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CUPRINS

THE EFFECTS OF ANTIDIURETIC HORMONE (VASOPRESSIN) ADMINISTRATION ON THE CONTRACTILITY OF THE ARTERIAL PREPARATIONS IN RATS COMPARED WITH PETS

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Abstract: *Vasopressin known as antidiuretic hormone is responsible for regulating plasma osmolality and volume. It acts as a neurotransmitter in the brain to control circadian rhythm, thermoregulation, and adrenocorticotrophic hormone release. The therapeutic use of vasopressin has become increasingly important in the critical care environment in the management of cranial diabetes insipidus, bleeding abnormalities, oesophageal variceal haemorrhage, asystolic cardiac arrest, and septic shock.*

The present study aims toward identifying similarities and differences between the resistance arteries belonging from various mammal species that are most involved in veterinary practice: rats, cats and dogs. Smooth muscle has been studied as circular preparations from rat aorta, cat and dog coronary gastric aorta. Force generation has been studied using isometric transducers while stimulation of preparations was made pharmacologically at various doses. Results were expressed as percentage of inhibition or stimulation of the control contraction.

Force generation, frequency and amplitude of spontaneous contraction have been recorded. Administration in isolated preparations where made using desmopressin acetate (Ferring) as vials of 4 mg/ml. The preparation is a synthetic analogue of the natural hormone 8-arginine-vasopressin, as arginine-vasopressine-monoacetatetrihydrate. Dosages varied from 10^{-12}M to10^{-8}M. Several methods have been tried for normalizing maximal isometric force developed by smooth muscle from various locations, vessel dimensions and animal species. We have measured in vitro the amount of force generated by arterial rings harvested from the same areas, very rigorously cleaned of adventice and surrounding tissues and the force, expressed as mN was ratioed to the wet weight of the preparations.

The results were statistically investigated using the t-test and ANOVA testing. In preparations of rat aorta and splachnic arteries from cat and dog, the vasopressin induced a tonic contraction, with an aspect of descending plateau.

In conclusion, vasopressin contraction has several special characteristics concerning its dynamics. The contractile plateau is kept only for about 10-15 minutes, after which it fades, and new administration in a space of approximately 16 minutes does not induce the initially effect (probable tachyphylaxis).

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

INTRODUCTION

Vasopressin, also known as arginine vasopressin (AVP), antidiuretic hormone (ADH) or argipressin is a neurohypophysial hormone found in most mammals (dog, cat, horse, etc). Its two primary functions are to retain water in the body and to constrict blood vessels. Vasopressin regulates the body's retention of water by acting to increase water reabsorption in the kidney's collecting ducts, the tubules which receive the very dilute urine produced by the functional unit of the kidney, the nephrons (Caldwell et al., 2006; Babar, 2013). It also, increases peripheral vascular resistance, which in turn increases arterial blood pressure. ADH plays a important role in homeostasis, by the regulation of water, glucose and salts in the blood. It is derived from a preprohormone precursor that is synthesized in the hypothalamus

and stored in vesicles at the posterior pituitary. Most of it is stored in the posterior pituitary to be released into the bloodstream. However, some AVP may also be released directly into the brain, and accumulating evidence suggests it plays an important role in social behavior, sexual motivation and pair bonding, and maternal responses to stress (Moncada et al., 1991; Rozenfeld et al., 2000).

Vascular reactivity is one of the three pillars on which lies the regulation of arterial pressure in living organisms. Arterial pressure is one of the main determinants of the activity state of various organs and systems both in healthy and in pathologically-altered states.

The aim of this study is to investigate the most common modifications encountered in the veterinary practice in the vascular arterial reactivity that could be involved in the pathogenesis of various animal species. We also wish to make a comparative investigation of the vascular reactivity at the arterial segments collected from different mammal species the veterinary pathology has most frequently to deal with, segments which are histologically and functionally similar.

The investigation of the methods that are at the basis of the arterial tonus adjustment and of the metabolism of the arterial smooth muscle fiber relied in the last two decades on the very well known isometric transducers pattern and on that of the annular preparation of different arteries. The arterial duct of election used for these types of investigations is the rat aorta, as it combines most of the stability, accessibility, disposability and controllability conditions required for a trustworthy investigation. The price is also an important criterion in this matter.

MATERIAL AND METHODS

The organ parts investigated were taken from the Medical and the Surgery Clinique's from the Faculty of Veterinary Medicine, from dead animals that were not subject of legal euthanasia nor had affections with vascular implications (Legea nr 471 din 9 iulie 2002).

The reactivity of the arterial rings was measured in terms of both absolute force, measured as force index and relative reaction towards a standardized witness. Also, where possible (considering the preparations availability) curves dose/effect were made, involving the majority of the known vasorelaxing and vasoconstrictor substances that are pharmacologically well characterized.

The comparative study was made on similar arteries in what their dimensions are concerned, being part of the resistance segment, in this situation branches from the gastric coronary artery or the superior mesentery which had similar dimensions: maximum length: 2 mm, Φ = 1 mm and weight: 10-15 mg. The force of contraction was quantified in N/mg wet weight (Rozenfeld et al., 2000).

After dissection the vessels were exsanguinated, washed in a solution of physiological salt, sectioned in fragments of 5-10 cm and then put into Krebs-Henseleit serum and transported in maximum 30 minutes to the place of the experiment. The aorta fragments were fixated through a metallic serfina on the bottom of the isolated organ baths, and the ring tensioned through the verniers of the tensiometrical stamps to an initial tension of 100 mN.

The vascular endothelium was removed by gentle rubbing with damp filter paper whenever the experimental characteristics required it. The presence of the functioning

vascular endothelium was pharmacologically verified using carbachol and through direct microscopy.

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

Isometric force transducers connected to a computerized system for data acquisition were used for detecting the contractions of the vascular smooth muscles. The preparations were allowed to equilibrate for 60-90 minutes under a rest tension of 100 mN.

The aorta rings were afterwards precontractated with phenylephrine (10^{-7} – 10^{-6}M) and K+ (40-70 mM) and treated with carbachol (10^{-6}M) for releasing endothelial NO (Jiang et al., 1991) The absolute magnitude of the contractions was of 175±25 mN for the phenylephrine (10^{-6} M) and K+ (40-70 mM).

RESULTS AND DISCUSSIONS

In the rat aorta preparations and in splanchnic arteries from cat and dog, vasopressin produced a tonic contraction, with the appearance of a descending plateau over a period of 10-15 minutes (Fig. 1). The force indices obtained at the vasopressin contraction are presented in table 1.

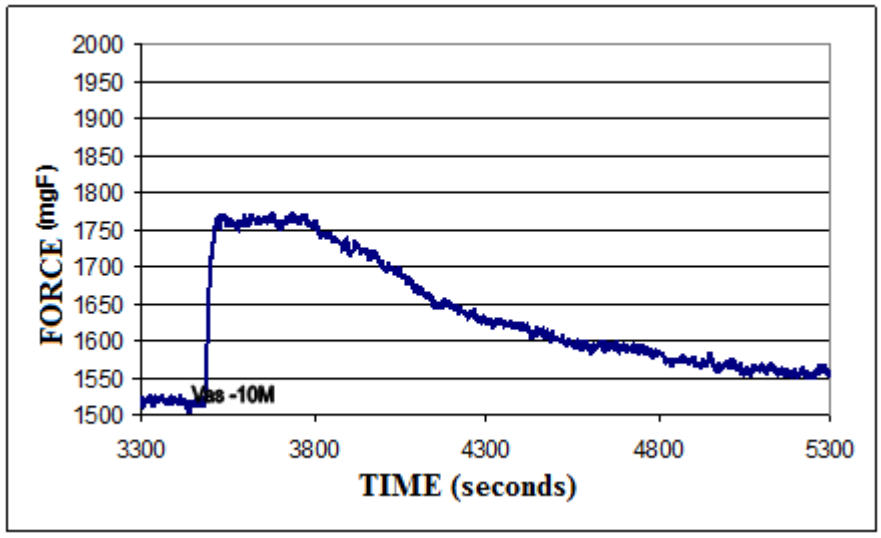

Figure 1. Characteristic aspect of vasopressin contraction on the rat aorta

Table 1.

Force index after administration of ADH 10^{-8} M

ANIMAL	FORCE INDEX (FI)
Rat	3,9 ± 1,25
Cat	4,1 ± 0,75
Dog	6,5 ± 1,5

Regarding the force produced, this is the most powerful of all preparations administered, except for the α-adrenergic agonist phenylephrine. From the dose-effect curve configuration (Fig. 2) it can be seen that very low doses (10^{-12} M – physiological) produce

little contractile effects, while with higher (pharmacological) doses, from 10-10 M, the contractile effects are close to maximum, having a curve with biphasic aspect. The curve aspect suggests the presence of quantum effects at the V1-type receptors.

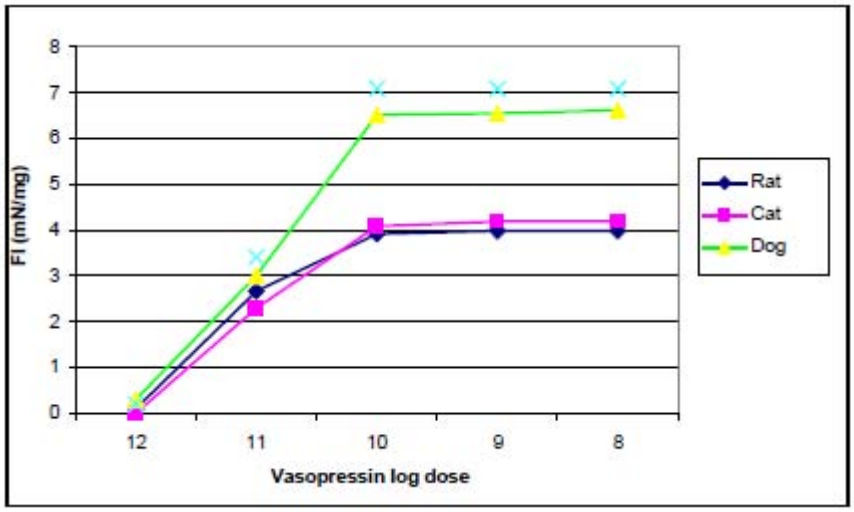

Figure 2. Vasopressin dose-effect curve in endothelized preparations from various species

Vasopressin contraction has a number of particular features concerning the dynamic. The contractile plateau lasts for only 10-15 minutes and after that it ceases and a new administration no longer produces the original effect (possibly tachiphylactic phenomena).

It also should be said that in dog's case, the effect was significantly stronger, considering the fact that the thickness of the muscular layer was smaller than that of the cat's, which leads to the assumption that dogs have a particular reactivity in what the vasopressin mediation is concerned (Fig. no 3).

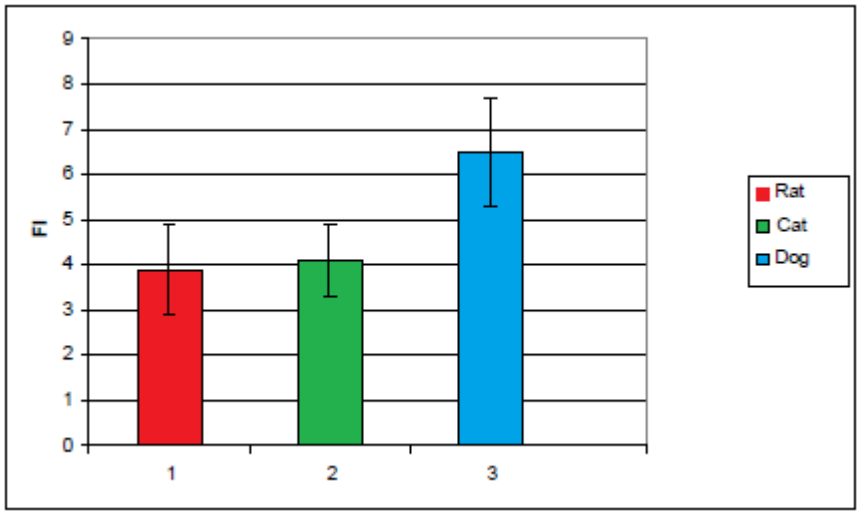

Figure 3. Vasopressin contraction force after administration of 10^{-10} M desmopressin. The differences between endothelized and de-endothelized preparations do not exhibit statistical significance

The ADH hormone is an essential neuropeptide for the cardiovascular homeostasis. Vasopressin was among the first peptide hormones ever described and it is clinically used for more than six decades, especially in treatment of diabetes insipidus and of upper gastrointestinal bleeding due to oesophageal varices (Caldwell et al, 2006). Vasopressin is also more and more often used in therapeutic management of shock, whether it is septic or vasodilator due to different reasons (Moncada et al, 1991).

The ADH is a nonapeptide having a disulphide bridge between two cysteine amino acids. It is synthesized in the paraventricular and supraoptic nucleuses of the hypothalamus, transported coupled to the neurophysins along the hypothalamohypophyseal tract to the neurohypophysis and stored in granules (Guimareas et al., 2001; Babar, 2013).

The effect of this hormone is achieved through two types of receptors, V1, found in the blood vessels (on the vascular smooth muscle) and which mediates the vasoconstriction through a cascade of mediators involving phospholipase C and release of calcium ions from intracellular stores through the inositol-phosphate system (Haulica et al, 2003).

The V2 receptors are located in the distal renal tubule and kidney collecting tube. Their effect is to stimulate the expression of aquaporin's (water channel proteins) in the tubule, thus allowing re-uptake of water and antidiuretic effect (Nielsen et al., 1995).

In normal conditions, ADH has little effect on arterial pressure and the doses at which its vasoconstrictor effect becomes noticeable are at least 10 times higher than normal plasmatic concentrations. However, in conditions of hypotension, its plasmatic concentration increases greatly and its vasoconstrictor effect allows keeping a high arterial pressure in the initial period. But as the ADH neurohypophysis reserves lessen, its plasmatic concentration decreases and its benefic effects – those of keeping the arterial pressure at quasi-physiological levels are fading.

The administrations in isolated preparations were made using the Desmopressin Acetate (Ferring) preparation in form of ampoules of 4 μg/mL. The preparation is a synthetic analogue of the natural 8-arginine-vasopressin hormone, under the form of arginine-vasopressin-monoacetate-trihydrate. The doses were administered starting from 10^{-12} M to 10^{-8} M.

CONCLUSIONS

Vasopressin reactivity produces the highest force, except for the α-adrenergic agonist phenylephrine. From the dose-effect curve configuration it can be seen that very low doses (10^{-12} M – physiological) produce little contractile effects, while with higher (pharmacological) doses, from 10^{-10} M, the contractile effects are close to maximum, having a curve with biphasic aspect. The curve aspect suggests the presence of quantum effects at the V1-type receptors.

In dog's case the effect was significantly stronger, considering the fact that the thickness of the muscular layer was smaller than that of the cat's, which leads to the assumption that dogs have a particular reactivity in what the vasopressin mediation is concerned.

REFERENCES:

1. Caldwell HK, Young WS, 2006. Oxytocin and Vasopressin: Genetics and Behavioral Implications. In Lajtha A, Lim R. Handbook of Neurochemistry and Molecular Neurobiology: Neuroactive Proteins and Peptides (3rd ed.). Berlin: Springer. pp. 573–607. ISBN 0-387-30348-0.
2. Babar SM, 2013. SIADH associated with ciprofloxacin. Ann Pharmacother 47 (10): 1359–63. doi:10.1177/1060028013502457. PMID 24259701.
3. Guimaraes S, Moura D, 2001. Vascular Adrenoceptors: An Update - Pharmacol Rev. 53: 319-356.
4. Haulica I, Bild W, Mihaila CN, Ionita T, Boisteanu CP, Neagu B, 2003. Biphasic effects of angiotensin (1-7) and its interactions with angiotensin II in rat aorta. - J Renin Angiotensin Aldosterone Syst. 4(2):124-8.
5. Jiang H, Halayko AJ, Rao K, Cunningham P, Stephens NL, 1991. Normalization of force generated by canine airway smooth muscles. - Am J Physiol. 260(6 Pt 1):L522-9.
6. Moncada S, Palmer RMJ, Higgs EA, 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev., 43: 109-142.
7. Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA, 1995. "Vasopressin increases water permeability of kidney collecting ducts by inducing translocation of aquaporin-CD water channels to the plasma membranes of the cells that make up the wall of the collecting ducts". Proc. Natl. Acad. Sci. U.S.A. 92 (4): 1013–7. doi:10.1073/pnas.92.4.1013. PMC 42627. PMID 7532304.
8. Rozenfeld V, Cheng JW., 2000 - The role of vasopressin in the treatment of vasodilation in shock states - Ann Pharmacother. 34(2):250-4.
9. * * - LEGE nr.471 din 9 iulie 2002 – Buletinul Oficial al României, privind aprobarea Ordonantei Guvernului nr. 37/2002 pentru protectia animalelor folosite în scopuri stiintifice sau în alte scopuri experimentale.

OBSERVATIONS CONCERNING THE USE A BIODEGRADABLE PROPOLIS EXTRACT, FOR METAPHYLAXIS AND TREATMENT OF PODAL DISEASES IN DAIRY COWS – PRELIMINARY STUDY

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Abstract: *Propolis is one of the few natural remedies that has maintained its popularity over a long period of time. The pharmacologically active molecules are flavonoids, phenolic acids, and their esters. These components have multiple antimicrobial effects on bacteria, fungi and viruses and in addition, propolis have anti-inflammatory, immunomodulatory activities, and antitumor activity. The majority of lameness (> 90%) involves the foot and the primary cause of lameness in most herds are confinement on concrete. Footrot, interdigital dermatitis, and digital dermatitis are diseases with an infectious component responsive to antibiotic treatment, particularly when identified early-on in the course of disease.*
The objective of this study was to identify the most prevalent podal diseases on a dairy herd, and based on well known Propolis properties to investigate the effectiveness of different therapeutic and metaphylactic protocol on podal diseases management(3) . We used a hygiene scoring assessment to determine the therapeutic and methaphylactic protocol in corelation with lamness scoring. Score the manure accumulation on the hoof and leg of the rear feet on a four-point scale where 1=clean, 2=splashes, 3=plaques but hair visible and 4=plaques and no hair visible.The protocol included the podal topical treatment(hoof functional and therapeutic trimming, bandages, medication application completed with footbathing.
In herds with fewer than 25% of cows scoring a 3 or 4 score, footbathing can be done as needed. Conversely, where herds are >75% 3 and 4 scores, then footbathing is probably a necessity 7 days per week, as methaphylactic protocol.

Key words: propolis, methaphylaxis, treatment, dairy cows

INTRODUCTION

Metaphylaxis and control of podal diseases in dairy herds is a challenging task, which include many uncontrolled factors and contributing factors like: nutrition, hygiene, cow comfort (freestall management), walking surfaces, time spent standing on concrete, hoof health, and hoof trimming.

