node, metastasis) classification, the female in the present study falls within the 4^{th} stage (T_4, N_1, M_1), as per the classification of Mangol et al. (1998) (Magnol et al., 1998).

Prior to the euthanasia, the female displayed severe dyspnea, a 4-day old anorexia, increased fever, abdominal colic and diarrhoea. Subsequent to the clinical exam and the additional investigations, a severe prognosis was established and the female was subjected to euthanasia.

After the lady dog's death the *necropsy* was performed, with an emphasis on the presence of pulmonary, hepatic, pancreatic, splenic and renal tumoral formations (macroscopically visible). From a pulmonary point of view, the study identified nodular formations of variable sizes, present both on the right, as well as on the left lung (on the left diaphragmatic lobe, size ≈ 1.5 cm, and on the right cardiac lobe, size ≈ 0.5 cm). These displayed a firm consistency, were compact and with a bacon-like appearance in section, against the lung's greyish-pink background. At the opening of the abdominal cavity a large amount of serosanguinolent liquid was observed (hemoperitoneum) (Figure 3).

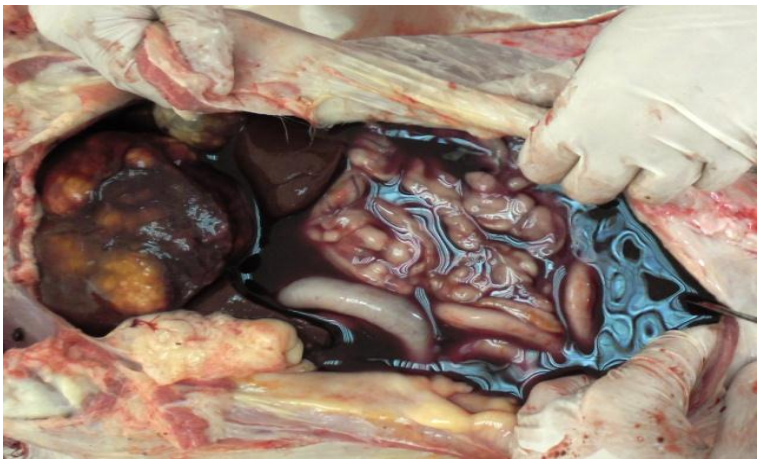

Figure 3. Abdominal laparotomy, emphasizing the hepatic nodular formations and the hemoperitoneum.

The necropsy identified multifocal modular hepatic formations, of variable sizes (ø 2-20 cm), disseminates within the entire liver mass. The right hepatic lobe was the most affected, being the host of a ≈ 20-cm nodule, followed by the right intermediary lobe (ø ≈ 8 cm) and the left lobe, which displayed two nodular formations measuring approximately 6 cm ø, situated at the lobe's poles (Figure 4). These presented a multinodular appearance in section, with a confluation tendency, with greyish-white bacon-like compact areas which alternated with small haemorrhagic areas.

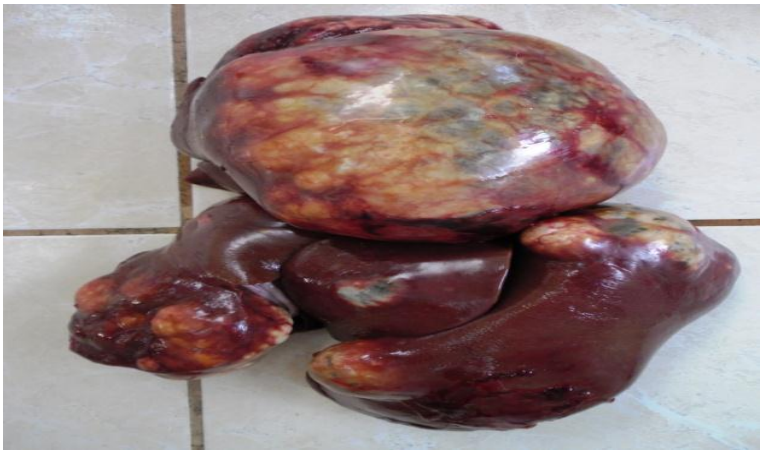

Figure 4. Multifocal nodular hepatic lesions, of variable sizes (hepatic metastases).

The sectioning of the large modules produced a viscous, dirty-greyish liquid. The pancreatic tumoral nodular formations were also disseminated within the entire organ mass. It was noticed, in the lateral-median area of the organ, a pedunculated formation approximately 4 cm in size, firm when palpated. The splenic region revealed a symmetrical splenomegaly with a stasis appearance and multiple greyish-white formations, compact in section, disseminated within the entire parenchyma. The renal region presented bilateral tumoral formations, the renal decapsulation was performed with ease, the left kidney displaying two tumoral formations, one approximately 2 cm in size, located at the cranial pole, with a cystic appearance (which comprises both the cortical area, as well as a part of the medullar area), a smaller one, ≈ 0.5 cm, situated on the dorsal side. A tumoral formation ≈ 1 cm was detected on the right kidney, close to the renal hilum, situated on the dorsal side. The lesional picture led to the suspicion of carcinomatous matastasis, having the mammary gland as a starting point, and was completed by a severe mesenteric lymphadenopathy.
The microscopic (histopathological) *exam* revealed the presence of a malign tumour, the diagnostic being the complex type mammary carcinoma, and confirmed that the remotely detected metastases have the same origin and the same histological pattern as the primary tumour.
The histologic aspects of the primary tumour (M_5 – inguinal mamma) and of the metastases were represented by the characteristic pattern of complex carcinomas, which comprises both epithelial proliferations, as well as proliferations of cells with a fusiform appearance, of mioepithelial origin, which affect both the mammary acini, as well as the ducts. The tumoral proliferation is associated with an inflammatory reaction (neutrophils, macrophages and lymphocytes). Notable aspects were the infiltrative nature of the tumoral cells, numerous clusters of necrosis within the tumoral mass, as well as a relatively high number of mitoses, all these representing high malignity criteria (Figure 5).

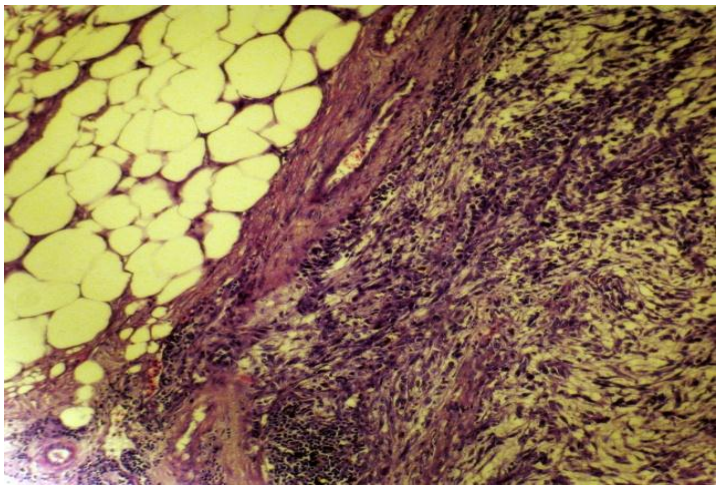

Figure 5. Histopathological exam, complex type mammary carcinoma, 100x.

From a histologic point of view, one can distinguish within the liver mass numerous carcinomatous clusters with a confluation tendency. Even if the tumoral formations seem to be delimited from the conjunctival stroma, it is penetrated by tumoral elements, which endows it with a marked infiltrative nature. The tumoral tissue morphology is heterogeneous, with areas, similar to the primary tumour, where the appearance of complex carcinoma prevails, with randomly positioned epithelial and mioepithelial cells (Figure 6).

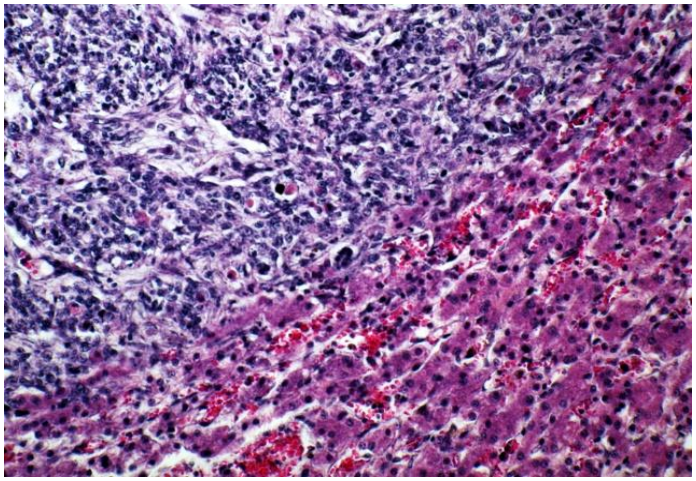

Figure 6. Histopathological exam, hepatic matastasis, 200x.

CONCLUSIONS

The present study was centred around a half-breed female, age 11, with mammary tumours at both chains and an extension of the metastases towards various organs: liver, lung, pancreas and kidney.
The ultrasound examination emphasizes areas with hypo- and hyperecogenity (suspicion of hepatic metastases).
The radiological exam revealed the presence of two radiopaque pulmonary areas of various sizes, included in the type of multiple metastases and, moreover, a sternal and mediastinal lymphadenopathy was identified, whereas the liver presented hepatomegaly and ascites radiological signs.
From a histopathological point of view, it was conformed that the metastases had the mammary gland as a starting point, with an emphasis on the type of complex mammary carcinoma.

ACKNOWLEDGEMENTS

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INFLUENCE OF AUTOLOGOUS PROSTATIC FLUID ADDED TO FROZEN-THAWED DOG SEMEN

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Abstract

The fertilizing capacity of dog spermatozoa depends on many factors, like: motility, plasmatic membrane integrity (viability), acrosome integrity. The role of the prostatic fluid in the fertilization process is still controversial. The aim of our study was to evaluate the effect of post-thaw dilution with autologous prostatic fluid on viability, motility and acrosome status of cryopreserved dog spermatozoa. Semen was collected from 6 Beagle dogs. The sperm rich fraction was frozen with a standard extender for dog semen containing Tris, fructose, glycerol and egg yolk (TFG-EY). For each dog, six straws were thawed: three straws were diluted 1:2 with autologous prostatic fluid, while the others were not diluted at all. Motility (CASA), viability and acrosome status (flow cytometry), morphology (Diff-Quick stain) were assessed at 5 minutes, 1 hour and 2 hours post-thaw (T0, T1, T2). There were no significant differences regarding the morphology of fresh and frozen semen. The addition of prostatic fluid significantly reduced the total and progressive motility and increased the percentage of reacted acrosomes at T0, T1 and T2 ($P < 0.05$). Although the addition of prostatic fluid did not affect the viability and the morphology of frozen-thawed semen, it reduced the motility and increased the percentage of acrosome reactions.

Key words: *cryopreservation, dog semen, prostatic fluid*

INTRODUCTION

Although there are many protocols developed for the cryopreservation of canine semen, fertilizing results still vary (Linde-Forsberg et al., 1999; Thomassen et al., 2006). There are a many known factors that influence these results: technique of semen collection, extender and the final concentration of spermatozoa (Okano et al., 2004; Pena and Linde-Forsberg, 2000), semen processing (Nothling and Shuttleworth, 2005; Rijsselaere et al., 2002), the combination of extender and cooling rate during the freezing procedure (Pena and Linde-Forsberg, 2000; Schafer-Somi et al., 2006; Silva and Verstegen, 1995; Sirivaidyapong et al., 2000), the thawing technique (Pena and Linde-Forsberg, 2000; Strom et al., 1997) and the use of a thawing medium (Oettlé, 1986; Okano et al., 2004; Pena and Linde-

Forsberg, 2000; Pena et al., 2003). Individual factors that make individual dogs or individual ejaculates more resistant to freezing and thawing damage of spermatozoa are also important (Holt, 2000; Pena et al., 2003; Thurston et al., 1999).

The prostate is the only accesory gland of the genital system in the dog. Consequently, the prostatic fluid is the main component of seminal plasma. The role of the prostatic fluid in the fertilization process is still controversial. In vivo experiments showed that prostatic fluid increased fertility of frozen-thawed semen: improved conception rate after intrauterine insemination (Hori et al., 2005), and yielded higher fertility rates after intravaginal insemination when prostatic fluid was added to frozen-thawed semen (Nothling et al., 2005; Nothling and Volkmann, 1993). In vitro studies showed a negative effect of prostatic fluid on sperm incubated at 37^{o}C (England and Allen, 1992). Prostatic fluid also has a detrimental effect on semen preservation if it is added to refrigerated semen (Rota et al., 1995b), refrigerated semen followed by freezing (Sirivaidyapong et al., 2001) and when it is added after thawing (Rota et al., 2007; Yamashiro et al., 2009). Conversely, other in vitro studies demonstrated no effect on frozen-thawed sperm (Koderle et al., 2009) or a positive effect of prostatic fluid added to frozen-thawed semen on fertility (Nothling et al., 2005; Nothling and Volkmann, 1993).

Spermatozoa undergo capacitation and acrosome reaction inside the female genital tract. One of the functions of dog prostatic fluid is to coat sperm membranes, thus masking progesterone receptors and delaying capacitation (Sirivaidyapong et al., 1999). The freezing–thawing process induces destabilization of sperm membranes, which is similar to capacitation (Rota et al., 1999).

The aim of our study was to evaluate the effect of post-thaw dilution with autologous prostatic fluid on viability, motility and acrosome status of cryopreserved dog spermatozoa.

MATERIALS AND METHODS

Semen was collected from 6 Beagle dogs (aged 4 to 7 years) by digital manipulation into pre-warmed tubes (+37^{o}C) and separated into the three different fractions (Kutzler, 2005). Each sperm rich fraction was assessed immediately after collection and the following parameters were determined: volume, motility (computer assisted sperm analyzer, CASA, IVOS, Hamilton Thorne, USA), concentration (Toma cell) and morphology (Diff-

Quick stain, 200 sperm cells under 100 magnification). Only good quality ejaculates were frozen.
The sperm reach fraction was diluted with a standard extender for dog semen containing Tris, fructose, glycerol and egg yolk (TFG-EY), cooled at 5^{o}C for 3 hours and the straws were placed at 7 cm above liquid nitrogen for 10 minutes before the transfer into liquid nitrogen.
The third fraction of each ejaculate (prostatic fluid - PF) was collected separately, centrifuged at 1118 x g for 10 minutes and the supernatant was frozen at -18^{o}C until use.
For each dog six straws were thawed: three straws were diluted 1:2 with autologous prostatic fluid, while the others were not diluted at all. Motility (CASA, IVOS, Hamilton Thorne, USA) (Rijsselaere et al., 2003), viability and acrosome status (flow cytometry) as described below, morphology (Diff-Quick stain) (Root Kustritz et al., 1998) were assessed at 5 minutes, 1 hour and 2 hours post-thaw (T_0, T_1, T_2).
Each semen sample was differentially stained with a combination of PI and PNA (both fluorophores purchased from IMV Technologies, L'Aigle, France). Two µl of PI were added to 2 µl of semen diluted in 191 µl of Easy Buffer® (IMV Technologies, L'Aigle, France) and incubated at 37^{o}C for 5 minutes. Five µl of PNA were added to the mix and incubated at 37^{o}C for 5 more minutes.
Flow cytometric analysis was performed using Guava EasyCyte® flow cytometer (IMV Technologies, L'Aigle, France). Each analysis consisted of a minimum of 5000 events and 500 spermatozoa, which were quantified simultaneously for green and red fluorescence. The side and forward scatter light scatter parameters were gated so that only those cells possessing the light scatter characteristics of spermatozoa were analyzed for fluorescence. Green (PNA) and red (PI) fluorescence were collected at 548 nm. Data were analyzed using the Data Acquisition and Analysis Software (Guava Technologies Inc.®, Hayward, USA).
The double stain allowed the identification of four sperm population: live and intact acrosome spermatozoa (L-AI), live and acrosome reacted spermatozoa (L-AR), dead with intact acrosome spermatozoa (D-AI) and dead with acrosome reacted spermatozoa (D-AR) (figure 1).

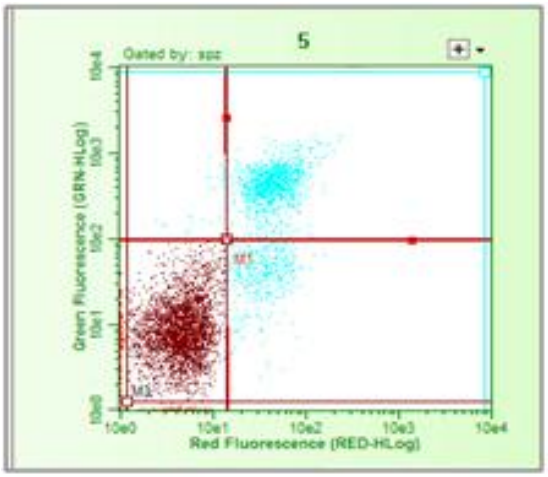

Figure 1. Histogram of a dual stained sample PI-PNA: low-left area – live-acrosome intact; low-right area – live-acrosome reacted; upper-left area – dead-acrosome intact; upper-right – dead-acrosome reacted.

RESULTS AND DISCUSSIONS

The experiment was replicated three times using two ejaculates from each of the six dogs. Statistical analyses were performed with IBM SPSS software (ver. 19 for Windows; IBM, New York, USA). The results are presented as mean values and a P value < 0.05 was considered statistically significant.
There were no significant differences regarding the morphology of fresh and frozen semen (with or without the addition of PF).

Treatment	Time	Total motility	Progressive motility	Live - AI	Live - AR	Dead - AI	Dead - AR
TFG- EY	T0	75,37 ± 9,13	71,34 ± 10,03	73,04 ± 10,03	9,76 ± 3,35	3,63 ± 1,92	13,56 ± 3,94
	T1	46,64 ± 19,56	42,51 ± 18,66	63,03 ± 5,37	12,71 ± 6,62	5,44 ± 3,63	18,99 ± 7,81
	T2	29,67 ± 19,38	28,13 ± 17,24	57,94 ± 5,45	12,88 ± 4,97	4,88 ± 2,78	24,30 ± 4,76
TFG-EY + PF	T0	63,08 ± 23,80	60,06 ± 23,80	64,21 ± 19,67	11,93 ± 7,95	6,70 ± 3,70	17,17 ± 13,69
	T1	34,93 ± 23,26	31,60 ± 22,85	51,23 ± 22,29	16,69 ± 9,62	8,36 ± 5,82	23,71 ± 17,33
	T2	23,96 ± 21,46	22,23 ± 20,91	37,03 ± 18,79	16,75 ± 9,47	10,30 ± 7,94	35,37 ± 18,00

Figure 2. Motility, viability and acrosome status data for the two groups of the experiment presented as mean±standard deviation.

The addition of prostatic fluid (PF) significantly reduced the total and progressive motility at T_0, T_1 and T_2. This finding partially disagrees with the results of a previous study (Nothling et al., 2005) that found no difference among thawed dog semen samples diluted with PF, saline or albumin-free TALP for the first 150 minutes. In our study, thawed semen samples from the TFG-EY group were not diluted at all and samples were evaluated for only 120 minutes. Our data contradicts the results of another study (Rota et al., 2007) where total motility of PF diluted samples were higher at T_0 and T_1. This study compared the PF diluted samples with Tris buffer diluted samples. The difference between our data and the two previous mentioned studies could be explained by the lack of diluter in the TFG-EY samples, so no external influence was exerted. Our results come in agreement to other studies where the addition of PF to ejaculated dog spermatozoa resulted in a more rapid decrease in the percentage of progressive motility compared to when no fluid (Gunzel-Apel and Ekrod, 1991), an egg yolk-Tris extender (Gunzel-Apel and Ekrod, 1991; Rota et al., 1995a) or minimal essential medium (England and Allen, 1992) were added to frozen-thawed ejaculated spermatozoa. In most of the studies that found a positive effect for the addition of PF used epididymal sperm (Nothling et al., 2007), not ejaculated spermatozoa that already had contact with PF like we did in this study.

The addition of PF significantly reduced viability at all times (T0, T1, T2). This partially agrees with the results of Rota et al. (2007) where PF did not prolong semen viability, without mentioning a decrease of this parameter. In this study the viability was estimated according to motility and no specific staining for viability was used.

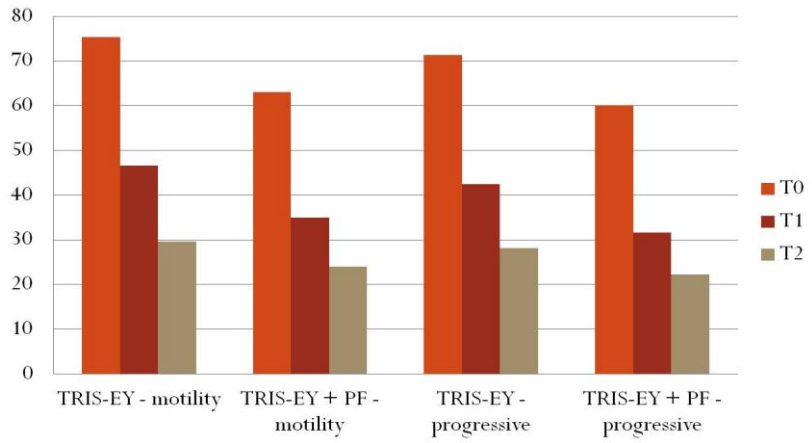

Figure 3. Comparison of total and progressive motility values

The addition of prostatic fluid significantly increased the percentage of reacted acrosomes at T_0, T_1 and T_2 ($P < 0.05$). This completely contradicts the results of another study (Rota et al., 2007) that found no influence of PF dilution upon acrosome status. The discrepancy could be explained by the method used to evaluate acrosome reaction: flow citometry in our study, Spermac stain for the other. Nöthling et al. (2005) suggested that PF might postpone the acrosome reaction based on the increased fertility obtained after intravaginal insemination, but without a specific method for quantifying this assumption.

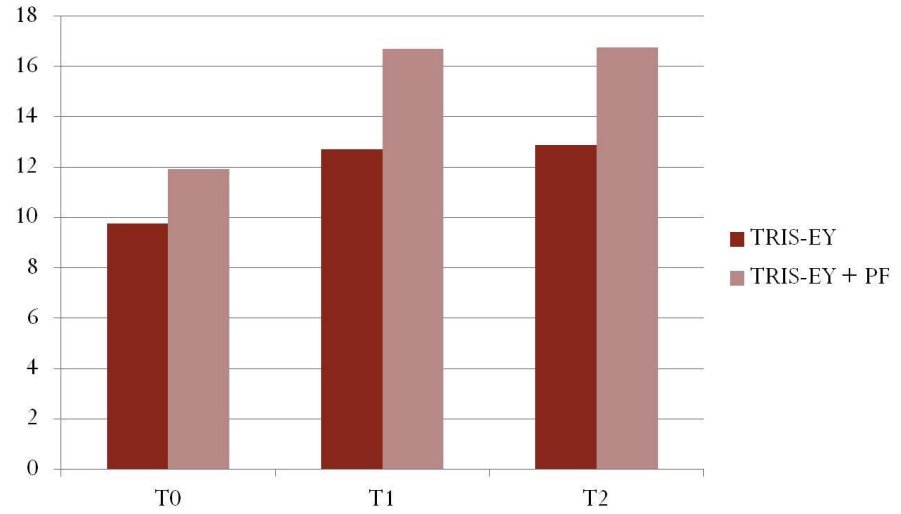

Figure 4. Acrosome reaction for live spermatozoa according to time dynamics

As laboratory analyses of semen can give only partial indication of semen fertility (Eilts, 2005; Nothling et al., 1997; Rijsselaere et al., 2005; Silva et al., 2006), our results and their impact on fertility should be further confirmed by a similar experiment with in vivo trials.

CONCLUSIONS

Although the addition of prostatic fluid did not affect the viability and the morphology of frozen-thawed semen, it reduced the motility and increased the percentage of acrosome reactions.

ACKNOWLEDGEMENTS

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THE EFFICACY OF DIFFERENT ACARICIDES AGAINST THE HARD TICK *DERMACENTOR MARGINATUS* ON INFESTED SHEEP

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Abstract

The aim of the present study was to evaluate the efficacy of four acaricids, from different pharmacologic groups, in control of tick infestation in sheep. Additionally, clinical specific aspects of the infestations were registered. The study was carried out during of March – April 2012, in a southern area of Romania (Colibasi village, Giurgiu County). The therapeutic efficacy of four different acaricides: Deltamethrin (pirethroid), Amitraz (formamidine), Diazinon (organophosphate), and Ivermectine (macrocyclic lactone) on natural tick infestation in sheep was evaluated. The animals (n=40) were divided into four groups (n=10/group) corresponding to the four used drugs. The first three products were used by dipping, in concentration of 0.05% for Delthametrin, 0.05% for Amitraz, and 0.04% for Diazinon. Ivermectine (1%) was subcutaneously injected (0.2 mg/kg). The animals were examined before and after treatment at 3, 5, 7, 14 and 21 days. The number of ticks per animal, the main elective body regions for tick attachment, and the associated lesions, were also registered. A total number of 1054 ticks (938 females, 116 males) were collected from infested sheep, all belonging to the Dermacentor marginatus species. The body areas highly infested were, in order of prevalence: the substernal area (63.6%), sides of the neck (14.2%) and the tail (7.1%). Lesions associated with tick infestation consisted of local irritations and inflammations, nodular dermatitis and micro abscesses. The best acaricidal efficacy was registered for Delthametrin (79.5%, at 3 days and 100% at 5 days p.t.) and Amitraz (90.4%, 96.4% and 100% at 3, 5 and 7 days p.t. respectively); both acaricids preserved full protection at 14 and 21 days after treatment. Ivermectin reached maximum efficacy at 7 days p.t. (92%), afterwards dropping at 79.8% at 21 days p.t..

Key words: *acaricids, Dermacentor marginatus, efficacy, infestation, sheep.*

INTRODUCTION

Ticks (Acari: Ixodidae) are ectoparasites with an important direct pathogenic role, but as well as vectors for many pathogens in human and animals (Estrada-Peňa et al., 2004). Tick infestation presents a serious challenge to farmers of ruminants in both developed and developing countries (Jongejan, 1999).

Ticks harm the hosts both directly and indirectly. Direct harm results from blood loss, tick burden as well as toxicoses. The bites can be injurious and cause severe hide damage including abscessation and can provide a route for secondary infection. Blood loss and reduction in weight gain resulting from tick feeding are among major factors that affect ruminant production in different parts of the world (Daynes et al., 1984).

Indirectly, ticks can cause economic loss because they play an important role as vectors of a wide range of pathogens to humans and domestic animals (Ioniță, 2004; Mitrea, 2011). Some arbovirosis, rickettsiosis, anaplasmosis, tularemiosis, babesiosis and theileriosis are pathogenic entities with great economic impact for animal production and some of them with zoonotic risks, too (Holdsworth et al., 2006).

Tick control is primarily based on the use of acaricides applied to animals on a systematic schedule, according to the local conditions. Various acaricides, including arsenic and DDT between the 1940s and 1970s, organophosphates between the 1980s and 1990s, and pyrethroids and amitraz from the mid 1980s to present time, have been used to control this economically important pest (Mitrea, 2011).

The major constraint of chemical treatment is selection for acaricide resistant tick strains. Inappropriate acaricide use (Bianchi et al., 2003) with incorrect concentrations probably contributes to the development of resistance, which leads to tick-control program failures (Pegram et al., 2000).

Hence, for an effective chemical control strategy, periodic monitoring of effectiveness is essential, especially in order to offer updated information on the efficacy of the different acaricides available on the market toward to provide an effective control against tick infestations on animals.

Therefore, the objective of the present study was to evaluate the efficacy of four acaricides, belonging to different pharmacological groups, in controlling naturally infestations with ixodide ticks of small ruminants. In the same time, the associated clinical aspects of tick infestations in animals were registered.

MATERIALS AND METHODS

The research was carried in a southern area of Romania (around Colibasi village, Giurgiu county), located between two rivers (Arges and Sabar). The climatic conditions (annual medium precipitations = 400-500 mm/m^2, medium temperature = 11ºC), local flora and fauna are favorable to the development of different species of hard ticks (Mitrea and Ionita, 2004).