Lame cows with podal diseases must be identified through locomotion scoring and be individually examined. Prevention and metaphylaxis are more economical and better than treatment, but close observation and prompt treatment of lame cows will decrease the duration and thus the cost of each case(6). Footbaths are used for methaphylaxys and prevention, not treatment of active painful lesions. It does not treat active lesions or infections, it helps control the spread of infection from cow to cow. Footbaths are often poorly designed and located. Footbath solutions may help clean the foot of manure and disinfect the interdigital space. Some solutions are probably primarily cleaning agents; other chemicals are disinfectants; antibiotics should only be used in outbreak situations where the

infection rate must be brought under control. There are several commercial products available, most of them are very expensive and not environment friendly. The Propolis based extract is an alternative, effective and environment friendly solution(3).

Propolis has several therapeutic properties, such as antibacterial, anti-inflammatory, healing, anesthetic, anticaricinogenic , antifungal, antiprotozoar and antiviral activities(5,8). Because of these properties the Propolis is a effective-cost potential anti-inflammatory agent for both acute and chronic stages of podal diseases in dairy cows.

MATERIAL AND METHODS

The observations from the study were performed on a herd with 200 milking cows from Holstein breed. Hygiene scoring and identifying the diseases in the herd, was followed by podal topical treatment(hoof functional and therapeutic trimming, bandages, medication application) completed with Propolis biodegradable extract footbathing. We scored the cows from the study based on the scoring chart according with manure contamination on the lower legs(Table 1.).

Hoof & leg hygiene scoring chart. Table 1.

Score 1	Score 2	Score 3	Score 4
-Legs and feet are clean; -Little or no manure contamination of lower limbs.	-Legs and feet are slightly dirty; -Lower limbs are lightly splashed with manure.	-Legs and feet are moderately dirty; -There are distinct placques of manure on the foot, progressing up the leg.	-Legs and feet are very dirty; -There are confluent placques of caked-on manure on the foot and higher up the lower leg.

All the lame cows were examined and the podal diseases were identified based on clinical signs and lameness scoring. The major podal diseases identified in the herd were: digital dermatitis, interdigital dermatitis, interdigital hyperplasia, sole ulcer, traumatic and septic pododermatitis, foot rot, exongulations, and others.

After hygiene scoring, podal diseases identifying, all the cows from the herds were trimmed during a week with a rotoclip disc, by restrain in the hoof trimming chute(9)(Fig.1).

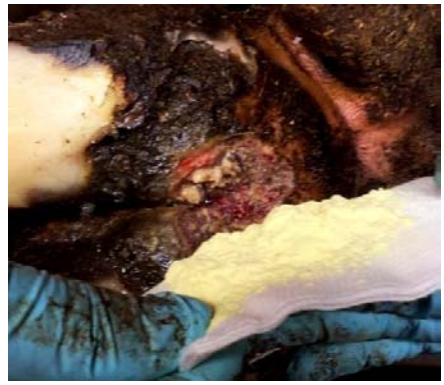
Fig.1. Cow restraing in trimming chute.

For this study we used two different discs : rotoclip aluminium disc with carbide steel blades fitted to them(Fig.2.) and tungsten carbide tipped chain saw disc. A correct functional hoof trimming were done with these disks using a standard sole thickness of 5 mm in the toe area(2).

Fig.2.Hoof trimming with rotoclip disc

After a regular functional trimming, all the cows with podal lesions were surgically debrided, topical Propolis spray or Oxytetracycline powder applied, after that were bandaged and wrapped with Vetotape, and sometime a block was applied(Fig.3.).

a.



b.

c.

Fig. 3.Topical treatment and bandaging.

Because of high incidence of podal diseases in the herd(especially digital dermatitis) correlated with hoof & leg hygiene scoring, the entire herd were passed through the footbath with Propolis extract three times per day after every milking.

Later the protocol of metaphylaxis, were reduced after 3 months at one day per week. Twin footbath were applied, because of high percentage of score 3 and 4 in the herds(Fig.4).

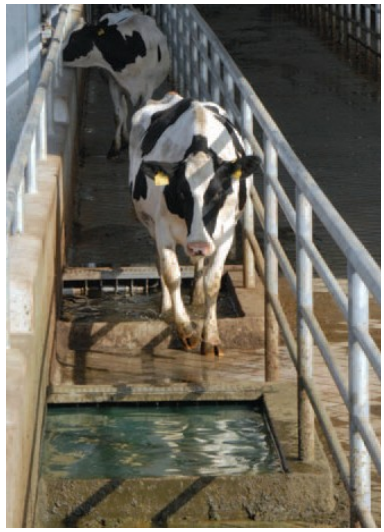


Fig.4. Twin footbath.

RESULTS AND DISCUSSIONS.

Distribution of the herd correlated according the hoof & leg hygiene scoring chart was as presented in the next chart(Chart 1)

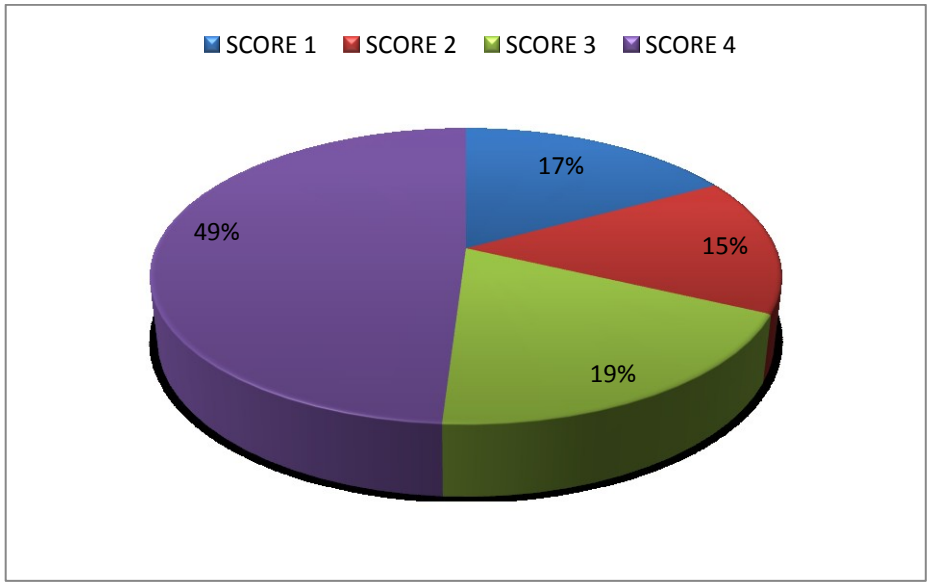


Chart 1. Hoof & leg hygiene scoring

From the previous chart is observing that at the beginning of the study the percentage of the score 3 and 4 were 66%. Because of this quite high incidence correlated with high proportion of the podal diseases with an infectious component in the herd, to trigger the

metaphylaxis effect were applied footbathing for a week, three times daily after each milking. The footbathing complemented the therapeutic measures as hoof trimming, topical medication applying and bandages. Because of this aggressive control protocol of podal diseases, the new clinical cases did not appear, and on top of this the number of cows with lameness decreased after first week of footbathing with Propolis extract(3). Increasing the quality of cleaning-scraping of manure operation, associated with appropriate bedding, improved the percentage of animals with score 1 and 2 at 70% which allowed to decrease the the number of metaphylaxis protocol at 3 days per week for the next months, and after to 1 day per week, three times daily.

The healthy status of the herd and the major podal diseases identified in the herd were distributed as are presented in the next table(Table 2).

Table 2. Status of podal health in the herd

Status or disease	Number of cows	Procentage	Ratio
Healthy cows	105	52,5%	**54,5%**
Digital dermatitis	58	29%	**45,5%**
Interdigital dermatitis	7	3,5%	
Interdigital hyperplasia	5	2,5%	
Sole ulcer	4	2,0%	
Traumatic and septic pododermatitis	9	4,5%	
Foot rot	3	1,5%	
Exongulations	3	1,5%	
Others	3	1,5%	

The high incidence cows lame with podal diseases with an infectious component in this study, justified the need of an metaphylaxis protocol to include an anti-infective agent. Because of restricted use of antimicrobial agents in dairy farms, we decided to use an natural product extract as is Propolis. Propolis has been used in popular medicine for a very long time; however, it was not a drug intended for all diseases. The aim of this study is to discuss the use of propolis with emphasis on its antimicrobial, anti-inflammatory and healing properties(5,7,8).Many other biological and pharmacological properties of propolis have been noted like open lesions healing and tissue regeneration, immunological properties and antioxidant and immunomodulatory actions. Consequently, these properties motivated the increasing interest on Propolis extract in this study.

The clinical results obtained in this study, translated by decreasing the number of cows in lameness in the herd and not expansion of podal diseases with an infectious component, proved the efficacy of Propolis extract footbathing in the metaphylaxis protocol.

The following are the most common hoof diseases that lead to lameness:

Digital dermatitis (papillomatous digital dermatitis, footwarts, heel warts,) is a superficial dermatitis that occurs most often on the rear feet at the commissure of the interdigital space near the heels. It is thought to be a multifactorial disease with bacterial involvement. Response to topical antibiotics is good but recurrence is common. *Interdigital phlegmon (foul in the foot, footrot)* is a bacterial disease that is generally caused by a synergism between two bacteria. It has a very characteristic smell and causes necrosis in the

interdigital skin. It can invade the deeper tissues if not treated early with antibiotics. *Sole and white line hemorrhages* originate from damage to corium with the blood being incorporated into the horn as it grows. These will become visible several weeks after the insult depending on the thickness of the sole and rate of growth. A sole abscess or white line abscess can occur if the hemorrhage becomes infected. *Sole ulcer* is a continuous opening in the sole horn that exposes the corium. The typical site is the rear middle part of the sole, which corresponds to the rear part of the pedal bone. The prognosis for sole ulcers depends on the damage to the horn producing tissue and the condition of the other claw. *Toe ulcer* occurs when the sole is worn too thin at the toe, or the toe drops inside the horn due to laminitis, or if the toe is accidentally trimmed too thin. Toe ulcers will always require an orthopedic block on the opposite claw. *White line disease* starts with fissures due to hemorrhage and poor quality horn formation. Rocks and gravel can become embedded in these and cause further problems. The problems with the stones or gravel are more likely the result of white line disease rather than the cause of the disease. (1,4,8).

CONCLUSIONS

1. The Propolis extract for footbathing are useful as a metaphylactic protocol for control of podal diseases in dairy cows
2. The efficacy of footbathing with Propolis depending by the hygiene score of the herd. The higher percentage of score 3 and 4, the higher will be number of days of foothbathing.
3. For obvious improvements of podal herd health status in herds with high incidence of podal diseases with infectious components, minimum a week with three times daily footbathing with Propolis extract are necessary.
4. Prevention and metaphylaxis protocol of footbathing with Propolis extract in dairy herds is completing and adequate hoof trimming, treatment, bandaging and an appropriate manure management.
5. Footbaths with Propolis extract are used for prevention and metaphylaxis, not for treatment of active painful lesions.

REFERENCES

1. Blowey R.,D.A. Weaver(2003) Color Atlas of Diseases and Disorders of Cattles,Mosby, p83-112.
2. Blowey R.(2007) Cattle lameness and hoofcare, Farming Press, Ipswich, U.K.
3. Castaldo S., Capasso F.(2002) Propolis, an old remedy used in modern medicine. *Fitoterapia*, 73, 1-6.
4. Hahn M.V.,B.T. McDaniel,J.C. Wilk(1986) Rates of hoof growth and wear in Holstein cattle,J.Dairy Sci.69:2148-2156
5. Marcucci MC.(1995) Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie*, 26, 83-99.
6. Masson F.J., J.D.Leaver (1988) The influence of dietary intake and hoof trimming on lameness in dairy cattle.Anim.Prod.47:185-190.
7. Ramos A. F. N., Miranda J. L.(2007). Propolis: a review of its anti-inflammatory and healing actions. *J. Venom. Anim. Toxins incl. Trop. Dis*., 13, 4, p. 700
8. Raven E.T.,R.T. Haalstra,D.J. Peterse(1989) Cattle footcare and claw trimming, Farming Press Ltd,Ipswich .
9. Stanek Ch., E. Mostl ,H. Pachtz, A.Sixt(1998) Claw trimming, restraint metods and stress in dairy cattlew,Proc.10th Int. Symp.Disorders Ruminant,p13-16.

POLIMORPHISM SCREENING USING A VNTR MOLECULAR MARKER SYSTEM IN ROMANIAN RESIDENT BISON INDIVIDUALS.

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Abstract:*Genomic DNA isolated from the blood sampled from 14 individuals of Bison bonasus was used in order to obtain a molecular fingerprint and based on that, to obtain a dendrogram exhibiting the phylogentic relationship among those individuals. By using the PCR technique and an VNTR (Variabile Number of Tandem Repeats molecular markers system: ISSR (Inter simple sequence repeats) was used in this study for establish set of DNA fingerprints. Based on molecular data a common binary matrix was developed. By using DendroUPGMA software, based on Jaccard coefficient the similarity indices and the genetic distances were calculated. Based on those data set a dendrogram containig the phylogenetic relationship among bison exemplars, was generated. By analyzing the molecular data it was observed that the degree of polymorphism was either absent or very low especially in this case, our recorded data suggest that among Romanian resident Bison exemplars, there is a high level of genetic similarity meaning that the individuals are most probably close blood relatives.*

Key words : *polimorphism screening, variabile number of tandem repeats*

Genomic DNA isolated from the blood sampled from 14 individuals of Bison bonasus was used in order to obtain a molecular fingerprint and based on that, to obtain a dendrogram exhibiting the phylogentic relationship among those individuals. By using the PCR technique and an VNTR (Variabile Number of Tandem Repeats molecular markers system: ISSR (Inter simple sequence repeats) was used in this study for establish set of DNA fingerprints. Based on molecular data a common binary matrix was developed. By using DendroUPGMA software, based on Jaccard coefficient the similarity indices and the genetic distances were calculated. Based on those data set a dendrogram containig the phylogenetic relationship among bison exemplars, was generated. By analyzing the molecular data it was observed that the degree of polymorphism was either absent or very low especially in this case, our recorded data suggest that among Romanian resident Bison exemplars, there is a high level of genetic similarity meaning that the individuals are most probably close blood relatives.

The wisent or European bison (Bison bonasus L.), was common in the Carpathian eco-region (about 210,000 km2 of mountain area) in Medieval Ages, it disappeared by late 18th century, due to overhunting and gradual habitat loss (Erlich and Erlich, 1981).). In the early twentieth century, only 54 individuals survived in the whole world population (Pucek, 1991). First attempts for its re-introduction are going back to the 1960s (Poland and Ukraine). In the late 1990s a restitution project was initiated and had involved five countries (Poland, Slovakia, Ukraine, Romania and Hungary) (Kajetan Perzanowski and Wanda Olech 2013). To date the specie accounts about 3600 exemplars with a high degree of inbreeding, as being

the descendants of only 12 individuals. Almost 40% live in small groups in captivity, and the rest live in a few, isolated, free-ranging and semi-free herds (Kajetan Perzanowski and Wanda Olech, 2007, Luenser, K). The genetic diversity among wisent populations is very limited making them vulnerable to diseases, affecting fertility and thus keeping the specie in the vulnerable estate (Małgorzata Tokarska, 2011, Radwan, J.,2010) . The loss of genetic variability can be prevented by allowing exchange of genes among sufficiently large numbers of animals (Kajetan Perzanowski and Wanda Olech, 2007, Tokarska, M 2009). All programs of re-establishing viable populations in the Carpathians are based on the genetic analysis of formerly released animals and of the ones to be released. (Wanda Olecha, Kajetan Perzanowski 2002).

The study presented in this paper is part of the genetic diversity scoring attempt of wisent individuals that were to be released in the in a protected area located Caras Severin county, Romania. Neutral genetic markers are an important tool for scoring genetic diversity and the similarity of living species. (Chambers GK, 2014) The molecular markers used in our DNA fingerprinting by PCR method experiments, in order to reveal the genetic diversity, were a type of VNTR (Variable Number of Tandem Repeats) molecular markers systems: namely ISSR (Inter simple sequence repeats). Inter simple sequence repeats (ISSRs) are dominant markers located between microsatellite sites in the genome, are often highly polymorphic at the species level and require no prior sequence information (Claes Ramel,1997; Heath DD,1993). Using VNTR markers, information about the population structure can be obtained and also spatial distribution, parentage, and movements of wisent small herd individuals. Those information are of high importance in re-constructing, preserving and protecting the European bison in natural environment. Obtained data were used to develop genetic diversity and similarity matrices that further allowed the construction of a genetic relationships dendrogram.

MATERIAL AND METHODS

Total genomic DNA was isolated and purified from 300 µl of total blood samples of 14 Bison bonasus L. individuals provided by World Wide Fund Romania the identity of individuals being their propriety. The quality and quantity of DNA was assessed by specrophotometric measurement (*NanoDrop 8000, Thermo Scientific*). ISSR molecular markers were chosen for the genetic diversity assessment. For the initial screening a set of 15

Table 1. ISSR primers sequences used in initial screening

primer code	**Sequence 5'...3'**
UBC810	GAGAGAGAGAGAGAGAT
UBC829	TGTGTGTGTGTGTGTGC
UBC834	AGAGAGAGAGAGAGAGYT
UBC836	**AGAGAGAGAGAGAGAGYA**
UBC840	**GAGAGAGAGAGAGAGAYT**
UBC 843	**CTCTCTCTCTCTCTCTRA**
UBC854	TCTCTCTCTCTCTCTCRG
UBC857	ACACACACACACACACYG
UBC880	**GGAGAGGAGAGGAGA**
UBC873	GACAGACAGACAGAC A

UBC885	**HBHAGAGAGAGAGAGAG**
UBC 886	VDVCTCTCTCTCTCTCT
A 12	**GAGAGAGAGAGACC**
A 21	**CACACACACACAAC**
A 17	**GACAGACAGACAGACA**

Single letter abbreviations for mixed-base positions: Y = (C, T), R = (A, G), B = (non A), H = (non G).

From those primers, based on the quality of amplification products, nine primers were chosen (marked in bold character, Table 1) to be used in scoring the genetic diversity.

PCR amplifications were carried out in volumes of 25 µl using 75 ng of DNA template. The composition of amplification mixtures were carried out following the producer instructions for GoTaq Green Master Mix (*Promega*, USA). The reactions were performed on a DNA Engine Peltier Thermal Cycler (MJ Research, U.S.A.) and the PCR program consisted of a first denaturing step for 5 min at 94°C, followed by 45 cycles of denaturation at 95°C for 45 sec, annealing at 48°C- 55°C for 45 sec and extension at 72°C for 2 min, the final step of extension at 72°C for 5 min., according to literature data (Pharmawati Made et al., 2005).

PCR products were run on 1.8 % agarose gels in TAE buffer at room temperature at a constant voltage of 100 V for 90 minutes. The PCR products were visualized and photographed under UV light (PhotoDocumentation System, UVP, England). The obtained data were analyzed with VisionWorksLC software.

The genetic diversity and similarity matrices and the dendrogram were assessed from a set of variables by using DendroUPGMA (http://genomes.urv.cat/UPGMA/index.php), software. The program calculates a similarity matrix and transforms similarity coefficients into distances and makes a clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

RESULTS AND DISCUSSIONS

Specific literature data does not contain information to state that VNTR markers systems were used in scoring genetic diversity and to molecular fingerprint the wisent population in our country. Considering this issue the first step of our study was a screening of ISSR molecular markers in order to choose those markers that will provide the most informative data. A set of 15 ISSR primers were chosen for an initial screening, in order to select polymorphic primers suitable to be used further in the experiment. A bulked sample consisting of 5 µl of each individual DNA was used as template for PCR amplification. For this screening experiment, a Gradient-PCR program, with annealing temperatures ranging from 47°C- 53°C was used. Based on the obtained screening result, 10 ISSR primers were selected to be used in the first step of the experiment (Figure 1), (Table 2).

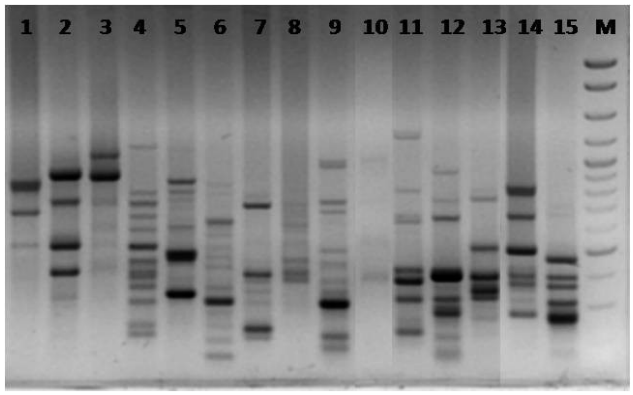

Fig. 1: PCR based screening with 15 ISSR primers:1 –UBC810, 2 –UBC829, 3 –UBC834, 4 - UBC836, 5 - UBC840, 6 - UBC843, 7 - UBC854, 8 - UBC857, 9 - UBC880, 10 - UBC873, 11 - UBC885, 12 - UBC886, 13 – A 12, 14 –A 21,15 – A 17, M- Molecular weight marker - GeneRuler Express DNA Ladder (*Fermentas*).

From the randomly selected ISSR primers only those that yielded a DNA fingerprint of good quality in terms of amplified sequences number and well defined PCR product (Figure 1), were considered as a valuable choice for further studies. Primers that yielded faint or low number of amplicons were eliminated from the experiment. Therefore, the DNA fingerprinting study was developed on the basis of 9 markers system (Figure 2, 3), (Table 2).

As o result of the genomic omogenity among studied individuals, four of used primers, namely A 17, UBC 880, UBC 843,UBC 886, did not reveal any polymorphism, in terms of amplified fragments, therefore their results were eliminated from the study (Figure 3). The others five primers: A 12, A 21, UBC 836, UBC 840 and UBC 885 provided enough data for the genetic diversity to be scored and a dendrogram to be developed (Table 3).

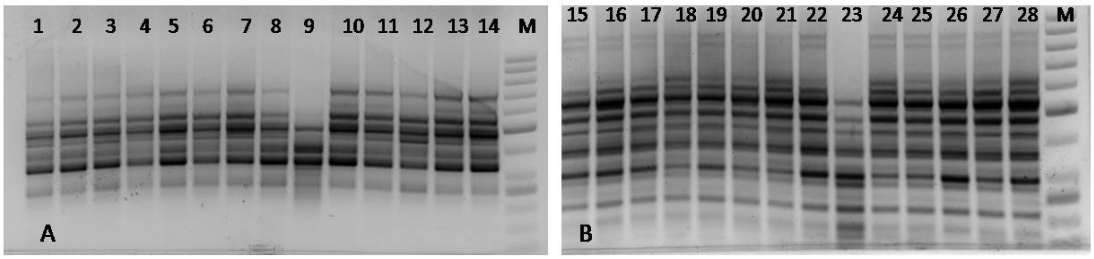

Fig. 3: Non- polymorphic DNA fingerprint obtained using ISSR primer: UBC 843 (panel A) and UBC 886 (panel B): 1 – 14; 15-28: wisent individuals; M - Molecular weight marker - GeneRuler Express DNA Ladder (Fermentas).

By using the primer A 12, a number of 16 amplicons were obtained with dimensions raging from 1800 to 210 base pairs, from which only two were polymorphic; both are situated in the area of low molecular weight fragments (Figure 2). Primer A 21 yielded 18 amplicons from which 11 were polymorphic, covering all areas of the molecular weights (Figure 3).

Table 3. Molecular markers and data collected in DNA fingerprinting experiment

Primer	**Sequence 5'…3'**	Fragment size range (bp)	Fraction polymorphic fragments
A 12	GAGAGAGAGAGACC	**1800 - 210**	**16 / 2**
A 17	GACAGACAGACAGAC A	-	-
A 21	CACACACACACAAC	**1810 - 205**	**18 - 11**
UBC836	AGAGAGAGAGAGAGAGYA	**1135 - 150**	**17 - 7**
UBC840	GAGAGAGAGAGAGAGAYT	**1450 - 160**	**19 / 13**
UBC880	GGAGAGGAGAGGAGA	-	-
UBC885	HBHAGAGAGAGAGAGAG	**1240 - 250**	**16 / 6**
total			**86 / 39 (45.3%)**

17 bands could be scored for primer UBC 836, with seven showing polymorphism. The polymorphic fragments are situated in the area of heavy and low molecular weight. For the primer UBC 840 19 amplicons were scored from which 13, most of them in the area of medium molecular weight fragments, are polymorphic. Primer UBC 885 yielded a total number of 16 amplified fragments. The six polymorphic bands were situated in all areas of the molecular weights.

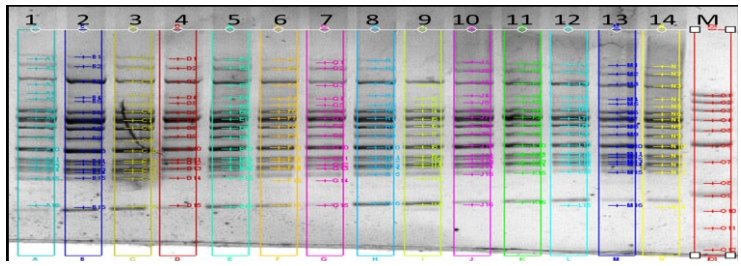

Fig. 2: DNA fingerprint obtained using ISSR primer: A 12: 1 – 14:wisent individuals; M - Molecular weight marker - GeneRuler Express DNA Ladder (Fermentas).

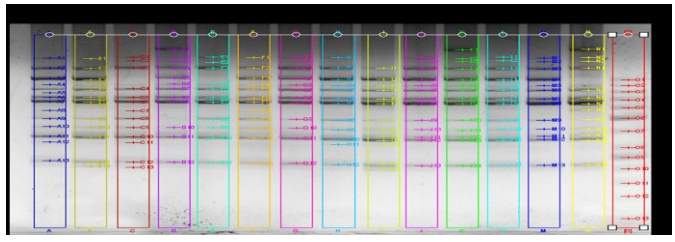

Fig. 3: DNA fingerprint obtained using ISSR primer A 21 : 1 – 14 wisent individuals; M - Molecular weight marker - GeneRuler Express DNA Ladder (Fermentas).

Data collected from all ISSR primers were used to develop a binary matrix of 86 scored PCR products, from which 39 were polymorphic. This matrix consisted of scores (1 for present and 0 for absent) for each of the 14 individuals DNA fingerprint obtained by using the 14 primers. The binary matrix was uploaded in the software as complete set of variables. The Jaccard coefficient has been used to compare among this set of variables and further, to construct the genetic distance and similarity matrices (Table 4, Table 5).

The genetic distances are used to approximate the genetic divergence between analyzed individuals. Thus, a value of 0 means the lack of genetic divergence, obtained only in the case when comparing an individual to itself, and the value of 1 which is a theoretical maximum threshold. The medium value is around 0.5. Analyzing the obtained matrix it can be observed that the wisent individuals are very closely related, as expected all the obtained values were beneath the medium threshold close to the lower value. The minimum value of 0.077 was obtained in this case is between individual „12" and „13". The higher value of 0.280 is recorded between individuals „14" and „9".

Table 4. Genetic distance matrix of the studied 14 wisent individuals

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.154	0.165	0.192	0.167	0.120	0.190	0.169	0.145	0.184	0.217	0.195	0.198	0.244
2		0	0.141	0.215	0.212	0.192	0.190	0.169	0.192	0.208	0.195	0.195	0.175	0.200
3			0	0.203	0.154	0.156	0.130	0.156	0.203	0.218	0.183	0.160	0.207	0.210
4				0	0.133	0.184	0.133	0.184	0.253	0.224	0.165	0.188	0.212	0.259
5					0	0.108	0.107	0.133	0.205	0.173	0.115	0.091	0.141	0.212
6						0	0.108	0.135	0.184	0.176	0.165	0.165	0.167	0.192
7							0	0.108	0.228	0.197	0.115	0.139	0.165	0.190
8								0	0.160	0.200	0.141	0.165	0.190	0.237
9									0	0.224	0.232	0.210	0.235	**0.280**
10										0	0.132	0.156	0.158	0.184
11											0	0.076	0.077	0.127
12												0	**0.077**	0.173
13													0	0.104
14														0

Table 5. Similarity matrix of the studied 14 wisent individuals

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	1	0.846	0.835	0.808	0.833	0.880	0.810	0.831	0.855	0.816	0.783	0.805	0.802	0.756
2		1	0.859	0.785	0.787	0.808	0.810	0.831	0.808	0.792	0.805	0.805	0.825	0.800
3			1	0.797	0.846	0.844	0.870	0.844	0.797	0.782	0.817	0.840	0.793	0.790
4				1	0.867	0.816	0.867	0.816	0.747	0.776	0.835	0.812	0.787	0.741
5					1	0.892	0.893	0.867	0.795	0.827	0.885	0.909	0.859	0.787
6						1	0.892	0.865	0.816	0.824	0.835	0.835	0.833	0.808
7							1	0.892	0.772	0.803	0.885	0.861	0.835	0.810
8								1	0.840	0.800	0.859	0.835	0.810	0.762
9									1	0.776	0.768	0.790	0.765	**0.720**
10										1	0.868	0.844	0.842	0.816
11											1	**0.924**	0.923	0.873
12												1	0.923	0.827
13													1	0.896
14														1

The similarity coefficients represent the degree of relation among the studied individuals, and are expressed with values raging from 0 to1. The absolute similarity is represented by 1, the lack of it being represented with 0 values. Considering a medium value of 0.5 the data are interpreted as it approaching to the mentioned extremes.

As in the case of genetic distance, the obtained data quite resembled. All the coefficients are above the medium value. The highest value of 0.924 is recorded between individuals „11" and „12", and the lowest of 0.720 is recorded between individuals „9" and „14".

On the basis of resulted matrices an UPGMA dendrogram was designed (Figure 4). The similarity coefficients are used to group the individuals in clusters. The length of the clusters is being dictated by the genetic distance coefficients. The dendrogram illustrates the genetic relationship among studied wisent individuals. Dendrogram analyses revealed that the 14 individuals are grouped in two different major clusters. The first cluster contains the majority of individuals from „1" to „9", individual „4" being the most distinct among them as it is clustered separately. The most related individuals seem to be „5" and „7". In the second major cluster the most distinct individual is „10" and the more related are individuals „11" and „12". Individual „13" is also very close to the cluster of the previous two. Overall, the obtained data suggest, as expected, a high degree of similarity among the analyzed wisent individuals. As specific literature data state, this situation is of concern for all wisent herds that live in semi-wild environment all cross Europe protected areas. Genetic diversity studies are helpful for generating the population of a new small herd and also, can provide useful information in sustaining artificial exchange of individuals between those herds.

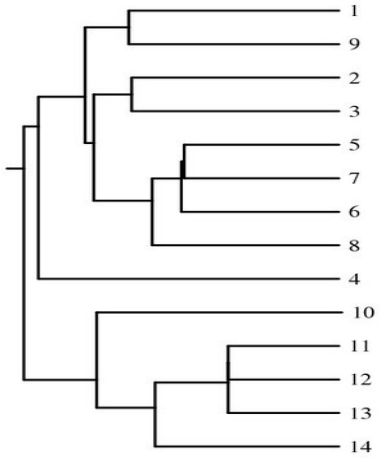

Fig. 4: Genetic distance Dendrogram of *Bison bonasus L.* individuals created by DendroUPGMA program using data from 5 ISSR molecular markers.

CONCLUSIONS

This paper presents a case study with the goal of developing a cheap molecular markers system that can become an important tool of fingerprint wisent individuals, thus combating severe inbreeding and, if applied on all individuals, can be used in scoring genetic diversity and phylogeny studies.

The proposed ISSR markers were proved to be reliable and provided accurate information in terms of establishing an individual molecular fingerprint but also in clustering individuals according to the degree of genetic similarity, despite the fact that there some non-informative molecular markers were anccounted. Even so, most accurate data will be obtained by similar studies with the help of an increased number of molecular markers systems. The developed similarity and genetic distance matrices differentiated among studied individuals according to their genetic inheritance. In order to develop the proposed method and further to create a genetic data base, higher number of VNTR markers systems must be analyzed in similar studies.

REFERENCES

1. Chambers G K, Caitlin Curtis, Craig D Millar, Leon Huynen , David M Lambert, DNA fingerprinting in zoology: past, present, future, Investigative Genetics, 2014, doi:10.1186/2041-2223-5-3 5:3;
2. Claes Ramel , Mini- and Microsatellites, Environmental Health Perspectives , 1997, Vol 105, Supplement 4;
3. Erlich, P.R., Erlich, A.H., 1981. Extinction: the Causes and Consequences of the Disappearance of Species. Random House, New York USA;
4. Heath DD, Iwama GK, Devlin RH, PCR primed with VNTR core sequence yields species specific patterns and hypervariable probes. Nucleic Acids Res., 1993, 21: 5782-5785;
5. Kajetan Perzanowskia and Wanda Olech, 2007, A Future for European Bison Bison Bonasus in the Carpathian Ecoregion?, Wildlife Biology 13(1):108-112;
6. Luenser, K., Fickel, J., Lehnen, A., Speck, S., & Ludwig, A. (2005). Low level of genetic variability in European bisons (Bison bonasus) from the Bialowieza National Park in Poland. *European Journal of Wildlife Research*, *51*(2), 84-87;
7. Małgorzata Tokarska ,Cino Pertoldi ,Rafał Kowalczyk , Kajetan Perzanowski, 2011, Genetic status of the European bison Bison bonasus after extinction in the wild and subsequent recovery, Mammal review, DOI: 10.1111/j.1365-2907.2010.00178;
8. Perzanowski, Kajetan, and Wanda Olech. 2013, "Restoration of wisent population within the Carpathian eco-region, Europe." *Global Re-introduction Perspectives: 2013. Further case studies from around the globe,* 190;
9. Pharmawati Made, Guijun Yan, Patrik M. Finnegan, Molecular Variation and Fingerprinting of Leucadendron Cultivars (Proteaceae) by ISSR Markers, Annals of Botany, 2005, 95: 1163–1170;
10. Pucek, Z., 1991. History of the European bison and problems of its protection and management. In: Bobek, B., Perzanowski, K., Regelin, W. (Eds.), Global Trends in Wildlife Management. Swiat Press, Krakow-Warszawa, pp. 164–184;
11. Radwan, J., Demiaszkiewicz, A. W., Kowalczyk, R., Lachowicz, J., Kawałko, A., Wójcik, J. M., Babik, W.,2010. An evaluation of two potential risk factors, MHC diversity and host density, for infection by an invasive nematode Ashworthius sidemi in endangered European bison (Bison bonasus). Biological conservation, 143(9), 2049-2053.
12. Tokarska, M., Marshall, T., Kowalczyk, R., Wojcik, J. M., Pertoldi, C., Kristensen, T. N., Bendixen, C. (2009). Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: the case of European bison. *Heredity*, *103*(4), 326-332.
13. Wanda Olecha, Kajetan Perzanowski 2002, A genetic background for reintroduction program of the European bison (***Bison bonasus)*** in the Carpathians, Volume 108, Issue 2, December 2002, Pages 221–228;
14. **http://genomes.urv.cat/UPGMA/index.php**

ELECTROENCEPHALOGRAPHY EVALUATION OF CEREBRAL BIOELECTRIC ACTIVITY IN DOGS WITH BABESIOSIS

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Abstract: *Canine babesiosis is a parasitic disease characterized by a cluster of clinical signs like: hemolytic anemia, jaundice, secondary nephritis, and occasionally neurological disorders and gastroenteritis.*
The aim of the current study is to describe short time electroencephalographic (EEG) changes in dogs with babesiosis presenting neurological signs.
EEG examination was performed on 6 dogs diagnosed with babesiosis and showing neurological signs (incoordination, paresis, paralysis, muscle tremor, nystagmus, loss of consciousness, tonic-clonic epileptic seizures and coma). EEGs were obtained via five subdermal stainless steel needle electrodes (F3, F4, O1, O2, Cz) placed as described by Redding (1978). The parameters used for each electroencephalographic recording were: sensitivity = 70μV/cm; time constant = 0.3 seconds; Hf = 70 Hz; Lf = 0.5 Hz; notch filter inserted; impedance of all electrodes < 10 kΩ.
The results of the present study revealed an EEG background activity characterized by the presence of the theta and delta rhythms, while alpha and beta waves were less encountered. In contrast with these the background activity showed an intersection of epilpeptiform interictal discharges (DIE) like: fast spike and atypical spike-wave complex in 2 cases, slow waves in 3 dogs and polyspikes just in one case.

Keywords*: babesiosis, electroencephalography, dogs, neurological signs*

INTRODUCTION

Canine babesiosis caused by different *Babesia* species is a parasitic disease with worldwide distribution and global significance. The severity of the disease depends on the species of *Babesia*, the presence of concurrent infections and the age and immune status of the host. (Irwin, 2009; Schoeman, 2009)

The most severe clinical and paraclinical findings in canine babesiosis are characterized by marked hemolytic anemia, severe acid-base abnormalities with frequent secondary multiple organ failure and complications such as acute renal failure (ARF), hepatopathy with marked jaundice, splenomegaly, hypoglycemia, acute respiratory distress syndrome (ARDS), gastroenteritis, additional immune-mediated red blood cell destruction (IMHA) and cerebral pathology. (Leisewitz et al., 2001, Keller et al., 2004, Jacobson, 2006)

Diagnosis of acute cases infected with *Babesia* species is based on the morphologic appearance of the parasite in the erythrocytes. (Solano-Gallego, 2011). Biochemical profile abnormalities are related both to the severity of the disease and the hypoxia degree. Common laboratory findings include elevation of liver enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) , hyperbilirubinemia -

more in the patients with marked icterus, hypoalbuminemia, electrolyte and acid–base abnormalities (mostly hypokalemia, hypercloremia and metabolic acidosis) (Schoeman, 2009, Furlanello et al., 2005).