The study was conducted during of March - April 2012 and included a number of 40 small ruminants (4 goats and 36 sheep - 34 females and 2 males), randomly assigned into four groups (A, B, C, D).
The animals, hybrids breeding in the area, with medium age of 2.08 years for goats and 5.3 years for sheep, identified with ear tags are breeds in extensive system, in population households.
The animals have been used pasture with approximately one month before starting the treatment, and they were continuing grazing also after they have been treated. No other treatments were applied.
The infestation degree of the animals studied was established by observing the ticks on the body of each animal.
Ticks were carefully collected from animals using forceps to ensure minimal mouthpart damage. Specimens were preserved in 70% ethyl alcohol, labeled, and were brought to the Parasitology Laboratory of Faculty of Veterinary Medicine, Bucharest, for taxonomic identification, using specific keys (Estrada- Pena et al., 2004).
The animals were clinically evaluated and various body parts of the infested animals have been examined. Subsequently, the animals were categorized as following: noninfested (no ticks on the body), low infested (mild) (1-20 ticks per animal), moderate infested (21-50 ticks per animal) and massively infested (over 50 ticks) (Teglas et al., 2005). The last three categories (mild, moderate, and high) were considered as "infestation."
Additionally, the associated lesions of the presence of ticks on animals were registered.
Group A, composed of 9 sheep and a goat, was treated with Ivermectine (IVM) 1% (Evomec – FarmaVet - Pasteur Institute) subcutaneous injections of 0.2 mg/kg, in a single dose. Group B, including 9 sheep (one male and 8 females) and a goat, was treated with Delthametrin (DMT) (Butox 50 – Intervet BSD) through bathing, in the concentration of 0.05%. Group C, including 9 sheep (2 males and 7 females) and a goat, was treated with Diazinon (DZN) (Diazinol – FarmaVet - Pasteur Institut) through bathing, in the concentration of 0.04%. Group D, comprised of 10 sheep, was treated with Amitraz (AMZ) (Taktic – Intervet BSD) through bathing, in the concentration of 0.05% (Crivineanu, 2008).
For bathing of each group, 60 liters of solution were used (approximately 6 liters for every sheep and 2 liters for goat).
After a single treatment with either of the above mentioned acaricides, the animals were examined for the presence of ticks on the body. The data are expressed as post-treatment tick burden on days 0, 3, 5, 7, 14 and 21.

The data collected were processed in Microsoft Office Excel 2007 program. The algorithm used to calculate the acaricide efficacy was:

$$\% \text{ Efficacy} = N_0 - N/ N_0 * 100,$$

where N_0 is the number of ticks before treatment and N is the number of ticks after treatment (Holdsworth et al., 2006).

RESULTS AND DISCUSSIONS

Dermacentor marginatus was the only tick species identified on the examined animals. A total of 1054 ticks were collected; of them, 938 were females and 116 males.

The overall tick infestation prevalence was 97.5% (39/40). The infestation degree was: low infested 64.1% (25/39), moderately infested 33.33% (13/39), and massive infested 2.56% (1/39).

The data regarding the parasitism dynamic intensity, for each lot after treatment are presented in Table 1.

In some subjects from group A, certain adverse reactions were noticed after 5-10 seconds of subcutaneously injected Ivermectine and persisted for about 45 seconds. Clinical signs consisted of bruxism, vaccilation, retropulsion, emprostotonus and circle movements. These aspects can incriminate the excipient of the product that produced an irritative reaction to the animals.

The highest intensity of the parasitism (56 ticks on a skin area with a diameter of 10 cm^2, in the neck region) was registered on an animal from group C, associated with obvious lesions like: thickened, low elasticity, hairless and pigmented skin.

Animals from group D presented the lowest degree of infestation, with a total number of 95 ticks collected.

Various body sites were categorized based on tick attachment preference on the infested animals: the substernal region (63.6%) was primary site of attachment, followed by neck region (14.2%), tail region (7.1%), internal side of the thigh (6.7%), legs (5.9%), and perineal region (2.5%) (Figure 1).

Table 1. **The ixodide tick infestation intensity on the four groups before and after treatment**

Day of examination	**Number of ticks**			
	Group A- Ivermectine	**Group B- Delthametrin**	**Group C- Diazinon**	**Group D- Amitraz**
Day of treatment	189	215	255	84
3rd day after treatment	104	44	43	8
5th day after treatment	35	0	7	3
7th day after treatment	15	0	0	0
14th day after treatment	12	0	8	0
21st day after treatment.	26	0	21	0
Total ticks	**381**	**259**	**334**	**95**

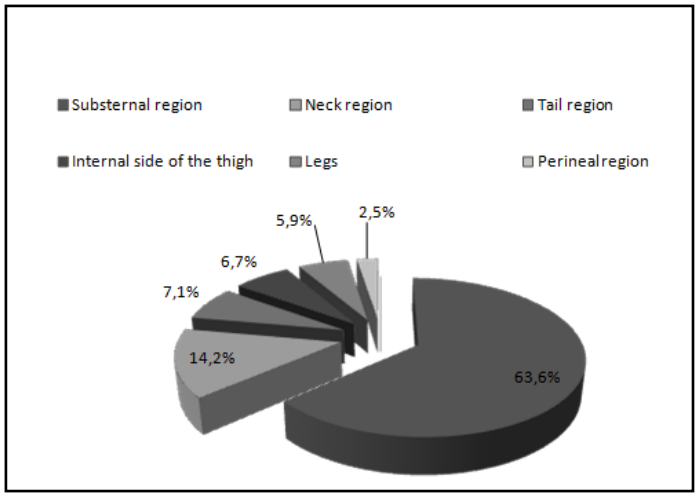

Figure 1.**The distribution of ticks according to their attachment preferences on the animal body** (in percentages)

Data regarding the efficacy of the acaricides used, expressed as percentage, at 3, 5, 7, 14 and 21 days post treatment, are presented in Table 2.

Table 2. **The efficacy dynamics of acaricides in relation to the day of examination**

Post-treatment	**Group A-Ivermectine**	**Group B-Delthametrin**	**Group C-Diazinon**	**Group D-Amtiraz**
3rd day after treatment	72,7%	79,5%	87,2%	90,4%
5th day after treatment	91%	100%	98%	96,4%
7th day after treatment	92%	100%	100%	100%
14th day after treatment	91,8%	100%	98%	100%
21th day after treatment	79,8%	100%	93,7%	100%

The control efficacy in terms of percent reduction in tick number decreased significantly at 7 days post-treatment, for all the four acaricides used (Figure 3).

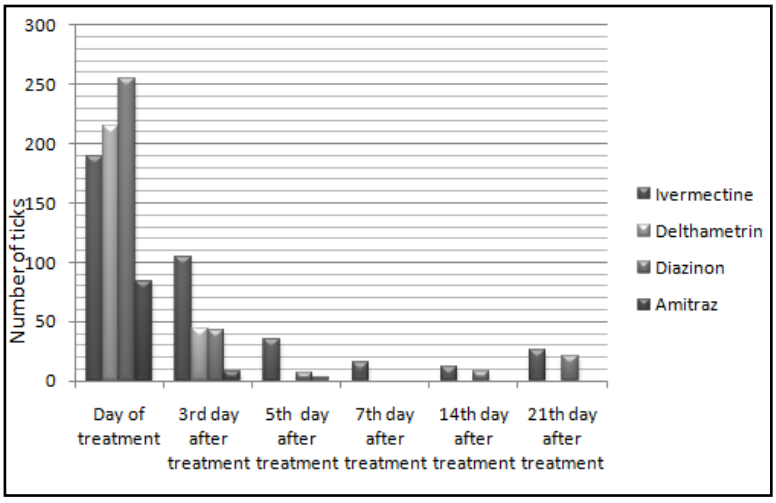

Figure 3. **Dynamics of the efficacy of the four acaricides**

In the IVM-treated group, the maximum reduction in number of ticks was found from days 5 to 7, until day 14, thereafter the tick infestation level started to increase. Therefore, IVM was not found to be effective in controlling the tick burden after 21 days post-treatment.

In the DTM-treated group, reduction in the number of ticks was significant higher, even from day 5 post treatment, being found effective even after 21 days post-treatment.
A similar, very good, efficacy was registered in the AMZ-treated group at 7 days post treatment, which has been maintained also at 21 days p.t.
For the DZN-group, the maximum reduction was obtained at 7 days p.t., however, at 14 and 21 days after the treatment the efficacy was lower.
Hence, the in vivo efficacy trials of DTM, AMZ, DZN (by bathing) and IVM (injectable) revealed better results for deltamethrin and amitraz.
The results obtained in the present study are in according with those of other authors. In a study carried out in Pakistan on 360 adult goats, the animals were submitted to treatments with pour-on Ivermectine and Cypermethrin 5% (other piretroid). The study revealed that Ivermectine reached maximum efficacy in the day 5 and 10 after treatment and continued until the 15th day. On the other hand, the group treated with Cypermethrin presented positive effects between day 1 and 5 of treatment, lasting over the 15th day. All substances used as bathing and as injections have better time coverage than substances used orally against endo- and ectoparasites (Sajid et al., 2011).
As lesions associated with tick infestations and their consequences, the following were registered: local swelling, followed by hypersensiti-vity reactions (type 1 and 4- due to foreign proteins found in the tick saliva), which manifest as local prurit (Coman, 2004); group stress; granuloma at the feeding site, which healed as a scar and lead to skin depreciation (Figure 2. A); alopecia on considerable areas with waistcoat depreciation (Figure 2 B); wet eczemas (Figure 2 C).
Other adverse effects, like septic complications, intoxications due to the anticoagulant substances secreted by the tick through its saliva and tick paralysis were reported in other studies (Barre et al., 2008; Sajid et al., 2011).

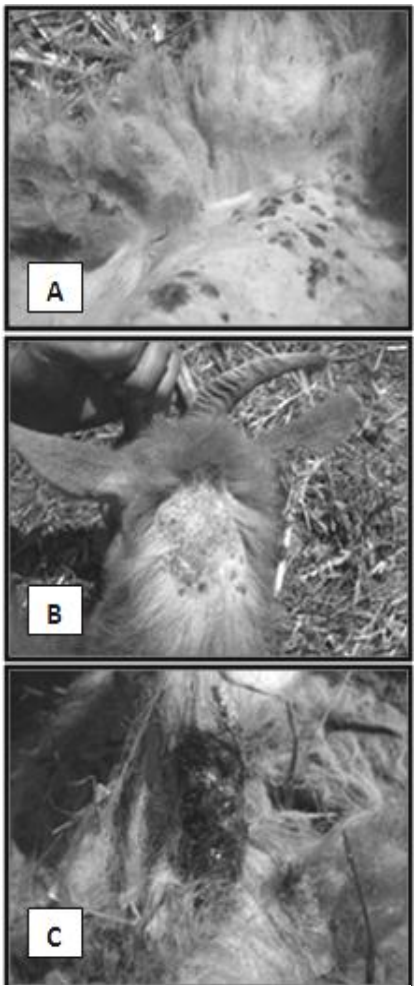

Figure 2. **Lesions associated with tick infestations**: A. healing phase granulomas; B: alopecia in the neck region; C: tail eczema

CONCLUSIONS

This study provides data on the efficacy of four different acaricides against *Dermacentor marginatus* infestation on small ruminants, revealing a maximum efficacy (100%) at 5 days after treatment for delthametrin and at 7 days after treatment for amitraz and diazinon. The therapeutic efficacy and protection against reinfestation was registered even at 21 days after treatment, when the last clinical examination was carried out.

These findings will help to promote an effective control of tick infestations on animals, based on a proper usage of the acaricides and on appropriate rotation sachems in order to avoid developing of chemoresistance.

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USE MOET PROGRAMME FOR DEVELOPMENT AND CONSERVATION OF SOME RACES OF BOVINE IN ROMANIA

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Abstract

In Romania there are populations of endangered cattle as Grey Steppe breed and Pinzgau of Transylvania and Romanian Buffaloes number is a marked decline. An important role in preserving the "ex situ" is biotechnology breeding populations vulnerable to conserve animal genetic information but not breeding material: gametes usually stored sperm as semen coming from a single parent (haploid) and embryos with 2 parents (diploid) population structure that allows preservation by freezing.
Material and method
MOET program implementation (Multiple Ovulation Embryo Transfer) to 4 gray cattle breed cow, repeated ovarian stimulation with FSH and PMSG intervals: 55, 60, and 115 and 157 days, and at 2 Holstein Friesian cattle every: 52 and 87 days.
Results
Following these repeated treatments Grey Steppe breed cows resulted in 99 of the corpus luteum (11CL/DR) from or taken Embryonic Formations 69 (7.66 FE / DR), of which 40 were embryos transferred and of these, 27 were frozen and stored in liquid nitrogen at -196 ° C. Following repeated application poliovulations FSH treatment every 52 to 87 days, 2 heifers HF, ovaries responded with a total of 31 CL 6.2-CL / DR to who prelevat12 FE and 2.4 FE / DR and 12 embryos were frozen blastocyst. FSH were poliovulations with a 4 cows and 6 Pinzgau of Transylvania Romanian buffaloes, a total of 10 female donors who have repeatedly poliovulations treatment, and the average number was the 8.4 CL / D, 1 CL lower than the average female with repeated poliovulations, the average number was 3.3 EFF EFF / D 3 also less than 6.7 EFF in the group of females with repeat poliovulations treatment.
Conclusion
We conclude that after several repeated hormone treatments poliovulations 6 embryo donor females found that health in general and particularly genital tract not suffer in comparison with the group of 10 females poliovulations once and morphology embryos was very good, allowing freezing and storage in gene banks

Key words: biotechnology, embryon, reproduction, bovine

INTRODUCTION

MOET raises fertility poliovulator only females treated several times to IA. Assuming that a cow M. T. 5 descendants are obtained by IA and are obtained by Moet 15, fertility has increased 3 times.
Scientific research in biotechnology breeding and their applications in practice zoo veterinary aimed mainly at increasing the number of livestock, livestock production, under increasing fertility, fertility and prolificacy. Preservation "ex situ" breeding animal biotechnology is to conserve animal genetic information but not breeding material: usually gametes, sperm stored as semen coming from a single parent (haploid) and embryos with 2 parents (diploid), which allows preservation of population structure by freezing (1.3). This method of protecting biodiversity is particularly useful for populations of rare or vulnerable state, not the obvious and immediate value in terms of trade and production. Of bovine monotocic species that an estrous cycle develops and open one mature follicle, ovulation latest development applied in the practice of multiple embryo donors became Moet. Moet relies on increasing donor embryo female fertility and hence the possibility of a higher selection intensity on the female sex bulls used in AI The multiple ovulation does not increase prolificacy donors because they do not girl born not many products, but only their fertility, their participation in designing a larger number of individuals in subsequent generations (5). Moet is a concept that can be used success, using embryo transfer in animals (6,8).