The electroencephalography is a noninvasive test on electrical activity of the brain, trying to assess the impact of different neurological anomalies on electrical activity of the brain.

The aim of the present study was carried out to evaluate cerebral bioelectric activity in dogs with clinical babesiosis presenting neurological signs.

MATERIAL AND METHODS

The study was made on 6 dogs diagnosed with babesiosis and showing neurological signs (incoordination, paresis, paralysis, muscle tremor, nystagmus, loss of consciousness, tonic-clonic epileptic seizures and coma), that were presented to Internal Clinic of Faculty of Veterinary Medicine, Iasi.

In order to uniform the batches regarding the subjects' fatigue degree, the tests were performed at the same moment of the day and in identical environmental conditions.

The electroencephalogram was performed under general anesthesia, using medetomidine (Domitor, Pfizer) in a dosage of 0.03 mg/kg administered intramuscularly, in order to eliminate the artifacts triggered by the muscular contractions. After the anesthesia is installed, that is when the animal is no longer able to perform voluntary moves (in about 10-20 minutes after administration), the patients were put in sternal-abdominal decubitus.

The acquisition of the biopotentials was made with Neurofax electroencephalograph (Nihon Kohden) for 30 minutes. Needle electrodes were introduced subcutaneously according to Redding and Knecht model (1984), using five electrodes (Fig. 1): two frontals (F3, F4), one central (Cz) and two occipitals (O1, O2) used in bipolar montage, the reference electrode being placed on the nasal bone. Electrodes' nomenclature is similar to the one described by 10-20 system in human medicine (Aminoff, 2005; Nordli et al., 2011).

The parameters used for each electroencephalographic recording were: sensitivity: 70 μV, time constant: 0.3 seconds, filter pass – down of 70Hz, filter pass – up 30 Hz and electrode impedance < 10Ω.

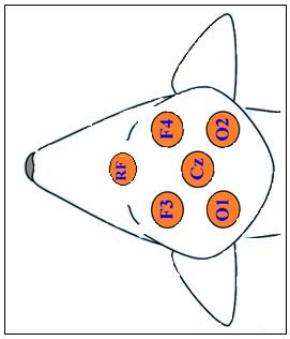

Fig.1 The electrodes positioned according to Redding and Knecht model (1984). F3 - frontal left, F4 - frontal right, Cz - vertex, O1- occipital left, O2 -occipital right, Rf-nose, between the eyes.

Recording sections were visually selected for analysis of background activity using Fast Fourier Transformation (FFT). Spectral bands were, 8.0-13.0 Hz for alpha, 13.0-30.0 Hz

for beta, 0.5-4.0 Hz for delta and 4.0-8.0 for theta activity. In order to minimize errors through different skull sizes, forms and thicknesses, the relative power of the spectral bands was calculated for every lead.

The visual analysis of the electroencephalographic tracks presumed the recording of the electroencephalographic pattern (any EEG characteristic activity), marking the background activity (any EEG activity that represents the frame where a normal or abnormal pattern appears), all the paroxysmal activities (spike, poly-spikes, sharp slow waves, wave-spike complexes, bursts of slow waves and spikes), as well as possible artifacts.

RESULTS AND DISCUSSIONS

The results of the present study revealed a modified EEG background activity in all dogs, characterized by the presence of the theta and delta rhythms, while alpha and beta waves were less encountered. All 6 dogs were presented an intersection of epileptiform interictal discharges (DIE) like: fast spike and atypical spike-wave complex in 2 cases without clinical epileptic seizures, slow waves in 3 dogs and poly-spikes just in one case (Fig.2).

At 2 dogs that died it was observed an increased reduction over EEG traces. In their case, it was noticed brain death characterized by the exclusive presence of amplitudes below 2μV. Generalized slow waves suggest a bilateral disturbance of brain function common in diffuse encephalopathy. When is observed an attenuation of EEG activity, the etiology of brain disorders cannot be determined, but is considered to be associated with a diffuse slowing and poor reactivity to somatosensory stimulation (Tatum, 2014).

At 2 dogs, EEG recorded an ictal period. (Fig.3) One dog was presented a Burst-suppresion (EEG activity characterized by rhythmic discharges with increased voltage alternating with periods of brain inactivity). This type of EEG activity may occur after administration of large doses of anesthetics or sedatives, hypothermia and coma. The presence of tissue hypoxia may lead to a poor prognosis. (Niedermeyer, 2009, San-juan 2009, Bauer 2013).

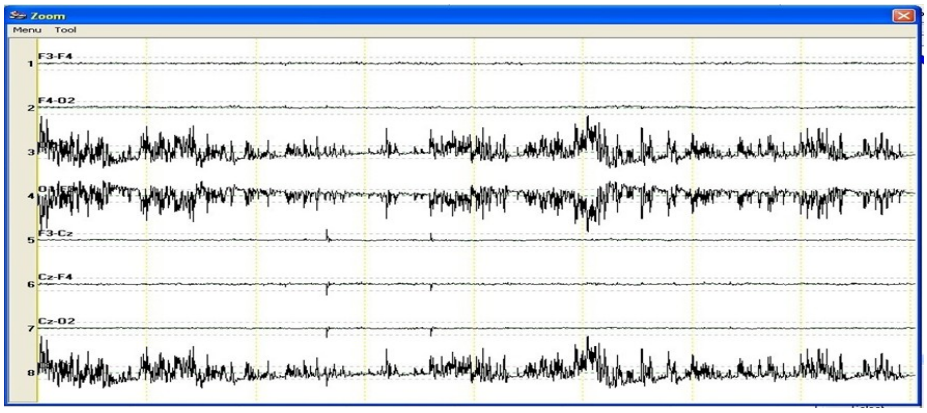

Fig.2 EEG of a Bordeaux Dog, 3 years old. Peaks at Cz derivations and hypervaulted beta at O1 derivations. Background activity more subdued.

The important causes of hypoxia include anemia, hypotensive shock, vascular stasis by sludging of erythrocytes, excessive endogenous production of carbon monoxide, and parasitic damage to hemoglobin (Ayoob et al., 2010; Taboada, 2006). The central nervous system, kidney and muscle are the organs most affected by tissue hypoxia (Jacobson, 2006). Hypoxia is thought to be more important than hemoglobinuria in damaging the kidneys of dogs with babesiosis (Lobetti et al., 1996; Mathe et al., 2007; Ayoob et al., 2010).

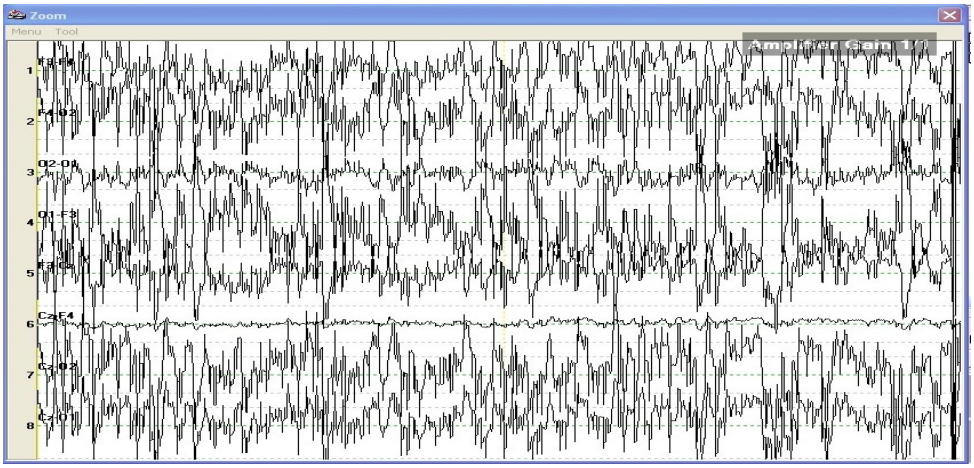

Fig.3 EEG of a German Shepard, 3 years old, generalized tonic-clonic crisis

Neurologic complications result from sludging of parasitized erythrocytes in CNS capillary beds, with congestion and macroscopic and microscopic hemorrhages. Another cause of neurological signs is severe hypoglycemia (Birkenheuer, 2014).

Thus, taking into account the previously stated, in the EEG of brain activity appears a cluster of anomalies which occur in hepatic and renal failure, hypoxia, cerebral edema, hypoglycemia or cerebral hemorrhages.

CONCLUSION

Examining the cerebral behavior of the dogs with babesiosis presenting neurological signs, the EEG showed epileptiform discharges in all patients.

REFERENCES

1. Aminoff, M.J., 2005. Electroencephalography: General Principles and Clinical Applications. In: Aminoff, M.J. (Ed.), Electrodiagnosis in Clinical Neurology. Elsevier, Philadelphia, pp. 37-84.
2. M. Armaşu, R.M.A. Packer, S. Cook, G. Solcan, H.A. Volk, An exploratory study using a statistical approach as a platform for clinical reasoning in canine epilepsy, The Veterinary journal, 2014, 202, 292-296
3. Ayoob, A.L., Hackner, S.G., Prittie, J., 2010, Clinical management of canine babesiosis. J VetEmerg Crit Care (San Antonio) 20, pp. 77-89.
4. Bauer G, Trinka E, Kaplan P.W., 2013 - EEG patterns in hypoxic encephalopathies (post-cardiac arrest syndrome): fluctuations, transitions, and reactions. J Clin Neurophysiol.; 30(5):477-89.
5. Birkenheuer, A. J. 2014, Canine and Feline Infectious Diseases, Chapter 75, Babesiosis. Elsevier, Philadelphia, pp. 727-738

6. Irwin, P.J., 2009. Canine babesiosis: from molecular taxonomy to control. Parasit. Vectors 2 (Suppl. 1): S4.
7. Furlanello, T. Fiorio, F.M., Caldin,G. Lubas,L. Solano-Gallego, 2005, Clinicopathological findings in naturally occurring cases of babesiosis caused by large form Babesia from dogs of northeastern Italy. Veterinary Parasitology 134, pp.77–85
8. Jacobson, L.S., 2006, The South African form of severe and complicated canine babesiosis: clinical advances 1994-2004. Vet Parasitol 138, pp.126-139.
9. Keller, N., Jacobson, L.S., Nel, M., De Clerq, M.,Thomson , P.N. & Schoeman, J.P. 2004. Prevalence and risk factors of hypoglycemia in virulent canine babesiosis. Journal of Veterinary Internal Medicine, 18:265–270.
10. Leisewitz, A.L., Jacobson, L.S., De Morais, H.S. & Reyers, F. 2001. The mixed acid-base disturbances of severe canine babesiosis. Journal of Veterinary Internal Medicine, 15:445–452.
11. Lobetti, R.G., Reyers, F., Nesbit, J.W., 1996 - The comparative role of haemoglobinaemia and hypoxia in the development of caninebabesial nephropathy. J. S. Afr. Vet. Assoc. 67, 188–198.
12. Mathe, A., Dobos-Kovacs, M., Voros, K., 2007 - Histological and ultrastructural studies of renal lesions in Babesia canis infected dogs treated with imidocarb. Acta Vet. Hung. 55, 511–523.
13. Niedermeyer E., 2009 - The burst-suppression electroencephalogram. Am J Electroneurodiagnostic Technol; 49:333–341.
14. Nordli D.R, Riviello J., Niedermeyer E., 2011. Seizures and epilepsy in infants to adolescents. In: Schomer D.L., Da Silva F.L. (eds.): Niedermeyer's Electroencephalography. Basic principles, clinical applications, and related fields.Philadelphia: Lippincott, 479–540.
15. San-juan OD, Chiappa KH, Costello DJ, Cole AJ, 2009 - Periodic epileptiform discharges in hypoxic encephalopathy: BiPLEDs and GPEDs as a poor prognosis for survival, Seizure, 18(5): 365–368
16. Schoeman, J.P. 2009. Canine babesiosis. Onderstepoort Journal of Veterinary Research, 76:59–66
17. Solano-Gallego L, Baneth G, 2011. Babesiosis in dogs and cats--expanding parasitological and clinical spectra. Vet Parasitol.181(1):48-60.
18. Taboada, J., Lobetti, R., 2006, Babesiosis, In: Greene, C.E. (Ed.) Infectious diseases of the dog and cat. Saunders Elsevier, Philadelphia, p. 722
19. Tatum WO., 2014 - Handbook of EEG Interpretation, Second Edition, ed. Demos Medical Publishing

INSULIN CHOICE AND TREATMENT PROTOCOL FOR "HEALTHY" DIABETIC FELINES

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Abstract:*Demand of exogenous insulin administration is currently limited by a series of factors, such as unbalanced pharmacodynamics, low therapeutic index, inconsistent intensity of effect, high physical instability and need for administration by injection. Still, the broad panel of insulin analogues available at the moment has a vital role in the treatment of diabetes mellitus, allowing individualized glycemic control in diabetic cats.*

The current paper describes the specific treatment protocol for clinically stable diabetic felines, focusing on two types of insulin frequently used in Romania,.

Data were collected from the medical records of cats presented at the veterinary teaching hospital of Faculty of Veterinary Medicine of Iasi, Romania. Cases were considered eligible for inclusion in the study if registered with a clear diagnosis of DM and were in a stable clinical condition. Therapeutic protocol focusing on dosing regiments for clinically stable diabetic cats, included two commercially available preparations: glargine and premixed isophane insulin. All cats were treated with both types of insulins and therapy was further continued with the preparation that provided the ideal control of blood glucose level.

Diabetes mellitus was diagnosed in 65 cats, of which only 18 were in a stable clinical condition. In 15 cats, ideal control was obtained with insulin glargine, with a median dose of 0.6 IU/kg (range: 0.2-1.1 IU/kg) administered twice daily. Only 3 cases were managed long term with 0.8 IU/kg (0.4-1.9IU/kg) premixed isophane twice daily. The premixed insulin never exceeded a period of action of 8 hours and allowed reinstallation of hyperglycemia on a time interval varying from 2 to 4 hours before the next administration.

Insulin glargine provided a good control of blood glucose level, with a very low incidence of subclinical hypoglycaemia episodes and was associated with a higher remission rate of diabetes. Premixed isophane insulin was associated with a blood glucose level to the lower normal range and with frequent hypoglycemia episodes. Also, due to the accelerated metabolism, isophane insulin activity did not reach the second administration in the day, thus cats were hyperglycemic for a varying length of time during the day.

Keywords: *feline diabetes, glargine, hyperglycaemia, insulin treatment, isophane,.*

INTRODUCTION

With an impressive body of research developed until present on diabetes mellitus (DM), specific treatment still relies on exogenous injectable insulin administration. Development and biosynthesis of recombinant DNA insulin analogs has provided various types of preparations, each with different pharmacokinetics, which allow adequate control of both basal and prandial glycemia. Recombinant DNA insulin analogs are defined as artificially modified forms of insulin, with different sequentiality and pharmacokinetics from natural endogenous insulin. For each type of insulin the hypoglycemiant effect, action onset and length of activity are different from one to another. Insulin analogues can be of bovine, porcine, or human synthesis, replicated on non-pathogenic bacterial or fungal cultures. Bovine insulin shares the highest degree of similarity with the feline insulin, with only a single amino acid variation on position 18 of chain A. Unfortunately, bovine preparations are

no longer available on the market and insulin treatment generally relies on either artificial, porcine or human insulin (Mooney and Peterson 2012). Compared to the human form, endogenous feline insulin presents variations on four amino acids, the former sharing more similarities with canine insulin, with a single variation on the last amino acid of the B chain (Lehninger 1982). Although the broad panel of insulin analogues available at the moment allows individualized glycemic control, insulin still remains a difficult to handle hormone. Its exogenous demand is limited by a number of factors, such as unbalanced pharmacodynamics, low therapeutic index, inconsistent intensity of effect, high physical instability and the need for administration by injection (Mayer, Zhang et al. 2007).

In cats, the purpose of insulin treatment generally targets bringing blood glucose levels below the renal threshold (<270 mg/dl) and maintain close to normal parameters for as long as possible during the day and between insulin doses. Insulin treatment is aimed at eliminating the toxic effect generated by high blood glucose (glucotoxicity) and remission of polyuria and polydipsia syndrome (PU/PD). In some cases, depending on the causal agent (obesity, iatrogenic diabetes mellitus and other associated diseases), an adequate insulin treatment protocol, can achieve DM remission. Briefly, remission can be translated as the resumption of pancreatic β cells secretory capacity, followed by total withdraw of the need for exogenous administration of insulin, with the possibility of relapse when causal agents reoccur.

The current paper describes the ideal administration protocol of two types of insulin frequently used in Romania for the therapy of feline DM, focusing on insulin dosing, type and regiments for clinically stable diabetic cats.

MATERIALS AND METHODS

Data was collected from the medical records of cats presented at the veterinary teaching hospital of Faculty of Veterinary Medicine of Iasi, Romania. Case registration revised the medical records of both male and female cats, with ages 4 weeks to 18 years. Cats were considered eligible for inclusion in the study if registered with characteristic clinical signs and a clear diagnosis of DM (Ettinger and Feldman 2005, Gunn-Moore 2005). Diagnosis was based on the clinical signs of PU/PD, polyphagia associated with weight loss, persistent fasting hyperglycemia over 270 mg/dl (80-120 mg/dl), glycosuria and data obtained on the general serum biochemistry (Blois, Dickie et al. 2010, Reusch 2011, Solcan 2011). Only cats in a stable clinical condition, without pre-renal azotaemia, non-ketotic and with a normal appetite, were considered eligible for the study. Therapeutic protocol included two commercially available preparations: synthetic long acting insulin glargine and a human origin premixed short and intermediate acting form, containing 70% human isophane insulin and 30% human soluble insulin. All cats were treated with both types of insulins and continued on the type of insulin that provided the ideal control of blood glucose level.

Insulin type and dose were administered according to the patient's clinical condition and physical characteristics. For both glargine and premixed preparations, dosing was started with 0.2 IU/kg/12 and increased gradually, with variations of 0.2/kg/dose. The new dose was maintained unaltered for a period of three to five days before further modifications (Jacquie Rand 2004), in order to balance insulin action and avert overlap. Dose reductions were preformed according the imminence of hypoglycemic crisis, varying from 30% to 50%

reduction. In cases where blood glucose level decreased below 40 mg/dl, insulin administration was withhold completely until clinically and biochemically stable (Ettinger and Feldman 2005, Roomp and Rand 2009, Hebert and Bulliot 2010). For every cat, insulin efficiency was established based on the blood glucose curve interpretation, for which the following parameters were considered: a. initial blood glucose; b. onset time of insulin action; c. the nadir expressed in mg/dl (lowest point of glucose after insulin administration); d. duration of action - estimated from the administration, trough the nadir and to exceeding 270 mg/dl. Samples for blood glucose curve construction were prevailed prior to feeding and insulin administration at 0 hours, followed by every 60 to 120 minutes sampling, for 12 hours. Glycemic monitoring was instituted for all newly diagnosed cats or the ones undergoing dose adjustments and/or changing of insulin type. Data from the monitoring of blood glucose were graphically represented and interpreted to adapt the type and dose of insulin. When it was not possible to perform blood glucose monitoring, insulin dose never exceeded 1UI/cat/administration/day, preventing this way a possibly fatal hypoglycemia event (Rucinsky, Cook et al. 2010).