MATERIALS AND METHODS

All stages and phases of MOET program took place in SCDCB Dancu Grey Steppe breed Iasi (7), in Hunedoara County of Transylvania Pinzgau breed, SCDC Şercaia for buffalo and I.C.D.C.B. Baloteşti for Holstein-Friesian.
In our research conducted over several years at different locations bovine and we tried to give an answer to: poliovulations number of repetitions to be performed without damaging the overall health potential donor female genitalia and especially to preserve biological quality embryos and from what age can induce poliovulations the vines.
MOET concept (Multiple Ovulation Embryo Transfer) after M.PARASCHIVESCU (5) was initiated by Nicholas and Smith (1983) who considered embryo transfer (ET) as applied biotechnology to optimize genetic improvement of dairy cattle. Is multiple ovulation embryo transfer

stage biotechnological which makes a repeated poliovulations donor female (9). Poliovulation is the phenomenon of ovarian stimulation with FSH hormones or P.M.S.G. luteal administered during the oestrus following which are initiated and developed more follicles than normal is usually one species monotocic (6.8).

Steps in application MOET

Step - 1. Taken and preparation of donor females (D) embryos, comprising:

- Phase field: identifying females and gynecological clinical exameul, have more than 60 days after calving, the estrous cycle at regular intervals and without uterine infections, can take blood samples to determine the metabolic and hormonal profile in order to correct ration (9)

-phase Laboratory to determine progesterone (P4) and estrogen (E) ELISA kit DRG using EIA156.) (8.9)

Step- 2. Inducer poliovulation the donor and IA:

Includes phase field (application protocols poliovulaţie hormones FSH).

Protocol No. ET. .. / 2012

FARM:

Race ..... no. registration: D1 = ... D2 = ... ; Last calving: D1 .....D2 ..... + minimum 60 days post partum

V1. E.T.R. time-PGF 2 α .. I - date estrus after PGF 2 α I: D1and D2 and after 24 hours of estrus D1 and D2 is PGF 2 α I . R1; R2; R3: R4: R5 .

V2.+11days of PGF if I fall in heat will be the second PGF 2 α in D1 andD2 Day of estrus after PGF 2 α II and after 24 hours of estrus D1 and D2 is PGF 2 α II R1;R2; R3; R4; R5 . + 10 days after oestrus in D1 and D2 be Varianta1 (V1) and Version 2 (V2)

Poliovulation treatment of donors:

Hormone: FSH Pluset - company Serono, Italy, Laboratory of Spain

Day-1:….2012-AM-h7,00 -4 ml., im FSH/D1+D2

- PM-h.19 - 4 ml., im. FSH / D1+ D2

Day-2:….2012-AM-h7.00 -3ml., im. FSH/D1+D2

- PM-h.19 -3 ml., im, FSH / D1+ D2

Day-3: .2012 - AM-h.7,00 -3 ml., im. FSH / D1 + D2 of PGF 2 α II / R1-R5

- PM-h.19 -3 ml., Im, FSH + PGF 2 α / D1+D2

Day-4: ..... 2012 comments:

Day-5: ..... 2012-hour appearance will mark the donors who come into oestrus oestrus will ad. a 5 ml of receiver before performing PM

18-I h has I.A. x Code…….Taurus……..
Day-6: ..... 2012 AM-h II IA
+ 7 days after IA
Day of collection: 2012
Step - 3. Prelevation embryonic formation of embryos from donor female uteru. Phase Field: embryonic formation was taking the 7-day after IA, non-surgical techniques via the cervix, repeated lavage each uterine horn part, using type Folley catheters and Madi Dulbeco washing with PBS-BSA 0, 4%, and embryo collection has a special filter 22μ diameter.
Step - 4. Examination room equipped with a TV stereolupe and software acquisition and storage of images. Phase Laboratory formations were identified embryonic samples were evaluation and rated by international standards IETS Manual (2.4).

RESULTS AND DISCUSSION

Results concretized by the number of good embryos and blastocyst morula obtained from each donor handmade and embryos frozen embryo transfers, are presented in Table 1.
From Table no. 1, it follows that: 4 Grey Steppe cattle breed, repeated ovarian stimulation with FSH and PMSG intervals: 55, 60, 115 and 157 days, and at 2 Holstein Friesian cattle every: 52 and 87 days. Following these repeated treatments Grey Steppe breed cows resulted in 99 of the corpus luteum (11CL/DR) from or taken Embryonic Formations 69 (7.66 FE / DR), of which 40 were embryos transferred and of these, 27 were frozen and stored in liquid nitrogen at - 196 ° C. Following repeated application poliovulations FSH treatment every 52 to 87 days, 2 heifers HF, ovaries responded with a total of 31 CL 6.2-CL / DR to who prelevat12 FE and 2.4 FE / DR and 12 embryos were frozen blastocyst. FSH were poliovulate with a 4 Transylvanian Pinzgauer breed cows and laughter consisted of 34 ovarian CL, respectively 8,5 / D, which were taken 10 FE 2.5-EF / D and were frozen and stored 7 embryos. Were also poliovulate with FSH of 6 Romanian buffaloes and ovarian laughter was the CL 50 or 8.4 / D, which were taken 23 FE or 3.8 FE / D and have, were frozen and stored 16 embryos.

Table no. 1

RESULTS OF COLLECTION EMBRYOS FROM DONOR

No Crt.	Matricola of the donor	Date of collection day 7th	Interval in days from removals and FSH / PMSG	No. C.L. and Embryonic Formations Collected		Quality embryos M= morula Bl= blastocyst E.D.= embryos degenerate			Embryos transfer	Embryos frozen in EG or GL
				Nr. C.L.	Nr. F.E.C.	M	Bl	E.D. Ov.		
1	9999 Rec.1	05.04.06	FSH1000	9	6	2	1	3	3	-
	9999 Rec.2	05.06.06	I-II=60	17	7	0	0	7	0	0
2	0005 Rec.1	05.06.06	FSH	6	5	-	4	1	4	
	0005 Rec.2	08.10.06	I-II=115	9	0	0	0	0	0	0
3	0007 Rec.1	08.10.06	FSH	11	9	-	7	2	-	7 - EG
	0007 Rec.2	15.03.07	I-II=157	16	16	3	9	4	-	12 -GLY
	0007 Rec.3	10.05.07	II-III=55	13	12	5	7	-	6	6 - EG
4	0006 Rec.1	15.03.07	PMSG	13+6chi	9	-	1	8	-	1 - GLY
	0006 Rec.2	10.05.07	I-II=55	5	5	1	-	4	-	1 - EG
	4 Donoros Grey Step		m=55 M=157	99 (11 /R)	69 (7,66/R)	11	29 = 40 (5,44 Bl / D)	29	13	27
1	3618HFViţea	11.06.12	FSH500ui	7	4		4	0	0	4- EG
	3618 HF-14 l	03.08.12	I-II=52	7	7		7	0	0	7- GLY
2	1092HF heifer	11.06.12	FSH500ui	4+11chi	0		0	0	0	0
	1092HF-15 l	03.08.12	I-II=52	9	0		0	0	0	0
	1092HF	30.10.12	II.III=87	4+10chi	1		1	0	0	1- EG
	TOTAL 2-D Heifers HF		m=52 M=87	31 (6,2/R)	12 (6/R)	0	Bl-12			12
	6 donors Repet poliovulation		m=52 M=157	CL.130 (9,28/R)	FER.81 (6,7/R)	11	Bl-41 Total 52	29	13	39
1	39721-Pz	04.04.11	FSH500ui	11	0		0	0	0	0
2	91641-Pz	04.04.11	FSH	12	6		3	3	0	3-E.G.
3	0749-PZ	15.04.11	FSH	0	0		0	0	0	0
4	9506-Pz	15.04.11	FSH	11	4		0	0	0	4-E.G.
	TOTAL 4 -D. Pinzgau de Transilvania			34 (8,5 /D)	10 (2,5/D)	0	Bl-3	3		7
1	2761- B.	28.07.10	FSH1000	8	5		0	5	0	5- GLY
2	0014- B	28.-7.10	FSH1000	5	3		0	3	0	3-GLY
3	0133-B	23.08.10	FSH1000	8	0		0	0	0	0
4	26888-B	23.08.10	FSH1000	12	7		0	7	0	0
5	93037-B	29.03.11	FSH1000	10	5		5	0	0	5-E.G.
6	26810-B	29.03.11	FSH1000	7	3		3	0	0	3- E.G.
	TOTAL 6 donors Buffalo			50 (8,3 /D)	23 (3,8/D)		Bl-8	15	0	16
	10- D. poliovulation			84 (8,4/D)	33 (3,3/D)		Bl-11	18	0	23 Total 62

Step - 5. Freezing embryos
Laboratory-phase: good and very good embryos in morula stage (M) or blastocyst (Bl) were processed by passing at least 5 successive baths BSA0 PBS + 4%, each time changing the pipette tip and the last bath was used trypsin were then împaietaţi in the freezing glycerol or ethylene glycol, the Mid sparkles between two bubbles and two columns environment.
What 27 Grey Steppe breed embryos were frozen in glycerol (Gly) 10% - 13 and Ethylene Glycol (EG) of 1.4 M -14. The straw will write the code number of staff authorized to transfer, date of collection, number of embryos and stage of development (M or BI), registration number and name of donor-breed, number and name mareicol bull-breed. Freezing these embryos was gray cattle breed with a portable freezer E.Robertson type that does not use electricity. (Foto.nr.1)

Foto.nr.1

Portable freezer type E.Robertson

Portable freezer equipment is:
- exterior that Dewar vessel serves only as a container for liquid nitrogen (LN2).
- Inner Dewar vessel having double walls and a controlled vacuum from walls, like a thermos, is the only piece absolutely necessary to freeze embryos.
To start freezing, inner Dewar vessel filled with 350 to 400 ml of ethyl alcohol or methyl and outer Dewar vessel immersed in liquid nitrogen LN2.
Straws embryos are placed in the bowl with alcohol and freezing diagram starts at room temperature 18 -20 ° C with a rate decrease of 0.5 to 0.7 of

temperature ° C / min up to - 6 ° C, when alcohol and sparkles vessel is removed from the vessel with nitrogen and induce crystallization (seeding) with forceps previously cooled in liquid nitrogen. When all the sequins were plucked and alcohol temperature reached - 7 ° C, the vessel with alcohol and sparkles again to reintroduce nitrogen vessel where cooling is continued until the temperature of -32 degrees. Then sequins to remove and plunge into liquid nitrogen at -196 ° C, then pass the goblet and stored in the container.

Others, 35 embryos, collected from other races donor HF, Pz and Romanian Ox were frozen in 1.4 M EG was used freezer type Freeze Control (Photo No. 2.) With a rate of decrease of 1 C / min to -7 C, when the seeding induced for 10 minutes, then the temperature of 0.5 C / min.până at - 35 C, then plunges directly into liquid nitrogen

Foto nr.2

Freezing of gametes and embryos automatic with freezer

FREEZE CONTROL®

CryoPreservation Systems

CONCLUSION

It was found that the health in general and genital apparatus especially the 6 female donor (4-gray cattle and 2-HF) embryos, had suffered from the 14 treatments with FSH hormone repeated at intervals minimum of 52 days and a maximum of 157 days and ovaries response was 9.28 on average CL / DR to the average of 8.4 CL / D of the 10 donor females once poliovulate (4-Pz., and 6 buffalo).

The 2 HF heifers that were repeated hormone treatments with FSH at a minimum intrval 52 days and maximum 87 days, were aged 14 months and 15 months-3618-1092 and post-treatment ovarian response was an

average of 6.2 CL / DR without further consequences of cyclic ovarian activity.

From 6 donors were sampled 52 high-quality embryos, 39 embryos were frozen: 20 to Gly and 19 in EG and stored in liquid nitrogen containers at -196 ° C, the genetic resource banks: SCDCB-Dancu, General Berthelot and CSCBA "Acad.David Davidescu" - Bucharest. Were immediately transferred to 13 recipient embryos Grey Steppe breed.

MOET (Multiple Ovulation Embryo Transfer) is a biotechnology applicable in optimizing embryo transfer genetic improvement for multiplication and preservation of breeds of cattle.

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EVALUATION OF MICROFLORA ASSOCIATED WITH CANINE OTITIS EXTERNA

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Abstract

This study was undertaken to characterize otic microflora encountered in dogs with clinical signs of otitis externa and to determine its role in causing the disease. For this purpose 73 otic samples from normal dogs and 149 otic samples from dogs with different clinical stages of otitis were microbiological evaluated. The most common pathogens in the etiology of otitis include members of Staphylococcus genus, Streptococcus genus and yeast from Malassezia genus. From normal dogs Malassezia canis was isolated as a pure culture or with staphylococci and streptococci in 32 samples, representing 43.8%, staphylococci were recorded at a frequency of isolation (in pure and mixed cultures) of 32.9% and streptococci were isolated in 17 pure or mixed cultures, representing 23.3%. Staphylococcus aureus, Pseudomonas aeruginosa and Proteus spp. were not isolated in samples taken from dogs without ear problems. From dogs with varied clinical stages of otitis, Malassezia canis reported a frequency of isolation (in pure or mixed cultures) of 33.3%, staphylococci were isolated in 22.4% and streptococci in 19% from samples, in pure or mixed cultures. Staphylococcus aureus and Pseudomonas aeruginosa were isolated both in pure cultures and mixed cultures and Proteus spp. only in mixed cultures.