RESULTS AND DISCUSSIONS

The subclinical form of DM was identified in 6 (9.2%) patients diagnosed in an early-stage of the disease. Individuals in this group were identified either as a result of routine controls or evaluations for other purposes. Cats were presented in a stable general condition with very discrete characteristic DM clinical manifestations such as slightly increased appetite and discrete PU/PD syndrome, all considered signs of good health by owners. A number of 12 cats (18.4%) were presented with noticeable clinical DM manifestations: marked PU/PD, increased appetite with progressive weight loss and frequent vomiting episodes. Some cases also presented mild metabolic acidosis and liver steatosis, but kept a well preserved appetite. The remaining 47 cats were not eligible for inclusion in the study as they presented advanced forms of DM, with anorexia, severe metabolic acidosis and hepatic lipidosis. All cats with the complicated form of DM were submitted to an intensive treatment protocol, with soluble insulin until clinically stable. Glycemic curve was the main tool to determine insulin onset of action, the duration of effect, its effectiveness in reducing blood glucose level and most important detecting overdosing and preventing hypoglycaemia episodes (Jacquie Rand 2004). Ideal insulin effect exerted a nadir at 6 to 8 hours after administration, was maintained within 80-180 mg/dl and below renal threshold (<270 mg/dl) until next administration. A post-insulin blood glucose lower than 60-70 mg/dl, was observed in cats eating a smaller amount of food, or in cases of insulin overlap and potentiating effect of the two doses administered in one day. A nadir of 60 - 70 mg/dl, although within the reference range for feline blood glucose, holds an increased risk for the onset of hypoglycaemia, due to further activity of insulin (Bergman and Ader 2000, Hoenig, Thomaseth et al. 2007, Reusch 2011). Persistent hyperglycemia and glucose levels above 170 mg/dl were associated to insulin resistance, insulin underdosing, overeating and caloric excess when owners failed to comply with the prescribed diet and incorrect handling and administration of insulin by the owner. Mishandling and/or vigorous shaking of insulin vials can lead to insulin inactivation and loss hypoglycemiant effect (Lehninger 1982) due to the breaking of disulfide bonds joining the two terminals chains of insulin molecule.

Supplementary care was undertaken in cats suspected to present stress hyperglycemia. These cases were admitted to the clinic for at least three days, than were reevaluated. Removal of cats from the usual environment, especially of those that rarely leave their normal habitat and examination in a noisy environment, are all factors with a direct contribution to stress and registration of higher blood glucose values than the real ones. Stress hyperglycemia is explained as an adaptive response of the body under extreme conditions and is based on a surge of adrenaline, sympathetic nervous system stimulation and increases of catabolic hormones (Tappy 2008). Of these, glucagon and epinephrine lead to dissolution of glycogen stores, increasing blood glucose and reducing insulin mediated processes. Thus, stress or "fight or flight" reaction require a greater amount of energy substrate for central nervous system and skeletal muscles at the expense of parenchymal organs (Tappy 2008, Van Cromphaut 2009). An important consequence of stress hyperglycemia lies in the misinterpretation of blood glucose curve, consecutive insulin overdose and increased risk of hypoglycemic episodes.

In 15 cats, ideal control was obtained with insulin glargine, with a median dose 0.6 IU/kg (range: 0.2-1.1 IU/kg) administered twice daily. Insulin glargine hypoglycemiant effect always reached the second administration during the day. One case presented an overlapping effect of insulin doses administered in one day and further treatment was administrated once daily. A number of 11 cases (73.5% of the glargine treated group) treated with glargine insulin, were heading for remission and required a lower dose and in 9 cases only once daily administration. One case with concurrent stage IV kidney disease presented chronic Somogyi effect. This can be defined as a counter-regulatory effect, induced by insulin overdose, an abrupt drop in blood glucose level, followed by a rapid increase in blood glucose level in order to overcome life threatening hypoglycaemia. In this case, the insulin dose was reduced to 50% and the type of preparation was also changed from the synthetic long acting form, to the human premixed. The cat still presented strong fluctuations of insulin requirements and could not be equilibrated properly on the premixed insulin either. However the protocol managed to stop the chronic Somogyi effect.

Only 3 cases were managed with premixed isophane twice daily, with a median dose of 0.8 IU/kg (range: 0.4-1.9IU/kg). Two cases were treated with the premixed preparation due to insulin resistance induced by hypersomatotropism. The premixed insulin never exceeded a period of action of 8 hours and allowed reinstallation of hyperglycemia on a time interval varying from 2 to 4 hours before the next administration. Always, when transitioning from the fast-acting insulin to the intermediate and slow acting insulin, the dosing was reduced to 50% and adjusted further based on the blood glucose curve.

Generally, insulin treatment required twice daily administration for both types of insulin (Ettinger and Feldman 2005). Blood glucose level of clinically stable cats included in the study, was achieved in the majority of cats with long acting glargine insulin and in a reduced number with premixed insulin (Caney 2013). Insulin glargine, as in other studies (Mooney and Peterson 2012, Bloom and Rand 2014), had proven a beneficial superior effect, entailing a significantly lower incidence of hypoglycaemia (Roomp and Rand 2009, Roomp and Rand 2013). Only two cases treated with insulin glargine had sporadic subclinical episodes of hypoglycemia. The blood glucose of cats was kept under renal threshold throughout the day and from morning to evening dose. The rate of remission of cast treated

with glargine was 73.5%. Some studies have reported a contribution of insulin glargine to diabetes remission in up to 90% of cases, after a median of six months after starting treatment. Insulin glargine use in the specific therapy of feline type II DM is increasing gradually, with very good results (Maggiore 2015). Once injected into the subcutaneous compartment, the insulin forms a micro-precipitate and slowly release insulin, without having a strong peak of activity (Mooney and Peterson 2004). Although in humans the time of action can be up to 26 hours, fast metabolism of cats reduces the activity time to a median of 12h. Thus, in most cases, twice daily administration is necessary to adequately control blood glucose. As also seen in the current study, the slow onset of action of insulin glargine and absence of a pronounced peak, is associated with a significantly lower incidence of episodes of hypoglycaemia. The disadvantage of long acting insulin is overlap effect observed in some cases and a sudden stop in its action which might impose difficulties in establishing the appropriate dose. Also, insulin glargine can been associated with chronic Somogyi effect, as observed in one case in this study. Premixed insulin proved adequate only for three cases in the current study. It provided a better control than glargine in the insulin resistant acromegalic cats and in the cat with stage IV chronic kidney disease. In these cases insulin glargine failed to lower blood glucose under the renal threshold and only mild control was achieved with the premixed insulin. The advantage of premixed insulin analogues is the achieving of a fast hypoglycemiant effect, necessary at the time of food intake. Thus, this type of insulin gives an immediate insulin impulse after food ingestion, preventing postprandial hyperglycemia. Premixed insulin provides a very tight glycemic control, bringing blood glucose level within normal parameters, or under limits. However, most studies recommend keeping blood glucose under the renal barrier, as bringing blood glucose at the lower level or under normal parameters, can be associated with an extremely high risk of severe hypoglycemia, especially in cats with a varying appetite (Jacquie Rand 2004). Also, studies regarding insulin treatment in cats generally do not advice isophane insulin, unless no other type is available or proven to work (Mooney and Peterson 2012, Maggiore 2015).

CONCLUSION

Both premixed and long-acting insulins were administered only to clinically stable cats or as called “healthy” diabetics, which did not require intensive care therapy. Insulin glargine had a better effect in lowering blood glucose under the renal threshold and had the ability to keep hyperglycemia episodes under control between doses. Glargine insulin slow onset of action and absence of a pronounced peak were associated with a significantly lower incidence of hypoglycaemia episodes. The high rate of remission observed in cats treated with glargine is also in the favor of this type of insulin as first choice treatment agent. Premixed insulin provided a tight control bringing blood glucose to the lower normal range, but was highly associated with hypoglycemia episodes. Also, due to the accelerated metabolism of cats, isophane insulin activity did not reach the second administration in the day, thus cats were hyperglycemic for a varying length of time during the day.

REFERENCES

1. Bergman, R. N. and M. Ader (2000). "Free fatty acids and pathogenesis of type 2 diabetes mellitus." Trends Endocrinol Metab **11**(9): 351-356.
2. Blois, S. L., E. L. Dickie, S. A. Kruth and D. G. Allen (2010). "Multiple endocrine diseases in cats: 15 cases (1997-2008)." J Feline Med Surg **12**(8): 637-642.
3. Bloom, C. A. and J. Rand (2014). "Feline diabetes mellitus: clinical use of long-acting glargine and detemir." J Feline Med Surg **16**(3): 205-215.
4. Caney, S. M. (2013). "Management of cats on Lente insulin: tips and traps." Vet Clin North Am Small Anim Pract **43**(2): 267-282.
5. Ettinger, S. J. and E. C. Feldman (2005). Diabetes Mellitus. Textbook of Veterinary Internal Medicine. R. W. Nelson, Elsevier Saunders. **II:** 1563-1591.
6. Gunn-Moore, D. (2005). "Feline endocrinopathies." Vet Clin North Am Small Anim Pract **35**(1): 171-210, vii.
7. Hebert, F. and C. Bulliot (2010). Guide Pratique de Medicine interne chien, chat et NAC, MED`COM.
8. Hoenig, M., K. Thomaseth, M. Waldron and D. C. Ferguson (2007). "Fatty acid turnover, substrate oxidation, and heat production in lean and obese cats during the euglycemic hyperinsulinemic clamp." Domest Anim Endocrinol **32**(4): 329-338.
9. Jacquie Rand, R. M. (2004). Feline diabetes mellitus. BSAVA Manual of Canine and Feline Endocrinology. M. E. P. Carmel T. Mooney, BSAVA**:** 129-149.
10. Lehninger, A. L. (1982). Principles of Biochemistry, International Union of Biochemistry and Molecular Biology.
11. Maggiore, A. D. (2015). "Canine and Feline diabetes mellitus: insulin therapy options." BSAVA Congress Proceedings: 110-111.
12. Mayer, J. P., F. Zhang and R. D. DiMarchi (2007). "Insulin structure and function." Biopolymers **88**(5): 687-713.
13. Mooney, C. T. and M. E. Peterson (2004). BSAVA Manual of Canine and Feline Endocrinology.
14. Mooney, C. T. and M. E. Peterson (2012). BSAVA Manual of Canine and Feline Endocrinology, BSAVA.
15. Reusch, C. (2011). "Feline diabetes mellitus." Veterinary Focus **21**(1): 9-16.
16. Roomp, K. and J. Rand (2009). "Intensive blood glucose control is safe and effective in diabetic cats using home monitoring and treatment with glargine." J Feline Med Surg **11**(8): 668-682.
17. Roomp, K. and J. S. Rand (2013). "Management of diabetic cats with long-acting insulin." Vet Clin North Am Small Anim Pract **43**(2): 251-266.
18. Rucinsky, R., A. Cook, S. Haley, R. Nelson, D. L. Zoran, M. Poundstone and A. American Animal Hospital (2010). "AAHA diabetes management guidelines." J Am Anim Hosp Assoc **46**(3): 215-224.
19. Solcan Gh. (2011) –Sistemul endocrin în Medicina internă a animalelor, coord. Falcă C., Vol II, Ed. Eurostampa, Timişoara , 248-306
20. Tappy, L. (2008). "Basics in clinical nutrition: Carbohydrate metabolism." European e-Journal of Clinical Nutrition and Metabolism **3**: 192-195.
21. Van Cromphaut, S. J. (2009). "Hyperglycaemia as part of the stress response: the underlying mechanisms." Best Pract Res Clin Anaesthesiol **23**(4): 375-386.

PITUITARY DWARFISM IN A GERMAN SEPHERD-AKITA MIX DOG

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Abstract:*A 4 month old unneutered male German shepherd-Akita mix dog was presented at the Veterinary Teaching Hospital of Iasi for stunted growth in spite of a slightly increased appetite and adequate dietary intake. The dog underwent physical, routine and specific clinicopathological investigations. Physical examination revealed stunted growth, muscle atrophy, delayed dental eruption, woolly hair coat, fox-like facial features and underdeveloped testis. The owner did not report any other changes in the general state of the dog. Routine clinicopathological examination did not reveal any abnormalities. Considering the case history, age, breed and the physical appearance of the dog, the most probable suspected disease was hormonal deficiency. Special laboratory investigations revealed a low level of GH and FT4, confirming congenital pituitary dwarfism. The recommended treatment in this case was thyroid hormone replacement and medroxy-progesterone acetate at three weeks interval, than every six weeks. Four weeks post-diagnosis, the dog was brought back to the clinic with a three day history of profuse watery diarrhea, severe dehydration, anorexia and hypothermia and died shortly after admittance. The current paper describes a case of congenital pituitary dwarfism in a four month old German shepherd-Akita mix, a disease with a rare incidence in dogs.*

Keywords: *congenital GH deficiency, dog, hypothyroidism, pituitary dwarfism.*

INTRODUCTION

Pituitary dwarfism is the result of defects in the organogenesis of the pituitary gland and consecutive isolated or combined hormonal deficiency. In most cases, this endocrinopathy can occur as a consequence of cysts formation in the anterior pituitary lobe, followed by pressure on the adjacent tissue and atrophy or hypoplasia. Pituitary developmental deficits can occur due to an autosomal recessive inherited abnormality(Mooney and Peterson 2012). Congenital dwarfism has been reported with a higher incidence in German shepherds, 20% of dogs in this breed being estimated to carry the faulty gene. Both parents have to be carriers of a single copy of the gene and with a recessive gene to give birth to an affected dog. If two carriers are mated, almost 50% of their pups will be carriers and on average 25% of their offspring will suffer from pituitary dwarfism (Kooistra, Voorhout et al. 2000).

Pituitary dwarfism in German Shepherds is also associated to cysts development in Rathke`s pouch as consequence of underlying genetic defects, but it is not yet clear if the cysts are the cause of this endocrinopathy(Voorbij, Leegwater et al. 2010, Voorbij, van Steenbeek et al. 2011). Other predisposed breeds include wolfhounds and a series of pastoral breeds. Pituitary dwarfism occurs rarely in felines (Mooney and Peterson 2012).

The most important aspect of pituitary dwarfism is growth hormone (GH) deficiency and secondary hypothyroidism, the cause of most significant clinical signs. The secretion of GH takes action in a pulsatile fashion, under the stimulant effect of GH-releasing hormone and inhibitory action of somatostatin, both secreted by the hypothalamus. Growth hormone exerts

both catabolic and anabolic effects(Mooney and Peterson 2004). Catabolic actions include insulin antagonism, with consecutive increase of lipolysis, gluconeogenesis and reduced glucose uptake in the intracellular space. On the other hand, the anabolic effects are controlled by insulin like growth factor-1 (IGF-1), secreted mainly in the liver, under the direct influence of GH. The IGF-1 is involved in processes of protein synthesis, chondrogenesis, growth and body size. Impaired thyroid function is the consequence of combined pituitary hormone deficiency. The physiopathology in this form of secondary hypothyroidism is different from primary hypothyroidism, and instead of being elevated, the level of TSH is on the lower limit as a consequence of reduced pituitary function(Mooney and Peterson 2004, Rijnberk and Kooistra 2010).

The current paper describe a case of congenital pituitary dwarfism in a four month old German Shepherd-Akita mix.

MATERIALS AND METHODS

A four month old unneutered male German Shepherd-Akita mix dog was presented at the Veterinary Teaching Hospital of Iasi for stunted growth in spite of a slightly increased appetite and adequate dietary intake. The dog was well proportioned to his body size but as reported by the owner, he was three to four times smaller compared to healthy littermates, in spite of receiving the same care, including type and amount of food and routine deworming and vaccination procedures. The dog underwent physical, routine and specific clinicopathological investigations. Considering the reported clinical signs, age, breed and the physical appearance of the dog, the most probable suspected disease was congenital pituitary dwarfism. Diagnosis protocol included physical examination, clinical findings, routine organ function evaluation and specific determinations, such as IGF-1 and thyroid hormones.

RESULTS AND DISCUSSIONS

Physical and clinical examination indicated a wide range of data. Musculoskeletal system evaluation revealed stunted growth, muscle atrophy, thin skeleton, delayed dental eruption and fox-like facial features, all characteristic signs of pituitary dwarfism. Also, on dermal examination, a series of specific features have been noted, such as thin skin, seborrhea sicca, woolly hair coat and retention of the lanugo hairs. Reproductive system was also affected, the dog was not cryptorchid as usually expected with pituitary dwarfism, but presented underdeveloped testis. No other changes of the general state of the dog were observed. Routine clinicopathological examination did not reveal any abnormalities regarding the kidney function, which is most often impaired in dwarfs due to the abnormal development of the glomeruli and reduced glomerular filtration rate(Mooney and Peterson 2012).

Basal IGF-1 level was well under the lower end of the reference parameters 12.57 nmol/l or 95.9 ng/ml (reference: 26.2-104.8 nmol/L or 200-800 ng/ml), confirming the suspicion of pituitary dwarfism. Secondary hypothyroidism was also diagnosed, based on a free thyroxine level of 8.8 pmol/l (reference: 10-45 pmol/l).

Figure 1: *Stunted growth, muscle atrophy, thin skeleton, fox-like facial features, woolly hair coat and retention of the lanugo hairs in a confirmed case of pituitary dwarfism*

Other recommended diagnosis procedures include determinations of basal specific GH, CT or MRI examination and pituitary function tests (Mooney and Peterson 2012). Even if directly affected by the disease, GH level is not always indicated as a sole diagnosis test. First, specific GH holds a low worldwide availability to clinicians and its determination usually requires shifting samples over borders. Also, GH is secreted in a pulsatile fashion and it can appear decreased in normal healthy dogs. However, GH determinations can be used within the pituitary function test, which is a definitive diagnosis procedure. The test is based on the administration of GH stimulants, such as GH-releasing hormone (GHRH) 1µg/kg i.v., alpha-adrenergic drugs, like clonidine 10 µg/kg i.v. or xilazyne 100 µg/kg i.v, followed by GH determinations at zero hours and then at 30 minutes. The expected response in a healthy dog is a two to four fold increase of the GH level, while a reduced increase is specific for pituitary dwarfism. Intracranial imaging by MRI or CT can reveal cysts and/or reduced volume (hypoplasia) of the pituitary gland (Meij, Mol et al. 1996, Hamann, Kooistra et al. 1999).