Key words: *canine, microflora, otitis, staphilococci, streptococci.*

INTRODUCTION

Canine otitis externa is one of the most common diseases encountered in veterinary practice and is estimated to affect between 5% and 20% of dogs (Gotthelf, 2005). Infectious otitis externa occurs as a secondary complication of primary factors that initiate inflammation within the external ear canal, such as hypersensitivity disorders (atopic dermatitis, food reactions, contact dermatitis), foreign bodies, ectoparasites, keratinization disorders, endocrine and autoimmune diseases (1,2,6).

Common pathogenic bacterial species include *Staphylococcus, Streptococcus, Pseudomonas, Proteus, Corynebacterium* and *Enterococcus* (2,4,5). The most common fungal pathogen in the etiology of otitis is

Malassezia spp., and rarely *Candida* or other saprophytic fungal organisms (7,8).
Considering multifactorial pathogenesis of otitis, treatments are varied and include topical therapy with antibiotic, antifungal or corticosteroid medication used alone or in combination (1,3,6). In canine otitis, the clinical diagnosis has an informative value, the adequate selection of a therapeutic method is conditioned by the results of the paraclinical diagnosis tests, imposed by the etiological polymorphism, especially microbiological examinations (2,8).
The purpose of the present study was to evaluate otic microflora diversity and involvement in emergence of various clinical forms of otitis.

MATERIALS AND METHODS

Cases were selected for study between January 2012 and October 2012 from dogs presented at Clinics of Faculty of Veterinary Medicine of Iasi, for ambulatory treatment, or hospitalization. Dogs of any age, breed or sex were eligible for enrollment.
The 73 healthy, normal dogs were selected as the control group, if there was no previous history of ear disease and no history of underlying disease (hypersensitivity disorders, keratinization disorders, endocrine and autoimmune diseases) and no clinical signs of ear or skin disease. Dogs in the control group were not currently on any medication other than preventive antiparasitic medication; dogs were not included if ototopical cleansers were used in the previous 2 weeks or systemic antibiotic/antifungal medication was administrated in the previous 4 weeks.
From dogs with different clinical stages of otitis, 149 samples were collected. Dogs were recruited based on history and clinical examination, which revealed the presence of characteristic signs (head shaking, local pain, pruritus, erythema, otorrhoea) in at least one ear. Complete physical and dermatological examinations were performed prior to collection of otic samples and physical examination findings were categorized to establish clinical stage of otitis. Dogs were not included if any topical or systemic therapy was administrated.
Samples were collected with a sterile culture swab introduced into ear canal and were sent to a private Microbiology Laboratory where they were processed according to conventional methods of isolation and identification.

RESULTS

From normal dogs (table 1) *Malassezia canis* was isolated as a pure culture or with staphylococci and streptococci in 32 samples, representing 43.8%, staphylococci were recorded at a frequency of isolation (in pure and mixed cultures) of 32.9% and streptococci were isolated in 17 pure or mixed cultures, representing 23.3%. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus* spp. were not isolated in samples taken from dogs without ear problems.

Table 1. Microorganisms isolation frequency from normal ear canal at dogs

Microorganism isolated	**No.**	**Frequency (%)**
Malassezia canis in pure culture	13	17.8
+*Staphylococcus* spp.	11	15
+*Streptococcus* spp.	8	11
+*Staphylococcus aureus*	-	-
+*Pseudomonas aeruginosa*	-	-
+*Proteus* spp.	-	-
Total	32	43.8
Staphylococcus spp. in pure culture	6	11
+*Streptococcus* spp.	5	6.9
+*Staphylococcus aureus*	-	-
+*Pseudomonas aeruginosa*	-	-
+*Proteus* spp.	-	-
Total	24	32.9
Streptococcus spp. in pure culture	4	5.4
+*Staphylococcus aureus*	-	-
+*Pseudomonas aeruginosa*	-	-
+*Proteus* spp.	-	-
Total	17	23.3
Staphylococcus aureus	-	-
Pseudomonas aeruginosa	-	-
Proteus spp.	-	-
TOTAL	**73**	**100**

Data in Table 2 reveals that *Malassezia canis* was isolated in 50 samples from dogs with varied clinical stages of otitis, representing 33.3%, of which 18 samples in pure culture. In mixed culture *Malassezia canis* was identified with staphylococci in 11 samples (7.4%), with streptococci in 9 samples (6%) and with *Staphylococcus aureus* in 8 samples (5.3%). *Malassezia*

canis recorded a frequency of isolation of 1.3%, both with *Pseudomonas aeruginosa* and with *Proteus* spp., respectively in 2 samples.
Staphylococci were isolated in 34 samples, representing 22.6%, from which 10% in pure culture. In mixed cultures *Staphylococcus* spp. recorded a isolation frequency of 6% with Streptococcus spp., 3.3% with *Staphylococcus aureus*, 2% with *Pseudomonas aeruginosa* and 1.3% with *Proteus* spp.

Table 2. Microorganisms isolation frequency from dogs with varied stages of otitis

Microorganism isolated	**Clinical stage**	**No.**	**Frequency (%)**
Malassezia canis in pure culture	C	18	12
+*Staphylococcus* spp.	E	11	7.4
+*Streptococcus* spp.	E	9	6
+*Staphylococcus aureus*	S	8	5.3
+*Pseudomonas aeruginosa*	S/U	2	1.3
+*Proteus* spp.	S/U	2	1.3
Total		50	33.3%
Staphylococcus spp. in pure culture	E	15	10
+*Streptococcus* spp.	E	9	6
+*Staphylococcus aureus*	S	5	3.3
+*Pseudomonas aeruginosa*	S/U	3	2
+*Proteus* spp.	S/U	2	1.3
Total		34	22.6%
Streptococcus spp. in pure culture	E	15	10
+*Staphylococcus aureus*	S	10	6.7
+*Pseudomonas aeruginosa*	S/U	3	2
Total		28	18.7%
Staphylococcus aureus in pure culture	S	2	1.3
Total		25	16.7%
Pseudomonas aeruginosa in pure culture	S	1	0.7
Total		9	6%
Proteus spp. in pure culture	S	-	-
Total		4	2.7%
TOTAL		**149**	**100%**

C – ceruminous otitis
E – exudative otitis
S – supurative otitis
U – ulcerative otitis

In pure and mixed cultures, *Streptococcus* spp. recorded a isolation frequency of 18,7%. In pure culture were isolated in 15 samples (10%), with *Staphylococcus aureus* in 10 samples (6.7%) and with *Pseudomonas aeruginosa* in 3 samples (2%).
In pure culture *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated in 2, respectively in 1 sample, recording a total isolation frequency (in pure and mixed cultures) of 16.7%- *Staphylococcus aureus* and 6%- *Pseudomonas aeruginosa*.
Proteus spp. was identified only in mixed cultures in 4 samples (2 with *Malassezia canis* and 2 with *Staphylococcus* spp.), representing 2.7%.
Data obtained reveals that in ear canal of dogs there are commensal and pathogenic conditioned bacteria so that, the isolated and identified bacteria in otic samples collected from dogs with various forms of otitis did not confirm the determinative role in external otitis emergence, but only its favoring role. Microorganisms that are normal resident flora or opportunistic pathogen rapidly colonize the ear canal when microclimate is alterated in early clinical forms resulting progressive deterioration.

CONCLUSIONS

The most common pathogens in the etiology of otitis include members of *Staphylococcus* genus, *Streptococcus* genus and yeast from *Malassezia* genus.
Malassezia canis was isolated (in pure or mixed cultures), in samples collected from normal dogs and also from dogs with varied clinical stages of otitis.
Staphylococcus aureus, Pseudomonas aeruginosa and *Proteus* spp. were not isolated in samples taken from dogs without ear problems.
From dogs with otitis *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated both in pure cultures and mixed cultures and *Proteus* spp. only in mixed cultures.
Comparative evaluation of ear flora (in health or disease) did not confirm the determinative role in external otitis emergence, but only its favoring role.

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VARIATIONS OF GLYCAEMIA AFTER ALFAXALONE INDUCTIONS IN RABBITS. PARTIAL RESULTS.

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Abstract

Alfaxalone is a neurosteroid with general anesthetic properties. His steroid structure, analog with progesterone, might have an influence on patient glycaemia. The aim of our study was to assess variations of glycaemia after alfaxalone induction.
For this study we used 7 female adult laboratory rabbits premedicated with fentanyl (0.0125 mg/kg) and droperidol (0.625 mg/kg) intramuscular. Alfaxan® was administered intravenously at a total dose of 3mg/kg by a constant rate infusion over 60 seconds using a syringe pump. Blood samples were drawn prior to and at 2, 4, 6, 10, 15, 20, 30 after induction by an indwelling catheter in the central auricular artery for instant blood glucose determination.Mean value of arterial blood glucose recorded was 154,36 mg/dl (81-262) with the highest peak at minute 4 after induction.
Glucose variation remained within normal limits with the highest value at minute 4. Other studies showed an increased in plasmatic glucose after progesterone administration. Women using the progesterone-T intrauterine device showed blood glucose increased after three hours. A study made on rats showed an increase of blood glucose 30 minutes after progesterone administration.
The theory that alfaxalone may influence plasmatic glucose have to be further studied on a higher number of animals, however is interesting the fact that in minute four after induction when rabbits suffered the most profound cardio-respiratory depression, blood glucose was at the peak. More extensive monitoring and a pharmacokinetic study are needed to comment accurately on alfaxalone effects on rabbits.

Key words: *alfaxalone, blood glucose, rabbit.*

INTRODUCTION

Alfaxalone is an analog of progesterone with neuron-active properties. It is registered for use in dogs and cats as Alfaxan®. It was also tested with good results on horses, ruminants, rabbits and other small mammals, reptiles and even amphibians. The study wants to demonstrate alfaxalone action over blood glucose level in rabbits because of its steroid structure.

MATERIALS AND METHODS

Six healthy young adult rabbit females conducted the research, all normal laboratory white breed. Food and water were not withheld prior to premedication. They were kept in a controlled environment. All rabbits were premedicated with the same dose of fentanyl 0.0125mg/kg (Sublimaze®, 50µg/mL) and droperidol 0.625 mg/kg (Droleptan™, 2.5 mg/mL) (Canellas J. et al., 1996; Strack L.E. et al., 1968; Tillman P. et al., 1983; Walden N.B., 1978) intramuscular in the lumbar region 15 minutes prior to cannulation and kept in a special contention box. Both ears were shaved and disinfected with chlorhexidine soap and alcohol to be prepared for vein and artery cannulation. For catheterization we used 22G catheters (blue) (Diehl K.H. et al., 2001; Morton D.B. et al., 2001; Sjøberg J.G. et al., 2003). Vein catheterization was realized over lateral auricular vein and was used for anesthesic induction. Arterial catheterisation was realised over central auricular artery and it was used for blood sampling (Tutunaru A.C. et al., 2012).

Induction was produced using alfaxalone (figure 1) 3 mg/kg (Alfaxan®, 10 mg/mL) was administered intravenously through a 22G catheter in the lateral auricular vein by a constant rate infusion over 60 seconds using a syringe pump (Marsh M.K. et al., 2009).

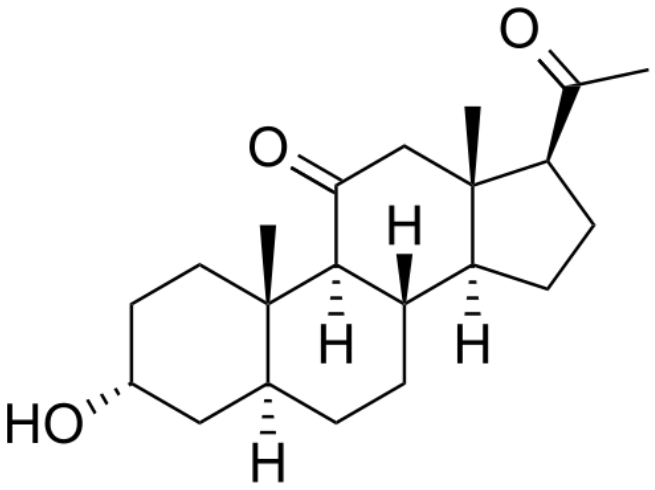

Figure 1. Alfaxalone chemical structure

All rabbits were intubated using blind technique with 3.5 mm endotracheal tubes and they breathed spontaneously room air. Respiratory function was assessed by measuring respiratory rate, the oxygen saturation of hemoglobin in the peripheral blood (SpO_2) and the end-tidal CO2 ($EtCO_2$). Cardiac function was assessed by measuring heart rate using a pulse oximeter. The level of anesthesia was monitored by evaluating ear and paw pinch reflex, as well as ocular signs such as nystagmus, exophtalmia and the loss of palpebral and corneal reflexes.
Arterial blood samples were taken befor alfaxalone induction and in minute 2,4, 6, 10, 15, 20, 25 and 30 after induction. Glicemia was assesed using a clasical glucometer (Ascensia® Contour® Blood Glucose Meter, Bayer®).

RESULTS AND DISCUSSIONS

Arterial blood glucose mean value was 154.36 mg/dL (range: 81-262) with the highest peak at minute 4 after induction (Figure 2).