Life expectancy in these cases does is 3 to 5 years old and by this age the dogs usually develop a series of complications secondary to progressive loss of the pituitary function, increasing volume of the pituitary cysts and chronic kidney disease. Even if treated properly, the prognosis remains guarded. Treatment protocol relies on hormonal replacement. Canine GH is not currently available, but porcine GH can be used as a treatment option, as the amino acid sequence is similar in both species. Human GH on the other hand cannot be used in dogs due to the different structure of the hormone between humans and dogs, which can lead to antibody formation (van Herpen, Rijnberk et al. 1994). Porcine GH can be administered subcutaneously in doses of 0.1 – 0.3 IU/kg, three times a week. Long term frequency of porcine GH administration can be reduced, based on the IGF-1 plasma concentration. Overdosing can lead to insulin resistance, thus blood glucose level should also be monitored along with the IGF-1. When GH is not available, progesterone can be used to induce the expression of the GH gene in the mammary gland of the dog, a process followed by GH secretion to the systemic circulation. Medroxiprogestesterone acetate 2.5-5 mg/kg can be administered subcutaneously every three weeks, and then every six weeks, resulting in increasing of body size and hair growth (Knottenbelt and Herrtage 2002). Progesterone can also lead severe side effects including insulin resistance, acromegaly, pyoderma, development of mammary tumours and cystic endometrial hyperplasia. Thus, all female dogs should be spayed before progesterone administration. Secondary hypothyroidism should be addressed with levothyroxine, 10 to 44 µg/kg administered orally every 12 h, based on free T4, total T4 and TSH (Mooney and Peterson 2012).

In the current case, the dog was presented back to the clinic, with a four day history of profuse diarrhea, anorexia, severe dehydration and lethargy and died shortly after admission. The owners did not agree to necropsy examination, thus confirming if there was pituitary hypoplasia or cysts, was not possible, nor the cause of death.

CONCLUSION

The current case presented a wide variety of clinical signs, all indicative for pituitary dwarfism. The German shepherd mix breed was also an indicative clue for the diagnosis. Although the physical features of pituitary dwarfism seemed obvious, the final diagnosis was based on low levels of IGF-1 and thyroid hormones determination. Treatment and hormonal replacement needs to be life-long. As medication on its own can lead to a series of complications protocol also requires periodical monitoring of hormone plasma levels. As in the current case, pituitary dwarfs have a low expectancy of life and even with proper treatment the long term survival rate does not exceed four to five years of age.

REFERENCES

1. Hamann, F., H. S. Kooistra, J. A. Mol, S. Gottschalk, T. Bartels and A. Rijnberk (1999). "Pituitary function and morphology in two German shepherd dogs with congenital dwarfism." Vet Rec **144**(23): 644-646.
2. Knottenbelt, C. M. and M. E. Herrtage (2002). "Use of proligestone in the management of three German shepherd dogs with pituitary dwarfism." J Small Anim Pract **43**(4): 164-170.
3. Kooistra, H. S., G. Voorhout, J. A. Mol and A. Rijnberk (2000). "Combined pituitary hormone deficiency in german shepherd dogs with dwarfism." Domest Anim Endocrinol **19**(3): 177-190.
4. Meij, B. P., J. A. Mol, H. A. Hazewinkel, M. M. Bevers and A. Rijnberk (1996). "Assessment of a combined anterior pituitary function test in beagle dogs: rapid sequential intravenous administration of four hypothalamic releasing hormones." Domest Anim Endocrinol **13**(2): 161-170.
5. Mooney, C. T. and M. E. Peterson (2004). BSAVA Manual of Canine and Feline Endocrinology.
6. Mooney, C. T. and M. E. Peterson (2012). BSAVA Manual of Canine and Feline Endocrinology, BSAVA.
7. Rijnberk, A. and H. S. Kooistra (2010). Hypothalamus-pituitary system Clinical Endocrinology of Dogs and Cats, Schlutersche, Hannover**:** 13-57.
8. Solcan Gh. (2011) –Sistemul endocrin în Medicina internă a animalelor, coord. Falcă C., Vol II, Ed. Eurostampa, Timişoara , 248-306
9. van Herpen, H., A. Rijnberk and J. A. Mol (1994). "Production of antibodies to biosynthetic human growth hormone in the dog." Vet Rec **134**(7): 171.
10. Voorbij, A. M., P. A. Leegwater and H. S. Kooistra (2010). "[Hypopituitarism associated dwarfism in German Shepherds, saarloos wolf dogs and Czechoslovakian wolf dogs. Access to genetic testing]." Tijdschr Diergeneeskd **135**(24): 950-954.
11. Voorbij, A. M., F. G. van Steenbeek, M. Vos-Loohuis, E. E. Martens, J. M. Hanson-Nilsson, B. A. van Oost, H. S. Kooistra and P. A. Leegwater (2011). "A contracted DNA repeat in LHX3 intron 5 is associated with aberrant splicing and pituitary dwarfism in German shepherd dogs." PLoS One **6**(11): e27940.

CHRONIC SINUSITIS IN DOGS

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Abstract:*This paper reveals the results of clinical, laboratory and therapeutic investigations in a case offrontal chronic sinusitis in a dog. The patient was brought in for consultation with two well defined collections in the left supraorbital region, which led to exophthalmia and bilateral muco-bloody rhinorrhea. The superior nasal cavity wall was soft at palpation, and the inferior one was bulging into the oral cavity.*
The laboratory results excluded the parasitical (Linguatula serrata) or fungal etiology (Aspergillus spp.), the clinical signs (lack offever, presence of appetite and absence of dental disease) excluded the possibility of a septic inflammation.
X-rays of the head and gross anatomy findings after maceration of the skull revealed serious lesions of the frontal, lacrimal, zygomatic, maxillary, ethmoid bones, offering a large communication with the cranial cavity, though the animal did not present any signs of postural and dynamic distress.
All these advocate in favor of a neoplastic process involving the frontal sinus and the nasal cavity.

Key words*: dog, sinusitis, nasal discharge, parasitological/ fungal examination, osteolysis*

Unlike in other domestic mammals, the paranasal sinuses in dogs are ventilated spaces connected to the nasal cavity. They are a series of cavities, arranged one after another, and all dogs have frontal and maxillary sinuses, which differ with age, breed and head appearance. They are well formed, completely separated and situated bilaterally (1).

In dogs, the sinus system is generally poorly developed. The frontal sinus extends from the medial margin of the orbit to the temporal line. The frontal sinus has 3 chambers which drain (just over the ethmoidal volutes) separately into the nasal cavity (1, 4). The maxillary sinus is a cavity which freely communicates with the nasal cavity, and is known as the maxillary recess. It is situated dorsally to the roots of the 3rd and 4th premolars, medially to the infraorbital canal. It is communicating with the nasal cavity through a narrow orifice (4).

Because of their topography and communication with the nasal cavity, the paranasal sinuses can be subject to trauma, infections, parasitical and fungal infestations, or tumor development (2, 3, 4). This paper reveals the results of investigations in a case of chronic sinusitis in a dog, with both sinuses and the nasal cavity involved.

MATERIAL AND METHOD

An 11-years-old common breed bitch is brought in for consultations at DUO-VET private clinic, with bloody nasal discharge, sneezing and noisy breathing, for over 4 months. Gradually, the left frontal region was deformed by two low consistency swellings, of continuously growing volume. Because of the pressure to the eye, the dog was exophthalmic, but with no signs of low visual acuity.

Because the nasal discharge and the sneezing meant serious efforts from the owner, he accepted exploratory surgery, for biopsy and, hopefully, for establishing the diagnosis. Pre-op, the teeth were examined, to overrule any infection, and samples were taken for

parasitical (Linguatulla spp.) and fungal (Aspergillus spp.) examinations.

The surgery was done under deep anesthesia, and the two swellings, which were soft at palpation (fig. 1), were opened in a classical manner, after incising the skin and epicrania fascia (fig. 2). They both appeared as bloody collections under pressure (fig. 3). The nasal bones were macerated, and the same content was coming out the tip of the nose. The soft palate was deformed to the inside of the mouth.

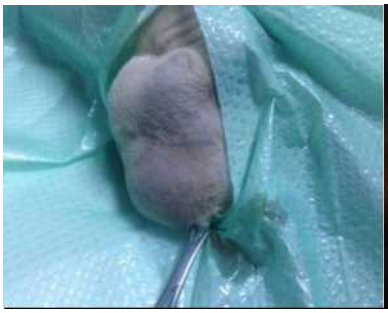

Fig. 1 - Pre-operative aspect of the collection

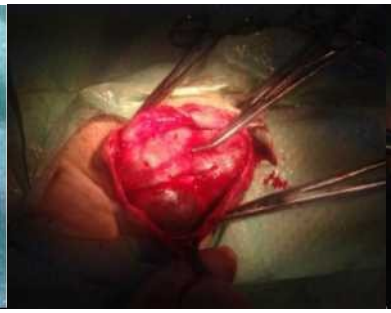

Fig. 2 - Intra-operative aspect

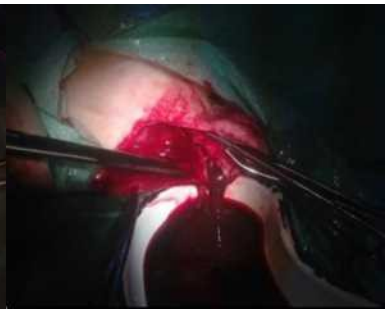

Fig. 3 - Bloody aspect of the collection

The exophthalmic eye was put back into the orbit. The nasal cavities were flushed with Saline solution, and the skin was closed with 2/0 non-absorbable suture, in a simple interrupted pattern (fig. 4).

Fig. 4 - Simple interrupted skin suture pattern

Antibiotics and iv fluids were given in post-op.

RESULTS AND DISCUSSIONS

The preoperative examination revealed at inspection the two swellings, extending from the frontal supra-orbital region to the nasal region. A discrete caudal deviation of the axis skull was noticed, but it was masked by the swellings. When opening the mouth, the animal showed no signs of discomfort; the soft palate was protruding towards the tongue, explaining why the animal avoided eating bones.

When examining the teeth from the left side, any problem with the premolars and molars was overruled, the dog showing no signs of dental disease.

Also, during this clinical examination, the abundant rhinorrhea was noticed and the

nasal discharge, of a bloody aspect, made the animal scratch its nose frequently. At palpation, the two deformed areas appeared as liquid collections under pressure. At this point, there was no additional nasal discharge, as seen when compressing the dorsal nasal cavity wall.

The direct examination, under magnification, of the samples taken from the nasal discharge or by aspiration from one of the collections, was negative for Linguatulla spp. For this parasite, the fecal exam was also negative. Fungal examination on Sabouraud agar plate, for identifying Aspergillus spp or other species, was negative. These laboratory investigations excluded the possibility of a fungal or parasitical nature of the disease.

Because the surgery allowed both collections to be drained, animal breathing was improved, normal feeding was resumed, and the vital prognostic was reserved, because the extent of the lesions could not be assessed. The overall clinical status of the patient was better, making the owner hopeful. Approximately three months after surgery, the nasal discharge reappeared and the two swellings began growing again. Respiratory distress was noticed, together with rhinorrhea. Although Hedlund, in 1994, shows that Aspergillus sinusitis can affect the 7-years-old dog, the laboratory findings showed no signs of fungal presence. The lack of dental disease and fever, and the aspect of the secretions do not indicate an inflammatory pathology. Frontal and nasal osteolysis do not overrule a neoplastic process, even if Hedlund (1994) says that sinus tumors are a mere 1% of the cancer pathology in dogs. The same author reveals that old dogs are affected (as in this case), and over 80% are of malignant nature.

The X-rays of the head, done after relapse, show frontal, nasal and maxillary osteolysis (fig. 5). The inferior contour of the frontal sinus is gone, and high radio lucent oval-rounded shaped areas are thought to be secondary to deep osteolysis.

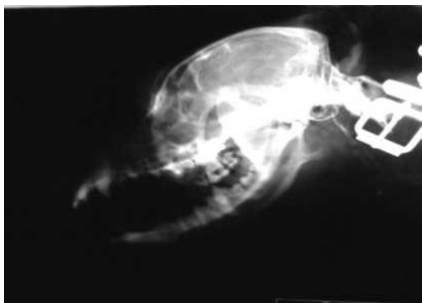

Fig. 5 - Osteolysis of the frontal, nasal and maxillary bones

This relapse, with a poor general status of the animal, led to euthanasia. After maceration of the skull (fig. 6), the osteolytical cavities (as seen on the radiographs) are confirmed.

Fig. 6 - Maceration of the skull left the bones clean and confirmed the severe osteolysis

The frontal bones are separated and deformed, the left sinus has thin and perforated walls. The orbital wall of the left frontal sinus is destroyed, probably after malignant development. The ethmoid bone is gone and a large communication with the cranial cavity is seen. Nevertheless, the animal showed no signs of static or dynamic postural impairment. The nasal bone and nasal conchae are also completely macerated. The osteolytical process on the left side started affecting the lacrimal, zygomatic and maxillary bones. The palatine processes are 80% destroyed, and the palatine, pterygoid bones and the vomer are missing.

All of these clinical, imaging and laboratory findings lead us to suspect a neoplastic process in evolution, with osteolysis and well defined cystic formations in the frontal and retrobulbar regions.

The osteolysis of the nasal cavity was complete, leading to serious impairment of breathing. Because the animal was presented in the last stages of the disease, there was no concluding if the process began in the frontal sinus or in the nasal cavity. The rhinorrhea is seen in both cases.

CONCLUSIONS

1. The development of collections in the frontal sinuses poses diagnostic issues, regarding the nature of the process.
2. The negative laboratory results for Aspergillus spp. or Linguatulla spp. are not in favor of a parasitical or fungal infestation.
3. The lack of fever, the presence of appetite and no dental disease exclude the inflammatory nature of the process.
4. The intra-operatory findings revealed two well defined cystic collections, with a bloody content and bone lysis of the anterior margin of the orbital and the nasal cavity.
5. The osteolytical lesions of the left frontal sinus and the surrounding bones (lacrimal, zygomatic and maxillary) were further distinguished through X-rays of the head or by maceration of the skull after death.
6. The evolution of the disease and all the clinical and laboratory findings advocate in favor of a neoplastic process.

REFERENCES

1. Cotofan V., Palicica E., Hritcu Valentina, Enciu V., 1999 - Anatomia animalelor domestice, vol. 1, ed. Orizonturi universitare, Timisoara, pag 143 - 173;
2. Hedlund C.S., 1994 - Surgical management of chronic nasal cavity and paranasal sinus disease, in Birchard and Sherding, Saunders Manual of Small Animal Practice. W.B. Saunders Company, Philadelphia, p. 541 - 547
3. Niculescu Al., Dida I., 1998 - Parazitologie veterinara, ed. Ceres, Bucuresti, p. 343 - 345;
4. Sharp N.J.H. et all., 1991 - Nasal cavity and frontal sinuses. Problems in Veterinary Medicine, Head and Neck Surgery, 3, 170.

INTRAMUSCULAR *SARCOCYSTIS* CYSTS DETECTION IN ANIMALS: A REVIEW ARTICLE

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Abstract:*Sarcosporidiosis is a cosmopolitan zoonotic parasitic disease caused by small intracellular apicomplexan protozoa of the genus Sarcosystis. This parasite has a heteroxenous life cycle, involving: a definitive host, where Sarcocysts spp. develops in intestine, infesting the enterocytes, and an intermediate host, in which invades the muscle cells in order to form the typical sarcocysts in tissue. Sarcocystis species parasitize almost all vertebrates and are widely distributed around the globe. So far, 189 species of Sarcocystis have been identified; however, for the both definitive and intermediate hosts are known only 46% of the species. The purpose of this literature review is to present reported data on the prevalence of intramuscular Sarcocystis cysts in animals. Data from various studies suggest different variations depending on the species and the area where research was performed, such as in cattle (24–80%), swine (0.80-43%), goats (52-79%), and sheep (18-100%). The field research refers to the degree of intensity and extent of the skeletal muscles microsarcocysts in the following anatomical regions: the intercostal muscles, the diaphragm, tongue muscles, and dorsal and hamstring muscles. From all the Sarcocystis species found in animals, only two of them (S. hominis and S. suihominis) have zoonotic potential, the man can be intermediate host for their species and also definitive host for species mentioned. Considering the fact that the sarcosporidiosis is high-impact diseases among animals, data discussed in this paper emphasize the presence of intramuscularly sarcocysts, as well as the zoonotic risk in several regions of the world in the last decade.*

Key words: *Sarcocystis spp.,* animals, intramuscular cysts, epidemiology

INTRODUCTION

Sarcocystis genus is a specific group of protozoa that infects intracellularly, and represents the end stage of the parasite asexual life, found in the tissue of its host (Mitrea, 2011). This parasite has a heteroxenous life cycle, involving: a definitive host, where *Sarcocysts* spp. develops in intestine, infesting the enterocytes, and an intermediate host, in which invades the muscle cells in order to form the typical sarcocysts in tissue (Dubey et al., 1989).

Sarcocystis spp. was reported in 1843 by Miescher, describing whitish cysts, threadlike, in striated muscles at the house mouse, lacking a scientific name, and after 20 years, the parasite was named as "Miescher's tubules" (Fayer, 2004). Over time, scientists have debated whether *Sarcocystis* species are fungi or protozoa. There have been theories that *Sarcocystis* were fungi, because only sarcocyst stage was known at the time, and when sarcocysts and their contents were placed in different culture media, mycelia and hyphae were sometimes found several days later. After the first reporting of *Sarcocystis*, the needle or crescent-shaped bodies (bradyzoites) in sarcocysts were studied by electron microscopy, and showed similarity with other aplicomplexan protozoa such as *Toxoplasma* and *Eimeria* (Fayer, 2004).

Although it is a parasitosis with discrete clinical events, the surveys show a special medical and veterinary significance. From all the *Sarcocystis* species found in animals, only two of them (*S. hominis*, from cattle, and *S. suihominis*, from swine) have zoonotic potential, the man can be intermediate host for their species and also definitive host for species mentioned (Dubey, et al., 1989).

The purpose of this literature review is to present reported data on the prevalence of intramuscular *Sarcocystis* cysts in animals.

Intramuscular *Sarcocystis* in animals

Sarcocystis species parasitize almost all vertebrates and are widely distributed around the globe. So far, 189 species of *Sarcocystis* have been identified; however, for the both definitive and intermediate hosts are known only 46% of the species (Odening, 1998), for example, in animals of the *Bovidae* family (domesticated *Bovidae*) have been identified 143 species of *Sarcocystis* (Heller et al., 2013).

Some species of *Sarcocystis* are always microscopic (e.g. *S. cruzi*), while other species becomes macroscopic (e.g. *S. gigantea* and *S. muris*). Common forms of sarcocysts are filamentous, elongated or globular. Macroscopic sarcocysts are almost always in skeletal muscle or esophageal muscles (Dubey et al., 1989). Parasites identification is based on observation of sarcocysts in muscle tissue. In the muscle preparations, sarcocysts size up to 1 cm long (Greiner et al., 1989).

As a result of the consumption of meat containing sarcocysts by the definitive host, the sexual phase of the life cycle of the parasite occurs in the host's intestinal wall and ends with the release of sporocysts with the elimination of faeces into the environment. Following ingestion of oocysts by the intermediate host, the parasites enter the asexual reproduction phase, which through four generations of merozoites leads to the formation of sarcocysts inside the muscle cells of the host (cardiac, striated, or smooth muscle) (Domenis et al., 2011). The field research refers to the degree of intensity and extent of the skeletal muscles microsarcocysts in the following anatomical regions: the intercostal muscles, the diaphragm, tongue muscles, and dorsal and hamstring muscles (Tăbăran et al., 2013).