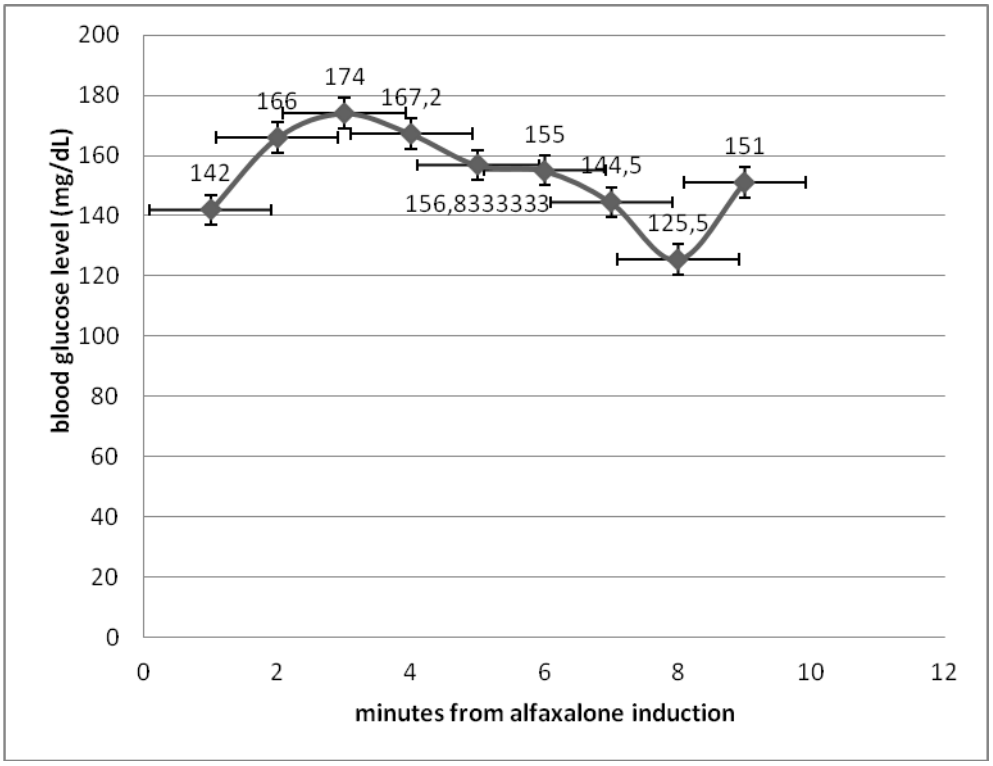

Figure 2. Blood glucose variation before and after alfaxalone induction

The mean respiratory rate recorded was 39 breaths/minute (range: 16-65). Two rabbits showed apnea after induction for ten seconds and respectively two minutes. Oxygen saturation (SpO_2) had a mean value of 91% (range: 42-100). The minimum values were recorded in those two rabbits which suffered of apnea in minute 2-4 after induction. Mean end tidal CO_2 recorded ($EtCO_2$) was 24.8 mmHg (range: 13-37).Mean heart rate recorded was 198 beats/minute (range: 115-300) with the lowest value at minute 4 after induction.

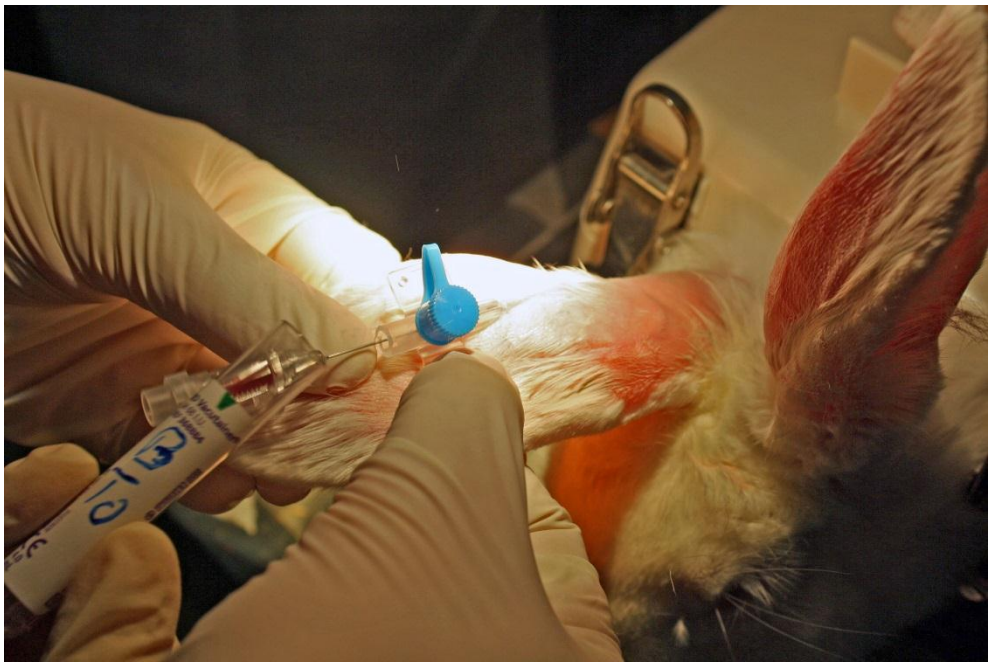

Figure 3. Blood sampling from central auricular artery using a vascular catheter

Alfaxalone is a synthetic neuron-active steroid. In this research we wanted to check for the first time if alfaxalone, as a steroid and a progesterone analog, can modify blood glucose levels. Glucose variation remained within normal limits with the highest value at minute 4, the minute with most pronounced cardio-respiratory depression. Amer et al. (1990) showed that on goats increased glucose levels results from administration of Saffan. Other studies showed an increased in plasmatic glucose after progesterone administration. Women using the progesterone-T intrauterine device showed blood glucose increased after three hours (Spellacy et al., 1978). A

study made on rats by Mei-Po and Yang M.M.P. (1970) showed an increase of blood glucose, 30 minutes after progesterone administration but this study also demonstrates that adrenal medulla is apparently involved in the hyperglycemic effect of progesterone. Another study on rats showed that Althesin® injected intra-peritoneal did not modify blood glucose concentration (Mezza et al., 1981).
The theory that alfaxalone may influence plasmatic glucose have to be further studied on a higher number of animals, however is interesting the fact that in minute four after induction when rabbits suffered the most profound cardio-respiratory depression, blood glucose was at the peak. More extensive monitoring and a pharmacokinetic study are needed to comment accurately on alfaxalone effects on rabbits.

CONCLUSIONS

We recorded a blood glucose peak even if it had not exceeded physiological limits correlated with alfaxalone maximum action over cardio-respiratory functions suggesting that alfaxalone influence glucose plasmatic level.
More pharmacological and pharmacokinetic studies are needed over alfaxalone induction in rabbits to demonstrate its effect over blood glucose.

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INFLUENCE OF HIGH TEMPERATURE ON REPRODUCTION IN SOWS

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Abstract

Temperature is the key factor to maintain thermal homeostasis at warm-blooded animals.
High temperature is a constant factor that influences reproduction at sows,where were found disturbances in the ovary, egg, embryo and fetus.
Existence of critical periods in reproductive process is more vulnerable to heat stress than others.
Excessive temperature of over 25 ° C,induce sows great harm materialized in weight loss during lactation, metabolic compensation efforts, reduced fertility, lactation capacity, the extension of prolificacy and unproductive.
Is also worth mentioning that there was a decrease in voluntary food intake.
Ambient temperature in the roof shelter of sows is recommended to be secured around 20 ° C, where maintenance is done in individual piggery without bedding.
Where is necessary to ensure bedding, temperature may be lower.
In conclusion, the temperature has an important role in improving reproduction indicators.

Key words: *indicators, reproduction, sows, temperature.*

INTRODUCTION

Temperature is probably the most critical factor in the physical environment for pigs, because pigs have a thermoregulatory inefficient (I.Dinu). Experiments in controlled environment conditions and semicontroled showed that temperature affects growth rate, feed efficiency, animal behavior, and reproductive efficiency by decreasing fecundity, prolificacy and birth (AT Bogdan, 1999).
In sows, high temperatures have adverse effects on reproduction, as

evidenced by the low levels recorded in worm season.So were made research on seasonal variations in conception rate, estrous cycle length and number of piglets about high temperature(Teague, Warnick, Tompkins, Swiestra, Holmes et al., Legault)
Depending on the severity of heat stress and humidity and degree of acclimatization, sows subjected to high ambient temperatures, showed anormal estrous behavior (Warnick et al., Teague et al.) or during ovulation (d'Arce et al .).
The results obtained by different researchers show that high ambient temperature adversely affects fertility and prolificacy sows.
So, if halls with average temperature in the shelter of 17-18 ° C the proportion of sows entering oestrus in a flock was 5% in summer to 28 ° C was 3.5 to 3.7% (J. Gadd,2005).
Monthly average temperature rise of 1 ° C reduce the duration of oestrus + / - 6hours or appearance of silent estrous .In regarding fertility, sows installed in January remained pregnant in 80%, while those mounted in august in proportion of 47%. Biggest influences on fecundity have high temperatures in the first eight days after mating, so that later to exercise on fetal development and parturition weight achieved.
The negative effects are amplified when high temperature is accompanied by direct solar radiation by housing animals in paddocks. Access at shadows may be the solution for the protection of direct sunlight, but not to the high air temperatures .
Controlled effect by intermittent sprinkling sows using automatic devices or by shower.Showers increase prolificacy in summer average of 2.35 piglets / sow (RJ Smits, 1999)
Provided antepartum temperature during parturition and 2-3 days postpartum will fit in values 24-26°C (PW Ferguson et al, 1985). Temperatures above 25 ° C reduce appetite in lactating sows, feed intake may decrease with 2kg/day decreases milk secretion and consequently piglets.
In problems in such conditions, watering the front of the sows in breastfeeding can be a salutary solution Klober Kelly, 1997

Table 1. Influence of air temperature on reproductive performance of sows
(after Teague and colab.)

Reproductive performance	**Air temperature**		
	26,7°C	**30**°C	**33,3**°C
Number of sows	74	80	80
Number of sows mounted	74	78	73
Number notentered in heat	0	2	7
Number returned in heat	2	8	8
Number notentered,notpregnent	5	2	3
Number pregnant at 25 days after mating	67	67 *	62
% pregnant at 25 days after mating	90,5	84,8	77,5

*) a sow died 3 days after mating

Increasing air temperature decrease affected the percentage of pregnant female at 25 days after mating.
Maintenance and temperature control are generally more difficult and more critical in higher temperatures than in the low temperatures.
Cooling using various environmental control facilities and spray with cold water are important in reducing the effect of high temperature on reproduction in sows.
This measures are required to be applied during the pre-assembled, installed and gestation in places with high temperatures.
In current conditions it is estimated that there are better ways to control animal environment, but also on the progress made in the field of genetics and improvement, by applying a suitable selection can be changed and type of animal product.
So the researchers are able to act on both units for concomitant improvement of temperature, and the animals can become more adaptive to these environmental conditions.

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This study was developed in the POSDRU/107/1.5/S/76888 contract.

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PROBIOVIT PHYTOTHERAPY EFFECTS OF DIARRHEAL SYNDROMES IN PIGS

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Abstract

Apiphitoterapy, branch of unconventional therapies provide health through detoxification and repair functions affected, reinstalling homeostasis of the body and its relationship with the environment. Getting Probiovit product apiphitoterapeutic of propolis and vegetal mass resulting from the process of winemaking was done by percolation battery hydroalcoholic extract in optimizing extraction parameters (time,temperature, granulation plant material, report solvent / plant material) in order to achieve optimal concentration of flavones, lectins, pectin. The preparation is a natural product derived from plants with anti-diarrheal properties. It is easy to administer at young piglets during treatment in diarrheal syndromes, hypotrepsic, and the stress of weaning piglets.

Key words: *diarrheal,piglets, probiovit,syndromes*

INTRODUCTION

Checking efficacy Probiovit (herbal extract) therapy nonspecific enteritis in piglets to control hypotrepsic and diarrheal syndrome.
Conducted research objectives consisted in:
Obtaining herbal product Probiovit powder and tincture and tested "in vitro" and "in vivo"
Lectins of this product isolation and study their effect in pathology of hypotrepsic syndrome and diarrhea in swine
Productive performance evaluation of piglets receiving Probiovit powder

MATERIALS AND METHODS

The experiment was conducted during the 2010-2011 academic year at the Congregation pig farm Jesus Popesti Leordeni.Young pigs included in the experiment was obtained in farm mothers F1 (Landrace & Large White) and terminal boars (50% and 50% Petrain Hamshire) transferred speakers growth at the age of 60 days.
In test plots were:
Group I. experimental - E 1 - 52 piglets treated with diluted Probiovit 50% in 0.85% saline, 1 ml / kg twice daily for 5 consecutive days oral.
Group II. witness - M1 - 54 piglets treated with streptomycin 0.1 g / kg and Efitard 10,000 IU / kg / day for 5 consecutive days intramuscular.
Group III. witness M2 - Composed of 20 piglets hypotrepsic 33 days of age were treated with saline 1 ml / kg;
Group IV. experimental E2 - hypotrepsic composed of 20 piglets treated with Probiovit 1ml/kg weight 50% dilution in saline 0.85% - for 5 consecutive days.
Group V. witness M3 - consisting of 6 animals - fed with a concentrated blend without added Probiovit.
Group VI. Experimental E3 - consisting of 6 animals - fed with the same mixture but added Probiovit dose of 10 kg / tone.

Table 1

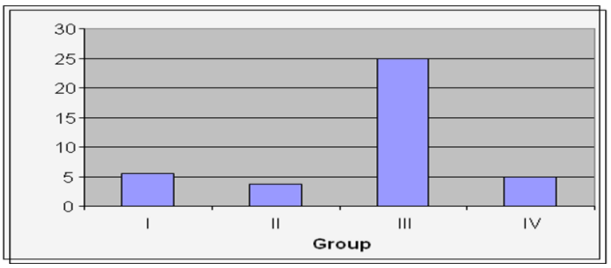
Group	**Average initial weight (g)**	**Final average weight (g)**	**Average growth for the period 10 days**	**Mortality rate (%)**
Group I	2210	2910	700	5,5
Group II Probiovit	2165	3320	1155	3,8
Untreated group III	2920	3420	500	25
Group IV Probiovit	2880	3380	500	5

Probiovit powder and tincture herbal product was investigated biochemical and toxicological and have identified and characterized lectins contained.Were made also metabolic profile investigations (biochemical and haematological) in piglets from lots located in experiment. Were performed on animals weighing experiment and evaluated average daily gain, average daily consumption, specific consumption, especially for clinically healthy piglets weaned lots.

RESULTS AND DISCUSSIONS

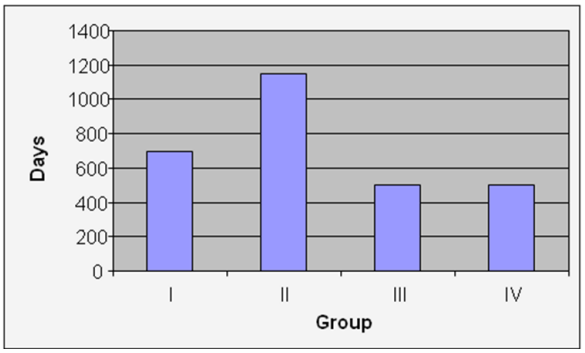
Therapeutic effectiveness in combating diarrhea and Probiovit product hypotripsic interpreted on the basis of assessing body weight and mortality rate (%)

Table 2.Increase growth medium weight piglets during the 10 days of experiment



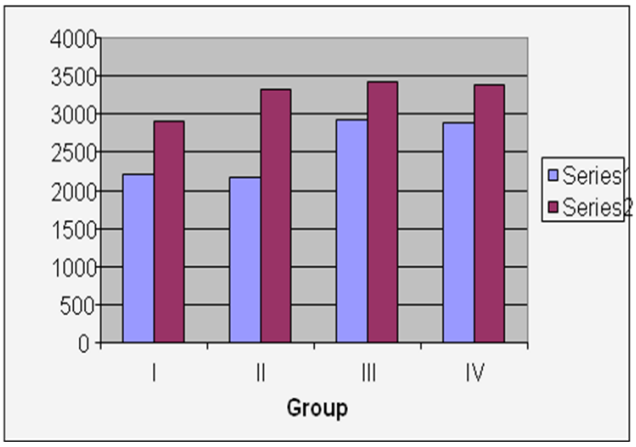
Group I - control group infants
Group II - experimental and infants (Probiovit)
Group III-witness hypotrepsic
Group IV - hipoterpsic experimental II (Probiovit)

Table 3.Evolution lots mortality of piglets treated / untreated Probiovit during 10 days of experiment



Group I - control group infants
Group II - experimental and infants (Probiovit)
GroupIII-witness hypotrepsic
Group IV - hipoterpsic experimental II (Probiovit)

Table 4.Evolution of the average weight of piglets in the experimental groups before and after the experiment



Group I - control group infants
Group II - experimental and infants (Probiovit)
Group III-Mart hipotrepsic
Group IV - hipoterpsici experimental II (Probiovit)

Table 5.Evolution of average daily gain (g) on stage and the entire experimental period 33-155 days (weaners)

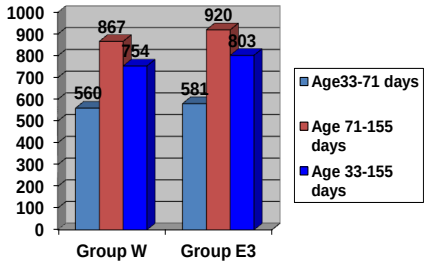
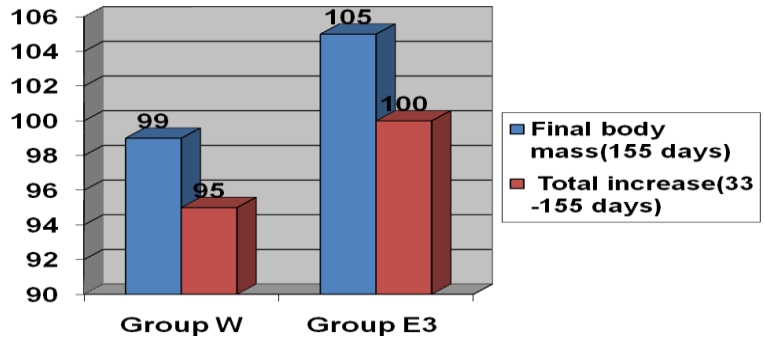


Table 6.The influence of Probiovit product on growth indices at pigs tested



CONCLUSIONS

This paper has established the presence of lectins (protein fractions with similar electrophoretic properties and activity agglutinated) in Probiovit product.

Given antidiarrheal effect exerted by Probiovit preparation, we believe that lectins interact with different bacterial, preventing their proliferation.

It also appeared that there were no side effects from treatment Probiovit,and suggests that lectin doesn't have negative effects.

Due to their properties, we concluded that it is possible to use purified lectins in preparation Probiovit therapy diarrheal syndrome.

Although mortality rates of piglets with neonatal diarrhea in experimental and control groups are similar, the average gain during the experiment at pigs treated with Probiovit is superior and the product must be used in the treatment of neonatal enteritis in piglets.

Percentage of mortality, although lower in group hypotrepsic piglets treated with Probiovit (5% vs. 25% for the control group) may not require use of the product in control piglets hipotripsic, final average gain is the same in both groups.

Inclusion in weaned piglets Probiovit product, the rate of 10 kg / tone of feed did not induce significant changes in hematological and biochemical parameters of blood.

Probiovit product administration in feed resulted an increase in body mass by 6.13% compared with controls, which is below the threshold of statistical significance.

The group on which the feed was added *Probiovit,* average daily gain was improved.

33-71 days during specific consumption was reduced by 9.3%, and the entire experimental period to 8.58 in the group to which the feed was supplemented with Probiovit being statistically significant.
The effects of product management and weaned piglets Probiovit to recommend it as a prophylactic and therapeutic product strictly diarrhea significantly more effective than currently used antibiotics.

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This study was developed in the POSDRU/107/1.5/S/76888 contract.

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COMPARATIVE THERAPEUTIC APPROACH OF CANINE TRANSMISSIBLE VENEREAL TUMORS (TVT)

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Abstract

Introduction*: In Romania, monochemotherapy using exclusively Vinca Rosea alkaloids created mutant cellular clones of Sticker sarcoma, activating MDR severe mutant genes.*
Materials and Methods*: During this study, a number of 10 dogs with TVT, of different breeds, genders and ages have been studied. Blood tests, X-Rays, coagulation profile, biochemistry of the blood, urine dipstick, abdominal ultrasound and cytology - FNA from the biopsy mass were performed. All the submitted samples were analyzed in reference laboratories from both Romania and Netherlands. The history, clinical and histological findings were all compatible with TVT. The approach was different in the two Clinics. In UK, the therapeutic approach was different, using Vincristine 0,7mg/m^2 week one, repeated every 7 days three more times. In Romania, monochemotherapy created mutant cellular clones of Sticker sarcoma. Therefore, polychemotherapy has been used (genital localization, expansive and proliferate pattern with no metastases). Preoperative, neoadjuvant polychemotherapy for cytoreduction, based on ciclophosphamide 50mg/m^2 or ifosfamide 200mg/m^2, cyclo dependent cytostatics, and 5-fluorouracil as an antimetabolite, 50mg/m^2 and Vincristine 0,7mg/m^2 week one, repeated every 14 days or after surgery to prevent recurrence.*
Results*: All cases treated in UK with Vincristine and all Romanian dogs treated with polychemotherapy shown remission of the penile masses and complete healing. In Romania, monochemotherapy created mutant cellular clones.*
Conclusion*: Numerous cases of TVT in the free dog population in Romania and uncontrolled breeding, along with the absence of neutering (castration) favored the spread of tumors and the transmission of resistance from one dog to another.*

Key words: *canine, polychemotherapy, Sticker, transmissible, TVT.*

INTRODUCTION

Transmissible venereal tumor (TVT) still bears the name of infectious sarcoma venereal granuloma, transmissible lymphosarcoma or Sticker tumor (Hasler and Weber, 2000).
TVT is a reticuloendothelial tumor of the dog, which is usually localized at genitalia, occasionally at the level of internal organs (Morrison, W.B., 1998).

It is a contagious cancer that is transmitted along with viable cells and fails to cross the barriers of the major histocompatibility complex between dogs and between family members in the Canidae family such as foxes, coyotes and jackals (Martins et all, 2005, Mukaratirwa and Gruys, 2003).
TVT was first described by Novinsky in 1876, which showed that the tumor could be transplanted from one susceptible host to another by inoculation of tumor cells (Rebbeck et all, 2009). The transmissible agent causing canine transmissible venereal tumor (CTVT) is thought to be the tumor cell itself (Murgia et all, 2006).
Numerous cases of TVT in the free dog populations of Romania have favored the spread of tumors and transmission of chemotherapy resistance from one dog to another. It usually responds very well to polychemotherapy, although there are areas with small populations which show satisfactory results to monochemotherapy (Rogers et all 1998).

MATERIALS AND METHODS

During this study, a number of 10 dogs with TVT, from different breeds, genders and ages have been studied. (Figures 1-4)
Blood tests, X-Rays, coagulation profile, biochemistry of the blood, urine dipstick, abdominal ultrasound and cytology - FNA from the biopsy mass were performed. All the submitted samples were analyzed in reference laboratories from both Romania and Netherlands. The history, clinical and histological findings were all compatible with TVT.
The approach was different in the two Clinics. In UK, the therapeutic approach was different, using Vincristine 0,7mg/ m^2 week one, repeated every 7 days for three more times. In Romania, at the Faculty of Veterinary Medicine in Bucharest, it was observed that monochemotherapy based exclusively on the Vinca Rosea alkaloids, among which the most widely used are Vincristine and Vinblastine, created mutant forms of Sticker's sarcoma cell clones with MDR gene activation (multidrug resistance) and serious side effects on the treated animal. Therefore, polychemotherapy has been used (genital localization, expansive and proliferate pattern with no metastases). Preoperative, neoadjuvant polychemotherapy for cytoreduction has been used based on cyclophosphamide 50mg/m^2 or ifosfamide 200mg/m^2, cyclo dependent cytostatics, and 5-fluorouracil as an antimetabolite, 50mg/m^2 and Vincristine 0.7mg/m^2 week one, repeated every 14 days or after surgery to prevent recurrence. A batch of Vincristine 0.7 mg/m^2 treated dogs using 3 doses in 7 days was created. (Table 1)

Table 1. The two treatment protocols in the Faculty of Veterinary Medicine Bucharest and
North Downs Specialist Referrals

CLINIC	NUMBER OF CASES	TREATAMENTS	TREATMENT LENGHT
Faculty of Veterinary Medicine Bucharest	**4**	***Cyclophosphamide 50 mg/m^2 or Ifosfamide 200 mg/m^2, and 5-Fluorouracil, 50 mg/m^2***	**At 14 days, alternating 3 treatments**
	3	***Vincristine 0,7 mg/m^2***	**3 treatments at every 14 days**
North Downs Specialist Referrals	**3**	***Vincristine 0,7 mg/m^2***	**4 treatments at every 7 days**

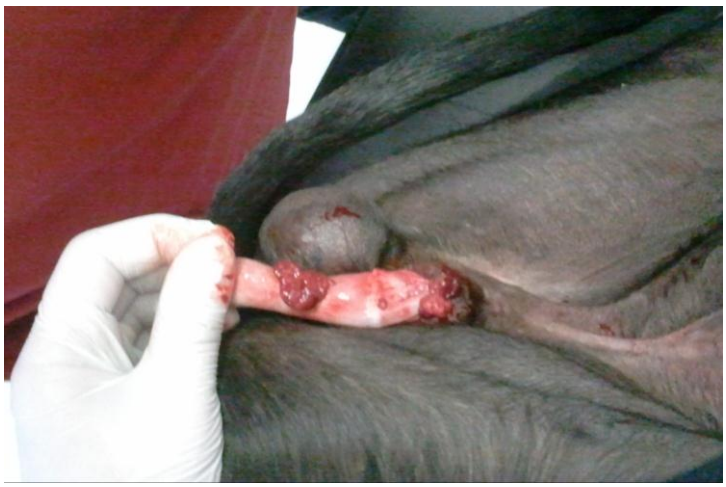

Figure 1. Sticker tumor at a 5 years old English Greyhound male (orig.)

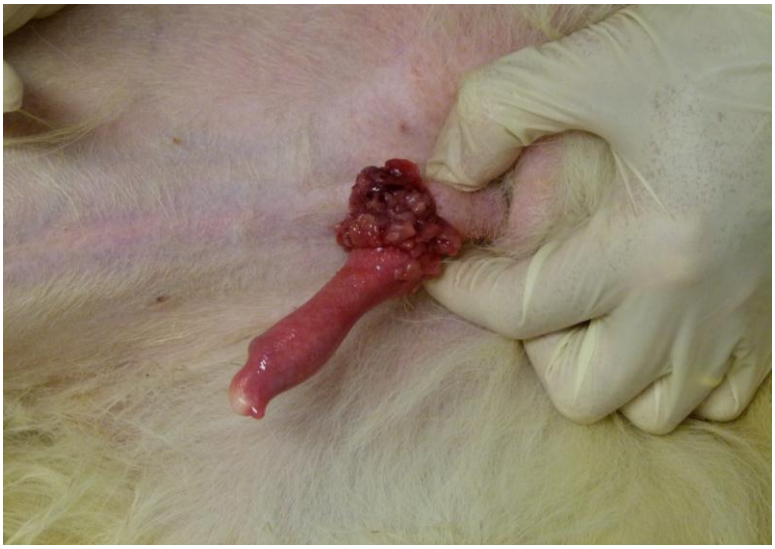

Figure 2. Sticker tumor at a 10 years old Samoyed male (orig.)

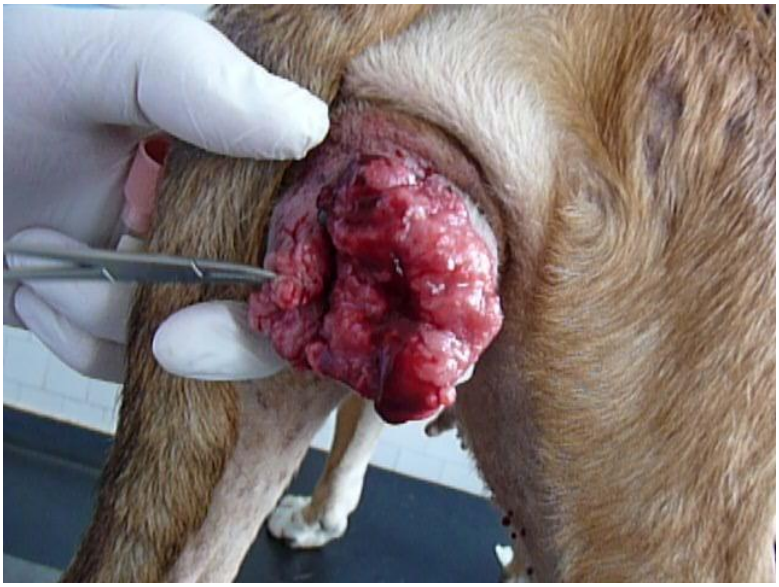

Figure 3. Sticker tumor at a 7 years old Amstaff female (orig.)