Data from various studies suggest different variations depending on the species and the area where research was performed, such as in cattle (24–80%), swine (0.80-43%), goats (52-79%), and sheep (18-100%).

***Sarcocystis* infection in cattle**

Heydorn et al. (1975) showed that conclusively there were three species of *Sarcocystis* in cattle and proposed new names for them: *S. bovicanis* having definitive host the canids, *S. bovifelis* having definitive host the felines, and *S. bovihominis* having definitive hosts the both primates and humans (Heydorn et al., 1975). The new names of these species have been rejected by the International Code of Zoological Nomenclature in favor of the existing designations: *S. cruzi, S. hirsuta* and *S. hominis* (Frenkel et al., 1979).

Exists a considerable confusion as to *Sarcocystis* species in buffalo (*Bubalus bubalis*), and in cattle (*Bos taurus*). This issue is of current interest, because some species such as *S. hominis* in cattle prove to be zoonotic, but the same cannot be said for the species of buffalo (Jehle et al., 2009; Yang et al., 2001a,b).

Although the *Sarcocystis* spp. infections were reported after more than a century in Hungary, the actual significance of infection in cattle with *Sarcocystis* is not known in the country, neither throughout Central and Eastern Europe. In 2014, in a study conducted by Hornok et al., there have been examined 151 cattle and 15 buffalos. In total, 100 of the 151 cattle (66.22%) were positive diagnosed for *Sarcocystis* infestation by PCR, while at buffalos has not been find any positive sample (Hornok et al., 2015).

A study conducted in north-western Italy, Domenis et al. (2011) identified the presence of *Sarcocystis* cysts in cattle by rapid histological examination of the diaphragm and cardiac muscle, and esophagus, and by conventional electron microscopy in combination with molecular

techniques. Through histological screening, 78.1% (300/384) of the animals tested were found to sarcocysts in at least one organ. The most frequent affected anatomical structure was esophagus (n=279), then the diaphragm muscle (n=228), and cardiac muscle (n=225). Through molecular techniques it was identified all 3 *Sarcocystis* species that are found in cattle (*S. cruzi, S. hominis* and *S. hirsuta*). The prevalence of these species detected in samples population was : 74.2% *S. cruzi* (n=285), 42.7% *S. hominis* (n=164), 18.5% *S. hominis*-like (n=71) and 1.8% *S. hirsuta* (n=7) (Domenis et al., 2011).

In Argentina, Moré et al. performed a study on 90 beef cattles, to diagnose infection with *Sarcocystis* sp. and to detect co-infections with *Neospora caninum* and/or *Toxoplasma gondii*, collecting the following sections: 50g of diaphragm muscle, 100g of myocardium (left ventricle), and 10 cm of esophagus, from each animal. After microscopic examination, this sarcocysts was found 100% in the myocardium, 71% in esophagum, and 28% in the diaphragm muscles. The characteristics of *Sarcocystis* cysts detected corresponded with *S. cruzi*: that shows thin walls (<1 µm thick), and villar protrusions had lengths between 6-13µm (Moré et al., 2008).

Sarcocystis infection in swine

Of the three of *Sarcocystis* species in pig (*S. miescheriana, S. porcifelis* and *S. suihomini*), only *S. suihominis* is important in public health. The vast majority infections in pigs are asymptomatic (Avapal et al., 2004).

In a study performed in Romania (Transylvania area) by Tăbăran et al. (2013) have been examined 150 of meat samples, of which 120 from domestic swine (diaphragmatic pillars) and 30 from wild boars (muscle tissue). The samples were examined for infection with *Trichinella* and *Sarcocystis*. Following the examination by classical trichinelloscopy were found a large numbers of infection with *Sarcocystis* spp cysts. Of the 150 meat samples, 97 (64%) were positive for *Sarcocystis*, of which 67 (56%) in domestic swine and 30 (100%) in wild boar. Comparing the statistics for these two species, it can be stated that the infection is higher in wild boar meat than it is in pig (Tăbăran et al., 2013).

In 1997, Saito et al. identified for the first time in Japan in pigs *S. suihominis*. In their study, cardiac and diaphragm muscle from the 600 breeding pigs reared in the same piggery were examined. Of the 600 pigs examined, 5 animals (0.83%) were positive for *Sarcocystis* cysts. Fresh cysts measured 1.080-2.040 x 106-107 µm length and 4-6 µm thickness (Saito et al., 1998).

Sarcocystis infection in goat

So far three species of *Sarcocystis* spp. have been identified in domestic goats: *Sarcocystis capracanis, S. hircicanis* and *S. caprafelis*. Also, *S. capracanis* and *S. hircicanis* produce microscopic sarcocysts (microcysts), while *S. caprafelis* produce macroscopic cysts (macrocysts). *S. capracanis* is the most pathogenic in goats, causing fever, weight loss, anorexia, tremors, irritability, abortion and death (Dubey et al., 1989).

In the study of Shekarforoush et al. in Iran, were examined 169 slaughtered goats, randomly selected. The animals were inspected for the presence of macroscopic sarcocysts, examining the tongue, heart, esophagous and various skeletal muscles, such as diaphragm, abdominal and intercostal muscles from each animal. 28 of the 169 goats (16.6%) were diagnosed as infected with macroscopic sarcocysts. Of the 169 goats, 168 were diagnosed as positive for *Sarcocystis* species by smear method, and all 169 samples were positive by digestion method. The sensitivity of the method spreading (smear method) compared to the digestion method in the

infection diagnosis was 94% in the heart, 92.3% in esophagus, 91% in tongue, and 84% in diaphragm (Shekarforoush et al., 2005).

***Sarcocystis* infection in sheep**

Four *Sarcocystis* species were reported in sheep: *S. gigantea, S. arieticanis, S. medusiforms*, and *S. tenella*, the later one being the most pathogenic in sheep (Heckeroth and Tenter, 1999; Dubey et al., 1989 a, c). Sheep can be infected by ingesting contaminated water or fodder with sporocysts. Clinical signs usually are reserved, but when are present include anorexia, fever, weight loss, anemia and even abortion (Silva et al., 2009).

The prevalence of *S. tenella* is variable throughout the world. In Asia is reported the highest prevalence: 33.9% in Iran (Daryani et al., 2006), 47.3% in Turkey (Őzkyhan et al., 2007), 93% in Ethiopia (Woldemeskel and Gebreab, 1996), 96.9% in Mongolia (Fukuyo et al., 2002).

In the study of epidemiology of sarcocystosis at sheep from Romania, conducted by Titilincu et al. (2008), were examined 48 sheep, with aged between 2-3 years. Of the 48 samples, 44 (91.7%) were found to be positive with *Sarcocystis* spp. The macroscopic examination were identified macrocysts only into the esophagus at 26.3% (10/38) examined carcasses. Microcysts were identified 81.6% (31/38) into esophagus, 79.9% (35/48) in myocardium, and 68.8% (33/48) in diaphragm muscles (Titilincu et al., 2008).

The increased prevalence is associated with the free access of dogs (definitive host) in sheep flocks, or arthropod that acts as mechanic vectors for sporocysts dog faeces excreted (Silva et al., 2009).

Heart, diaphragm and skeletal muscles are the organs most often parasitized by *Sarcocystis* spp. in the intermediate host (Buxton, 1998; Daryani et al., 2006). In a study conducted by Silva et al. (2009), were detected co-infection with *S. tenella* and *T. gondii* in the hart and diaphragm muscles, the animals not showing clinical signs (Silva et al., 2009).

Several studies in human population have been carried out for determination of human sarcocystosis. Prevalence data for *Sarcocystis* infection reflect mainly case reports and findings by physicians, public health workers and scientists with specific interests. Consequently, many infections are not reported (Fayer, 2004).

Humans can be in the same time intermediate host for their species and definitive host for *S. bovihominis* and *S. suihominis*. Consumption of raw meat or insufficiently prepared from pigs or cattle infected with *S. hominis* and *S. suihominis* produce the human infection (Van Knapen et al., 1987).

The anatomic regions most infected with *Sarcocysts* spp. in humans are skeletal and cardiac muscles, but there were, also, found in the larynx, pharynx and esophagus (Lele et al., 1986).

In conclusion, data from these studies concerning the presence of sarcosporydiosis are relevant to human and animal health, because, the incidence of *Sarcocystis* spp. in animals and humans has an increased extensivity with of sanitary and socioeconomic implications (Domenis et al., 2011).

REFERENCES

1. Avapal, R.S., Sharma, J.K., Juyal, P.D., 2004. Pathological changes in *Sarcocystis* infection in domestic pigs (*Sus scrofa*). Veterinary Journal, 168 (3): 358–361.
2. Buxton, D., 1998. Protozoan infections (*Toxoplasma gondii, Neospora caninum* and *Sarcocystis* spp.) in sheep and goats: recent advances. Veterinary Research, 29 (3-4): 289–310.

3. Daryani, A., Alaei, R., Dehghan, M.H., Arab, R., Sharif, M., Ziaei, H., 2006. Survey of *Sarcocystis* infection in slaughtered sheep and buffaloes in Ardabil, Iran. Journal of Animal and Veterinary Advances, 5 (1): 60–62.
4. Domenis, L., Peletto, S., Sacchi, L., Clementi, E., Genchi, M., Felisari, L., Felisari, C., Mo, P., Modesto, P., Zuccon, F., Campanella, C., Maurella, C., Guidetti, C., Acutis, P.L., 2011. Detection of a morphogenetically novel *Sarcocystis hominis*-like in the context of a prevalence study in semi-intensively bred cattle in Italy. Parasitology Research, 109: 1677-1687.
5. Dubey, J.P., Speer, C.A., Fayer, R., 1989a. *Sarcocystis* of animals and man. CRC Press, Inc., Boca Raton, Fla.
6. Dubey, J.P., Speer, C.A., Munday, B.L., Lipscomb, T.P., 1989c. Ovine sporozoan encephalomyelitis linked to *Sarcocystis* infection. Veterinry Parasitology, 34 (1-2): 159–163.
7. Fayer, R., 2004. *Sarcocystis* spp. in human infections. Clinical Microbiology Reviews, 17 (4): 894-902.
8. Frenkel, J.K., Heydorn, A.O., Mehlhorn, H., Rommel, M., 1979. Sarcocystinae: *Nomina dubia* and available names. Z. Parasitenk. 58 (2): 115–139.
9. Fukuyo, M., Battsetseg, G., Byambaa, B., 2002. Prevalence of *Sarcocystis* infection in meat-producing animals in Mongolia. Southeast Asian Journal of Tropical Medicine and Public Health, 33 (3): 490–495.
10. Greiner, E.C., Roelke, M.E., Atkinson, C.T., Dubey, J.P., Wright, S.D., 1989. *Sarcocystis* sp. in muscle of free-ranging Florida panthers and cougars (*Felis concolor*). Journal of Wildlife disease, 25 (4): 623-628.
11. Heckeroth, A.R., Tenter, A.M., 1999. Comparison of immunological and molecular methods for diagnosis of infections with pathogenic *Sarcocystis* species in sheep. The Tokai Journal of Experimental and Clinical Medicine, 23 (6): 293-302.
12. Heller, R., Frandsen, P., Lorenzen, E.D., Siegismund, H.R., 2013. Are there really twice as many bovid species as we thought? Systematic biology, 62 (3): 490-493.
13. Heydorn, A.O., Gestrich, R., Mehlhorn, H., Rommel, M., 1975. Proposal for a new nomenclature of the *Sarcosporidia*. Z. Parasitenk. 48 (2): 73–82.
14. Hornok, S., Mester, A., Takács, N., Baska, F., Majoros, G., Fok, E., Biksi, I., Német, Z., Hornyác, Á., Jánosi, S., Farkas, R., 2015. *Sarcocystis*-infection of cattle in Hungary. Parasites and Vectors, 8: 69.
15. Jehle, C., Dinkel, A., Sander, A., Morent, M., Romig, T., Luc, P.V., De, T.V., Thai, V.V., Mackenstedt, U., 2009. Diagnosis of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) in northern Vietnam. Veterinary Parasitology, 166 (3-4): 314–320.
16. Lele, V.R., Dhopavkar, P.V., Kher, A., 1986. *Sarcocystis* infection in man. Indian Journal of Pathology & Microbiology, 29 (1): 87-90.
17. Mitrea, I. L., 2011. *Parazitologie și boli parazitare*. Ed. Ceres, București
18. Moré, G., Basso, W., Bacigalupe, D., Venturini, M.C., Venturini, L., 2008. Diagnosis of *Sarcocystis cruzi*, *Neospora caninum*, and *Toxoplasma gondii* infections in cattle. Parasitology Research, 102: 671-675.
19. Odening, K., 1998. The present state of species systematics in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidie). Systematic Parasitology, 41, 209-233.
20. Őzkyhan, M.A., Karaer, Z., Ilkme, A.N., Atmaca, H.T., 2007. The prevalence of *Sarcocystis* species in sheep slaughtered in municipality slaughterhouse in Kirikkale. Turkish Society for Parasitology, 31 (4): 272–276.
21. Saito, M., Shibata, Y., Ohno, A., Kubo, M., Shimura, K., Itagaki, H., 1998. *Sarcocystis suihominis* detected for the first time from pigs in Japan. The Journal of Veterinary Medical Science, 60 (3): 307-309.
22. Silva, R.C., Su, C., Langoni, H., 2009. First identification of *Sarcocystis tenella* (Railliet, 1886) Moulé, 1886 (Protozoa: Apicomplexa) by PCR in naturally infected sheep from Brazil. Veterinary Parasitology, 165 (3-4): 332-336.
23. Shekarforoush, S.S.S., Razavi, S.M., Dehghan, S.A., Sarihi, K., 2005. Prevalence of *Sarcocystis* species in slaughtered goats in Shiraz, Iran. The Veterinry Record, 156 (13): 418-420.
24. Tăbăran, A., Mihaiu, M., Dan, S.D., Mihaiu, R., Cordiş, I.V., Cordea, D., 2013. Incidence of *Sarcocystis* spp. infestation in pork and wild boar samples in Transylvania. Porcine Research. International Journal of the Bioflux Society, 3 (2): 36-39.

25. Titilincu, A., Mircean, V., Blaga, R., Bratu, C.N., Cozma, V., 2008. Epidemiology and etiology in sheep sarcocystosis. Bull. UASVM Vet. Med. 65, 49–54.
26. Van Knapen, F., Bouwmann, D., Greve, E., 1987. Study on the incidence of *Sarcocystis* spp. in Dutch cattle using various methods. Tijdschr. Diergeneeskd, 112 (19): 1095–1100.
27. Yang, Z.Q., Zuo, Y.X., Ding, B., Chen, X.W., Luo, J., Zhang, Y.P., 2001a. Identification of *Sarcocystis hominis*-like (Protozoa: Sarcocystidae) cyst in water buffalo (*Bubalus bubalis*) based on 18S rRNA gene sequences. The Journal of Parasitology, 87 (4): 934–937.
28. Yang, Z.Q., Zuo, Y.X., Yao, Y.G., Chen, X.W., Yang, G.C., Zhang, Y.P., 2001b. Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. Molecular and Biochemical Parasitology, 115 (2): 283–288.
29. Woldemeskel, M., Gebreab, F., 1996. Prevalence of sarcocysts in livestock of northwest Ethiopia. Journal of Veterinary Medicine. Series B, 43 (1): 55–58.

PREVALENCE OF ENDOPARASITES IN CATS FROM TWO URBAN AREAS IN SOUTHERN ROMANIA: PRELIMINARY DATA

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Abstract: *Domestic cats can act as reservoir for many intestinal parasites. Furthermore, these animals play an important role in parasite transmission to human and other animals by contamination of the environment with faecal parasite stages. The purpose of this study was to investigate the endoparasite community of domestic cats from two urban areas in Southern Romania. For this, a total number of 120 cats were included in the study. From them, fecal samples were collected and examined, first grossly, then using a sodium chloride flotation technique followed by microscopy for identification of parasitic elements (eggs, oocysts). For a subset of 69 samples a Baermann method was performed for detection of lungworm nematodes. The overall prevalence of gastrointestinal parasites was 26.7% (32/120; 95%CI:0.19–0.35) and 2.9% (2/69; 95%CI:0.003–0.10) for lungworm, respectively, in the examined samples. Five endoparasite species, including helminthes (nematodes, cestodes) and protozoa were detected. The most prevalent species were Toxocara cati (12.5%) and Isospora felis (12.5%), followed by Aelurostrongylus abstrusus (2.9%), Ancylostoma tubaeforme (1.7%), and Dipylidium caninum (0.8%). For two samples (1.7%), mixed infection (T. cati + Ae. abstrusus and T. cati + A. tubaeforme) was diagnosed. In conclusion, the results are particularly of veterinary importance as these parasites commonly affect the animal health, suggesting the need for parasitological control. In addition, due to the zoonotic potential of some of these parasites, potential risks for the human health have to be considered.*

Key words: cats, endoparasites, urban areas, risk factors

INTRODUCTION

Endoparasitoses of cats are caused by a large range of parasitic species from different taxonomic units, such as protozoa and helminths. These parasites are the main cause of various clinical signs, from gastrointestinal disorders in cats (Calvete et al., 1998), to anemia or anorexia in the more severe cases (Traversa, 2012). Furthermore, stray cats play an important role in parasite transmission to human and other animals by contamination of the environment with faecal parasite stages (Khademvatan et al., 2014).

It is well known that *Toxocara cati* and *Ancylostoma tubaeforme* are responsible for human visceral/ocular and cutaneous larva migrans, respectively but also *Dipylidium caninum* can infect humans (Robertson and Thompson, 2002; Deplasez et al., 2011). Other non-zoonotic parasites are also important in cats (i,e, *Isospora*) because can cause digestive disorders, like diarrhea and malabsorption in its hosts (Borji et al., 2011; Barutzki and Schaper, 2013).

Therefore, the importance of parasite control is not only to cure clinical signs in infected cats, but also to minimize the contamination risks and transmission of these parasites to other animals or to humans (Borji et al., 2011).

The aim of this study was to investigate the endoparasite populations of domestic cats from urban areas in Southern Romania.

MATERIALS AND METHODS

In the present study, a total number of 120 cats (69 females; 51 males), originated from two urban areas [B (n=102) and C (n=18)] in Southern Romania were copro-parasitological investigated. Data about their age (kitten ≤ 1 year; adult >1 year), outdoor access, and anthelmintic treatments were recorded, as provided by the owners.

Fresh fecal samples, collected from the cats, were examined, first grossly, then using a sodium chloride flotation technique followed by microscopy for identification of parasitic elements (eggs, oocysts). For a subset of 69 samples a Baermann method was performed for detection of lungworm nematodes.