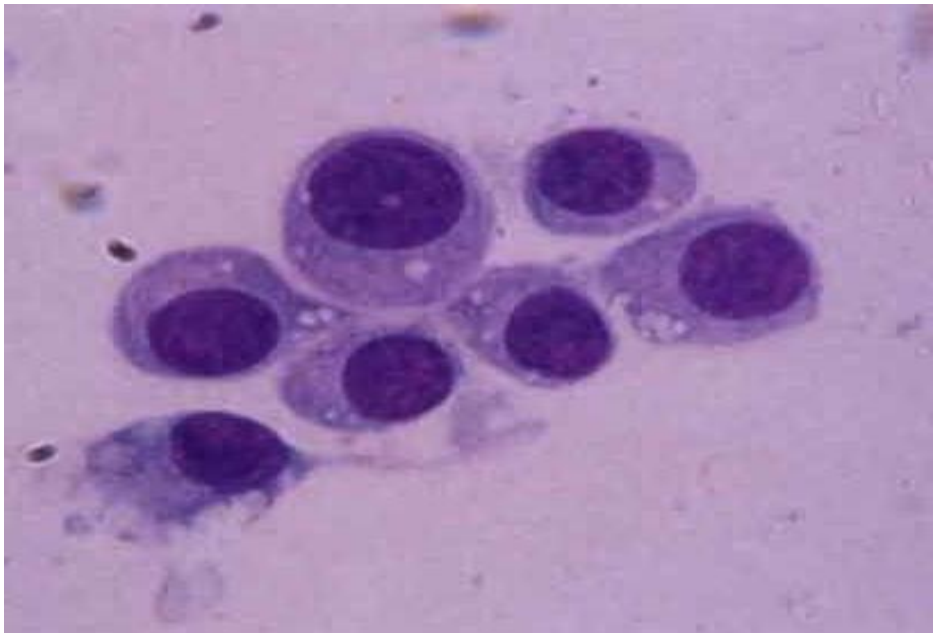

Figure 4. TVT round or slightly polyedric cells, with thin cytoplasm in which vacuoles and a round, hyperchromic nucleus with a single nucleolus and a low number of mitotic elements are visible (orig.)

RESULTS AND DISCUSSIONS

In Romania, monochemotherapy based solely on Rosea Vinca alkaloids created mutant cell clones and produced incomplete healing and recovery with local or general metastasis. All cases in our study treated unilateral with Vincristine, generated returns.

All cases treated in the UK, with Vincristine ended in complete remission and healing masses.

Out of the 4 cases in Romania treated using preoperative neoadjuvant multi-agent cytostatic, a total of three cases (75%) were completely cured and had no recurrences. (Figures 5, 6)

Table 2. The two treatment outcomes using the protocols in the Faculty of Veterinary Medicine Bucharest and North Downs Specialist Referrals

CLINIC	**NUMBER OF CASES**	**TREATAMENTS**	**TREATMENT OUTCOME**
Faculty of Veterinary Medicine Bucharest	**4**	***Cyclophosphamide 50 mg/m^2 or Ifosfamide 200 mg/m^2, and 5-Fluorouracil, 50 mg/m^2***	**Completely healed (3, 75%)**
	3	***Vincristine 0,7 mg/m^2***	**Incomplete healing or recurrence (3, 100%)**
North Downs Specialist Referrals	**3**	***Vincristine 0,7 mg/m^2***	**Completely healed (3, 100%)**

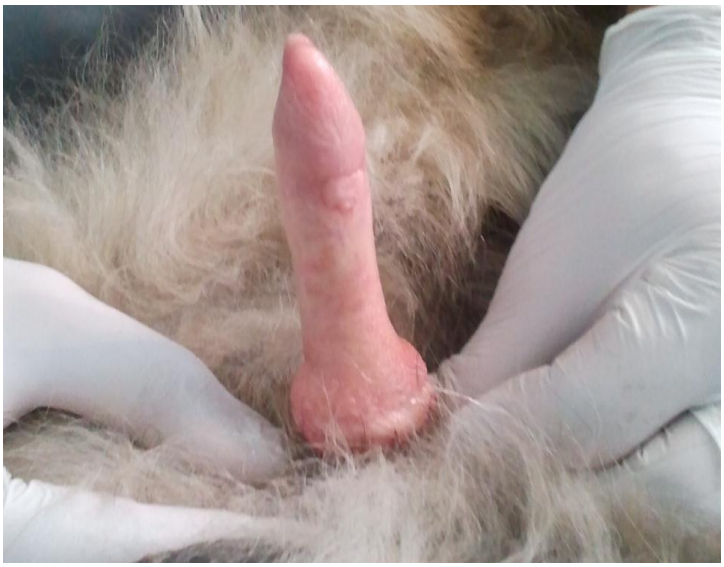

Figure 5. Sticker tumor remission at a 10 years old Samoyed male after treatment using polychemotherapy (orig.)

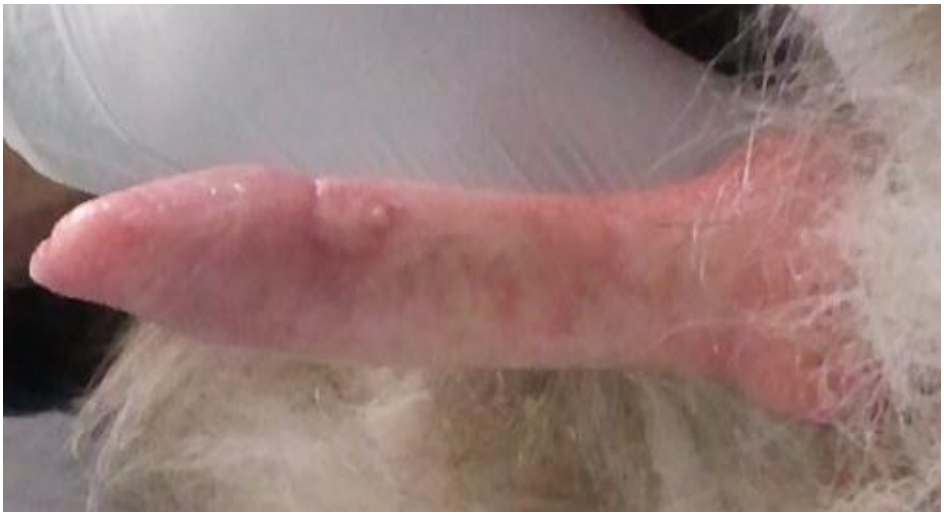

Figure 6. Sticker tumor remission at a 10 years old Samoyed male after treatment using polychemotherapy - detail (orig.)

CONCLUSIONS

Numerous cases of TVT in Romanian free dog populations and uncontrolled breeding, along with monochemotherapy based solely on Vinca rosea alkaloids, among which the most widely used are Vincristine and Vinblastine, created mutant forms of Sticker's sarcoma cell clones with MDR gene activation (multidrug resistance) and serious side effects on the treated animal.

Lack of castration favored tumor spread and resistance transmission from one dog to another.

Vinca alkaloids intervene in the S phase of the cell cycle blocking the specific mitotic division spindle of normal eukaryotic cells. Cancer cells do not have spindle of division, multiplying exclusively by amitotic direct division, thus having no Vincristine or Vinblastine sensitivity. Thus, Vincristine alone cannot be considered a cure for TVT.

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URETEROENTEROSTOMY IN THE DOG AND THE RESPONSE OF THE HEMATOLOGICAL PARAMETERS AFTER SURGERY

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Abstract
Monitoring the blood parameters in dogs undergoing ureteroenterostomy surgery reveal major changes in their values. Two weeks after surgery, creatinine, hemoglobin and total protein levels are within normal limits in case 1 and 2. In exchange, urea remains high compared to normal values even two weeks after the surgery.

Keywords: *uretero-enterostomy, dog, urea, creatinin, total protein.*

INTRODUCTION

Preoperative assessment of renal function in dogs undergoing surgery for ureteroenterostomy and post-op assessment through the ability to eliminate urine through the intestine, permeability of the ureteral in the anastomosis to the intestine, absorption of urinary components in the gut with consequent increases in blood levels was performed by monitoring the parameters: urea, creatinin, hemoglobin and total protein.

MATERIALS AND METHOD

Three clinically healthy dogs respective three females aged 1.2 (case 1), 1.5 (case 2) and 6 years (case 3) underwent surgery for ureteroneterostomy. The animals were subjected to a diet food (not fluid) for 12 hours before surgery. Since the ureteroenterostomy operation has functional consequences on the urinary organs, we collected blood and urine to assess the integrity of the urinary function, based on laboratory tests (Eberhard, 1998; Capatana et al., 1994).
We determined the blood concentrations of urea, creatinin, hemoglobin and total proteins. Of the urine samples were determined: density, pH, proteinuria. We examined the urinary sediment (both organized and unorganized) as well as the macroscopic aspect.
Before the operation we determined bleeding and clotting times.

RESULTS AND DISCUSSIONS

Before ureteroenterostomy operation, in the case of the three animals on which the experiment was performed, we used the assessment of the kidney function through blood parameters determined by laboratory examination in order to evaluate the condition of the urinary tract (Gherghariu et al., 2000).

Table 1. Normal values of blood parameters monitored

Blood	Urea mg/dl	Creatinin – mg/dl	Hemoglobin – g/dl	Total protein – g/dl
Normal values	20 - 50	0,5 – 1,6	12 - 18	5,5 – 7,5

Table.2. Preoperative values of blood parameters in case 1

Blood	Urea – mg/dl	Creatinin mg/dl	Hemoglobin – g/dl	Total protein – g/l
5.03.2002	39,2	1,44	19	6,6

In case 1 (table 1.), the values obtained were compared with normal blood values and the following results were found: urea, creatinine and total protein fall within physiological limits, hemoglobin is slightly low (above the maximum physiological value of 1g/dl).

Table.3. Preoperative blood parameters values in case 2

Blood	Urea – mg/dl	Creatinin mg/dl	Hemoglobin – g/dl	Total protein – g/l
13.03.2002	13,7	0,59	17,1	6,6

In case 2 (table 2.), preoperative urea is low, with 6.3 mg / dl compared to the lower limit, and creatinine, hemoglobin, total proteins fall within the normal range.

Blood parameter values in case 3, preoperatively, are as follows: urea is within normal limits, creatinine slightly increased, slightly decreased hemoglobin, total proteins slightly decreased.

Postoperative laboratory examinations were performed to assess the ability to eliminate urine out through the intestine, the permeability of the ureteral anastomoss to the intestine and possible absorption of urinary components in the gut with consequent increases in blood levels.

Table 4. Evolution of blood parameters, in case 1 postoperative

Nr.	Blood parameters		Surgery day 5.03.	6.03	8.03	10.03	19.03
1	Urea	mg/dl	39,2	112,3	210,5	153,3	97
2	Creatinin	mg/dl	1,44	1,22	1,58	1,41	1.28
3	Hemoglobin	g/dl	19	16,4	16,6	16,5	16,3
4	Total protein	g/dl	6,6	6,8	7,0	7,2	7,1

Table 5. Evolution of blood parameters, postoperatively in case 2

Nr.	Blood parameters		Surgery day 13.03.	14.03	16.03	18.03	27.03
1	Urea	mg/dl	13,7	125,5	212,7	193,2	75,5
2	Creatinin	mg/dl	0,59	1,24	1,60	1,57	1,15
3	Hemoglobin	g/dl	17,1	15,3	16,8	16,5	13,2
4	Total protein	g/dl	6,6	6,7	8,0	6,8	5,94

Table 6. Evolution of blood parameters after surgery in case 3

Nr.	Blood parameters		Surgery day 12.03.	13.03.	15.03.	17.03.	26.03.
1	Urea	mg/dl	24,5	161,8	317,5	-	-
2	Creatinin	mg/dl	1,7	1,68	3,01	-	-
3	Hemoglobin	g/dl	11,2	13,3	16,7	-	-
4	Total protein	g/dl	5,3	7,9	9,8	-	-

Before the ureteroenterostomy operation, the blood urea falls within physiological limits, slightly decreased in case 2. Following surgery, blood urea increased to very high values compared to the physiological limits,

reaching a maximum value about three days after surgery, and then begins to decrease, reaching two weeks after surgery almost to the upper limit, but still at increased values compared to normal (table 4,5,6). In the 6 years old dog, the general condition worsened progressively, so that on the third day after surgery the animal produced hypothermia (36.2), clonic contractions of the head muscles with pronounced trismus, neck muscle myoclonus, all these symptoms are correlated with blood urea values of 317.5 / which is a huge value compared to the normal (table 6.)(Jubb et al., 1985). On the fourth day after surgery, the animal died. Correlated with other determinations, creatinine and total proteins, this increase in the urea was due on the one hand to the disturbance of urinary flow in the anastomosis and on the other hand to the absorption of urinary components in the gut, of course to little extent.

In all three animals subjected to ureteroenterostomy surgery, creatinine increased postoperative, reaching a maximum on day 3, very close to the upper limit in the first case (table 4), at the upper limit in case 2 (table 5) and slightly above in case 3 (table 6). Monitoring the postoperative creatinine values, they are found to be within the normal range, respective a good renal tolerance of the renal parenchin of the surgery on the lower urinary tract (Gherghariu et al., 2000).

In all three cases which were evaluated postoperative, hemoglobin decreased slightly after surgery due to intraoperative bleeding, but returned rapidly to values falling within physiological limits.

Postoperatively, it is interesting to see the increase in the values, which exceeded the upper limit in cases 2 and 3, but gradually return to normal after the 4^{th} day after surgery.

CONCLUSIONS

After ureteroenterostomy surgery in dogs, monitoring the blood parameters reveals four major changes of values.

Two weeks after surgery, creatinine, hemoglobin and total protein are within normal limits in case 1 and 2.

Urea remains high compared to normal values even two weeks after the surgery.

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