RESULTS AND DISCUSSION

The coproscopical examination of the samples showed infestations with one protozoa and four helminthes species; an overall prevalence of gastrointestinal parasites of 26.7% (32/120; 95%CI:0.19–0.35) and 2.9% (2/69; 95%CI:0.003–0.10) for lungworm, respectively was registered. The most prevalent species were *Toxocara cati* (12.5%) (Fig. 1A) and *Isospora felis* (12.5%) (Fig. 1B), followed by *Aelurostrongylus abstrusus* (2.9%) (Fig. 1C), *Ancylostoma tubaeforme* (1.7%) (Fig. 2a), and *Dipylidium caninum* (0.8%) (Table 1).

For most fecal samples, infestation with a single parasites species was found. However, for two samples (1.7%), mixed infection (*T. cati* + *Ae. abstrusus* and *T. cati* + *A. tubaeforme*) was diagnosed.

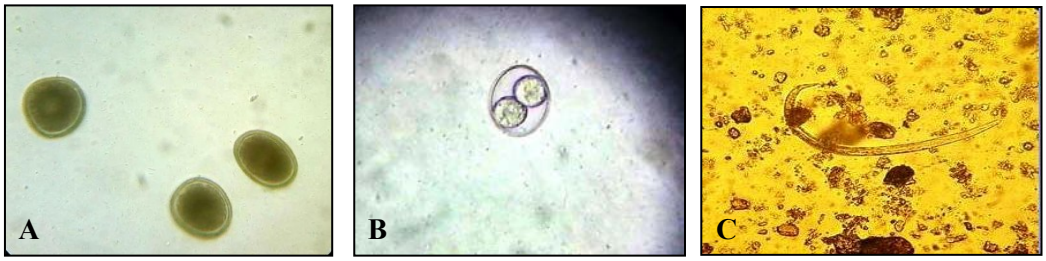

Figure 1. Parasitic stages microscopically identified using flotation technique (A, B) and Baermann method (C) in feces of cats from urban areas in southern Romania: **A)** eggs of *Toxocara cati* (x10); **B)** oocysts of *Isospora felis* (x20); **C)** larva of *Aelurostrongylus abstrusus* (x20)

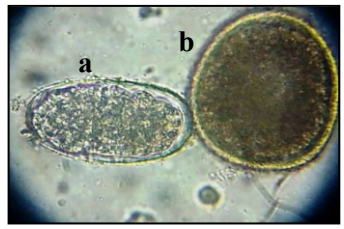

Figure 2. Mixed infection with a. *Ancylostoma tubaeforme* and b. *Toxocara cati* (parasitic stages identified using flotation technique; x20)

Infestations with helminthic stages were found in 14.1% (17/120) of the domestic cats included in the study. The most common helminth was *T. cati*, found in 12.5% (15/120) of the examined samples. Protozoa infections were found in 15 (12.5%) samples of the total cats examined.

Table 1. The frequency and prevalence of individual parasites identified by coproscopy in domestic cats from two urban areas in Southern Romania

Parasite species	Frequency (number positive/number examined)	Prevalence (%)
Isospora felis	15/120	12.5
Toxocara cati	15/120	12.5
Aelurostrongylus abstrusus	2/69	2.9
Ancylostoma tubaeforme	2/120	1.7
Dipylidium caninum	1/120	0.8

These findings, analyzed according to the age of animals, show a higher prevalence (44.0%) of endoparasites in kittens compared to the adult cats (17.7%).

Single parasitic infection predominated with a prevalence of 25.0%, while multiple parasitic infections had a prevalence of 1.7%. There was no significant difference in terms of parasitic infection between male (29.4%) and female (24.6%) cats (Table 2).

Similar studies, performed by Barutzki and Shaper (2003, 2011) shown that puppies and young animals under one year are more often infected with endoparasites than adult animal; this is in agreement with the results obtained in our study

A study contucted by Cantó et al. (2013) shows a prevalence of 36% of single infections and 7% of mixed infections; these results are accordingly with those obtained in our study. Engbaek et al. (1984) suggests that mixed infections are rare in cats since these have a higher resistance of them.

Table 2. Prevalence of gastrointestinal parasites found in cats from two urban areas in Southern Romania, according to their age and gender

Parasite species	Age		Gender	
	Kittens ≤1 year *n=41*	Adult >1year *n=79*	Male *n=51*	Female *n=69*
Toxocara cati	10 (24.4%)	5 (6.3%)	9 (17.6%)	6 (8.7%)
Ancylostoma tubaeforme	2 (4.9%)	0	1 (2.0%)	1 (1.4%)
Dipylidium caninum	0	1 (2.3%)	0	1 (1.4%)
Isospora felis	7 (17.1%)	8 (10.1%)	6 (11.8%)	9 (13.0%)
Total animals infested	**18 (44.0%)**	**14 (17.7%)**	**15 (29.4%)**	**17 (24.6%)**

Comparing our data with data reported from other similar studies, the prevalence values for some parasite species are similar, while for other species various results are obtained, according to different risk factors.

A study conducted in Ontario (Shukla et al., 2006) has reported a similar prevalence of *T. cati* (12.2%). In a recent study conducted in Europe by Beugnet et al. (2014), it is shown that cats with infrequent outdoor access (11.5%) were significantly less frequently infested with *T. cati* than cats having frequent access (23.0%) to the outdoors. In Romania, Mircean et al. (2010) reported a prevalence of *T. cati* (10.2%) from urban cats lower than that of the present study.

With regard to *Isospora* spp., the prevalence found in this study (12.5%) agrees with the data obtained by Mircean et al. (2010), they reported that the prevalence of *I. felis* infection was higher in cats less than 1-year old (11.6%) than adult cats (1.2%).

Khademvatan et al. (2014) detected *Isospora* spp. with a prevalence of 21.4% in the collected samples from different geographical areas of Iran.

The low prevalence of *A. abstrusus* (2.9%; 2/69) observed in the present study is in agreement with similar studies from our country (5.6%) (Mircean et al., 2010) or European countries (4.1%; Beugnet et al. (2014); however, a recent study from Italy reported a higher prevalence (26.5%) for this parasite (Genchi et al., 2014).

A. tubaeforme was found in a lower prevalence (1.7%) comparing to other studies in Romania (10.1% found in Transylvania (Mircean et al., 2010). In other countries, prevalence of this species found by coproscopical examination was variable: 0.3-0.4% in Germany (Barutzki and Schaper, 2003; 2011), 11.1% in Hungary (Capari et al., 2013), or 19.1% in Portugal (Waap et al., 2014).

The overall frequency of *Dipylidium caninum* (0.8%) obtained here is higher to those obtained by Barutzki and Schaper (2011) (<0.1%) in a previous study performed in Germany, but lower than the prevalence obtained by Kademvatan et al. (2014) (2.9%) from stray cats (Iran) and much lower than that obtained by Mohsen and Hossein (2009) in Iran (68.1%).

As the studies showed, endoparasites are common in domestic cats. Since, some of the parasites may impact the health of animals and some are known agents of zoonotic diseases, measures should be taken to appeal more attention to cats by both veterinarians and owners.

CONCLUSIONS

1. The coprological investigation performed on cats from two urban areas in Southeastern Romania showed a prevalence of 26.7% for gastrointestinal parasites and 2.9% for lungworm, respectively.
2. The proportion of helminthic infections (14.1%) did not differ significantly than those of protozoan (12.5%).
3. Kittens had a higher prevalence (44.0%) of parasitic infection than adult cats (17.7%).
4. A higher prevalence of *T. cati* was registered in kittens (24.4%) than in adult cats (6.3%).
5. These results are particularly of veterinary importance as these parasites commonly affect the animal health, suggesting the need for parasitological control. In addition, due to the zoonotic potential of some of these parasites, potential risks for the human health have to be considered.

REFERENCES

1. Barutzki, D. and Schaper, R., 2013. *Age-dependant prevalence of endoparasites in young dogs and cats up one year of age*. Parasitology Research, 1:119-131.
2. Barutzki, D., Schaper, R., 2003. *Endoparasites in dogs and cats in Germany 1999-2002*. Parasitology Research, 3:S148-150.
3. Barutzki, D., Schaper, R., 2011. *Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010*. Parasitology Research, 109:S45-S60.
4. Beugnet, F., Bourdeau, P., Chalvet-Monfray, K., Cozma, V., Farkas, R., Guillot, J., Halos, L., Joachim, A., Losson, B., Miró, G., Otranto, D., Renaud, M., Rinaldi, L., 2014. *Parasites of domestic owned cats in Europe: co-infections and risk factors*. Parasites and Vectors, 7:291.
5. Borji, H., Razmi, G., Ahmadi, A., Karami, H., Yaghifoori, S., Abedi V., 2011. *A survey on endoparasites and ectoparasites of stray cats from Mashhad (Iran) and association with risk factors*. Journal of Parasitic Diseases, 35(2):202-206.
6. Calvete, C., Lucientes, J., Castillo, J. A., Estrada, R., Gracia, M. J., Peribáñez, M. A., Ferrer, M., 1998. *Gastrointestinal helminth parasites in stray cats from the mid-Ebro Valley, Spain*. Veterinary Parasitology, 75(2-3):235-240.
7. Cantó, G. J., Guerrero, R. I., Olvera-Ramires, A. M., Milián, F., Mosqueda, J., Aquilar-Tipacamú, G., 2013. *Prevalence of fleas and gastrointestinal parasites in free-roaming cats in central Mexico*. PloS One, 8(4):e60744.
8. Capari, B., Hamel, D., Visser, M., Winter, R., Pfister, K., Rehbein, S., 2013. *Parasitic infections of domestic cats, Felis catus, in western Hungary.* Veterinary Parasitology, 192(1-3): 33-42.
9. Deplasez, P., van Knapen, F., Schweiger, A., Overgaauw, P. A., 2011. *Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis*. Veterinary Parasitology, 182(1):41-53.
10. Engbaek, K., Madsen, H., Larsen, S. O., 1984. *A survey of helminths in stray cats from Copenhagen with ecological aspects*. Zeitschrift für Parasitenkunde, 70(1):87-94.
11. Genchi, M., Ferrari N., Fonti, P., De Francesco, I., Piazza, C., Viglietti, A., 2014. *Relation between Aelurostrongylus abstrusus larvae excretion, respiratory and radiographic signs in naturally infected cats.* Veterinary Parasitology, 206(3-4):182-187.
12. Khademvatan, S., Abdizadeh, R., Rahim, F., Hashemitabar, M., Ghasemi, M., Tavalla, M., 2014. *Stray cats gastrointestinal parasites and its association with public health in Ahvaz city, South Western of Iran.* Judishapur Journal of Microbiology, 7(8):e11079.
13. Mircean, V., Titilincu, A., Cozma, V., 2010. *Prevalence of endoparasites in household cat (Felis catus) populations from Transylvania (Romania) and association with risk factors*. Veterinary Parasitology, 171(1-2): 163-166.
14. Mohsen, A., Hossein, H., 2009. *Gastrointestinal parasites of stray cats in Kashan, Iran.* Tropical Biomedicine, 26(1):16-22.
15. Robertson, I. D., Thompson, R. C., 2002. *Enteric parasitic zoonoses of domesticated dogs and cats.* Microbes and Infections, 4(8):867-873.
16. Shukla, R., Giraldo, P., Kralis, A., Finnigan, M., Sanchez, A. L., 2006. *Cryptosporidium spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario*. Canadian Veterinary Journal, 47(12):1179-1184.
17. Traversa, D., 2012. *Pet roundworms and hookworms: a continuing need for global worming*. Parasites and Vectors, 10;5:91.
18. Waap, H., Gomes, J., Nunes, T., 2014. *Parasite communities in stray cat populations from Lisbon, Portugal*. Journal of Helmintology, 88(4):389-395.

SAMPLING AND USE OF SWINE SMALL INTESTINE SUBMUCOSA IN CORNEAL RECONSTRUCTION ON RABBIT

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Abstract: *Swine small intestine submucosa (SIS) is easier to store and purchased than others biomaterials. It is used in veterinary ophthalmology for reconstruction of cornea with good results. Studies reported that SIS is a non-immunogenic biomaterial, with the ability to act as a scaffold for tissue regeneration in different organs, including the eye. Studies about the composition of SIS showed that the fully hydrated material has a content of 90% water, the remaining 10% is composed of proteins, lipids and carbohydrates.*

We evaluated efficacy of the material in five cases, all rabbits with corneal damages. The study was performed between 01 octomber 2013- 10 december 2014, in Surgery Clinic of the Faculty of Veterinary Medicine, in Cluj-Napoca. Four of the cases had corneal wound, a case has been diagnosed with corneal burn. In all five cases surgery was performed by suturing a SIS graft on the corneal defect. SIS was sampling from a healthy donor pig, prepared and stored at 4 ° in neomycin sulphate 10%. The surgical procedure was performed under general anesthesia, selected protocol for all five cases was Xylazine +Ketamine and retrobulbar block with Lidocaine 4%. Grafts were fixed in the eye with 8-0 polyglactin and 10-0 nylon in simple interrupted sutures. After the surgery chloraphenicol 0,5 % eye drop was topical administered three times per day for 7 days. Animals were evaluated at 3,7,15 until 60 days postoperatively. Four eyes healed with discrete to mild corneal scarring and very good visual result. Minor complication occurred in one eye, with partial integration of the SIS.

***Keywords*:** cornea, rabbit, SIS.

INTRODUCTION

Severe corneal defects should be treated surgically for optimum results. There are many techniques for surgical management of this pathology but all require expensive equipment and specialist skills. In veterinary ophthalmology is used successfully conjunctival pedicle grafts, but that can impair vision when the problem is in the axial cornea. The ideal biomaterial for corneal reconstruction should support of epithelial migration and adhesion, permeability to solutes and stability to corneal proteases. To find the perfect material for the reconstruction of cornea numerous noncorneal grafts have been employed with good result: equine pericardium (Barros et al., 1993), equine amniotic membrane (Barros et al., 1998) human amniotic membrane (Kim and Tseng, 1995), equine renal capsule (Andrade et al., 1999) and peritoneum.(Garcia et al., 1996).

Porcine small intestinal submucosa (SIS) is a natural biomaterial used with success in tissue engineering, investigated for the first time as a vascular graft by Badylak in 1989. The material ranges in thickness from 0.05 mm to 0.22 mm (Shi and Ronfard, 2013). SIS is primarly composed of type I collagen fibres, but also contains collagen type III, IV and VI. Glycoprotein like fibronectin and laminin, found in his structure, mediate the cell adhesion to the extracellular matrix (Shi and Ronfard, 2013). The growth factors reported in SIS are fibroblast growth factor (FGF-2), transforming growth factor-beta1(TGH-β1) and vascular endothelial factor (VE-GF) (Shi and Ronfard, 2013).

SIS acellular collagen matrix acts as a medium for corneal remodelling and reconstruction. His structure make this material an excellent choice for tissue engineering and clinical aplications (Lewin 1999, Featherstone and Sansom 2009).

The purpose of this study was to ilustrat the method of prelevation and conservation of fresh SIS for the use in surgical treatment of severe corneal disorders on rabbit.

MATERIALS AND METHODS:

This study was conducted in Surgery Clinic of the Faculty of Veterinary Medicine, in Cluj-Napoca, between 01 octomber 2013- 10 december 2014. We evaluated efficacy of the material in five cases, all rabbits with corneal damages. Four cases presented unilateral corneal wounds associated with suspected trauma or foreign body. One rabbit had bilateral corneal alkali burns (Fig 1). Surgery was performed after topical treatment failed. Complete bilateral ophthalmologic exam and fluoresceine test was performed in each case. All eyes were examined by indirect ophthalmoscope before and after flouresceine test for accurate evaluation of corneal lesion. Bacteriologic for anaerobic and aerobic culture was performed in two cases and reveled no growth of microorganism.

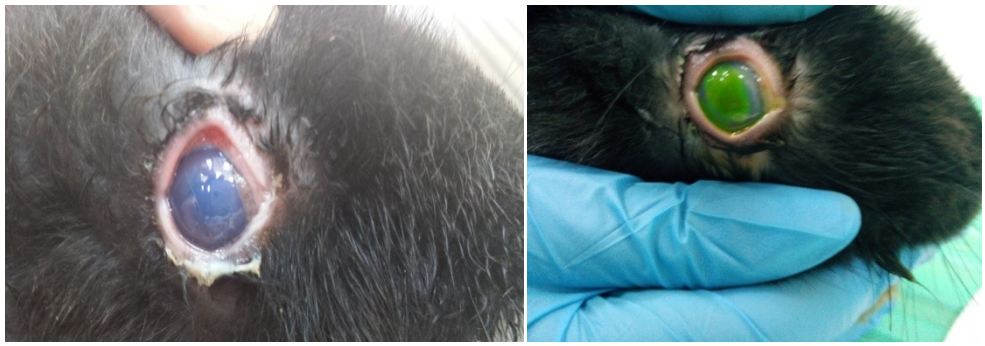

Fig 1. Corneal alkali burn before and after fluoresceine test (case number 5)

The samples of small intestinal submucosa were prevailed for a slaughter house. Within 2 hours of donor pig dead a segment of proximal jejunum was obtained and stored on ice until we arrived at the clinic and prelevated the submocusa. All mesenteric tissues were removed and the segment of jejunum was everted. The tunica mucosa was removed using a longitudinal wiping motion with a scalpel handle and moistened gauze. The segment was returned to original orientation and the tunica serosa and tunica muscularis were removed from the intestine by similar method. The remaining thin, whitish, translucent tube consisted of the tunica submucosa . The SIS was rinsed in sterile normal saline several times. Submucosa was cut with scissors to obtain a single layer of material then rinsed again with saline sterile solution. After, we divided in 40mm to 40 mm sections. SIS was stored in neomycin sulfate 10 % at 4 ˚ C until use.

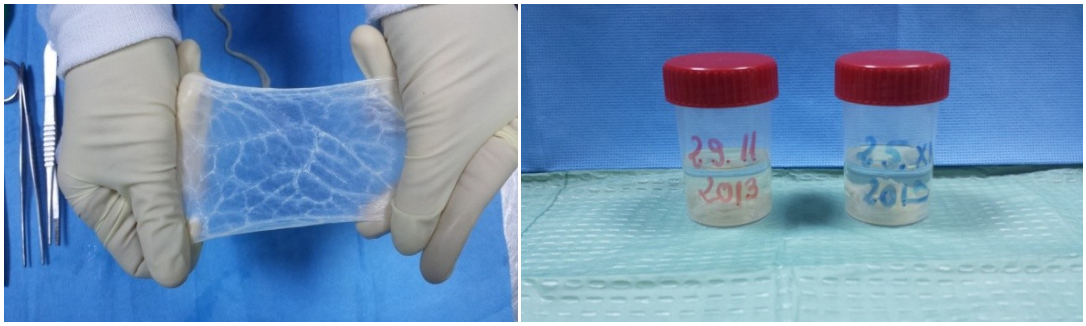

Fig . 2 SIS after preaparation (left) and store in neomycin sulfate 10% (right)

In every case, surgery was performed using a operating microscope. General anesthesia was induce with a combination of xylazine HCl (Narcoxyl® 2) at a dose of 5 mg/kg, im, followed by ketamine HCl (Imalgene ®) at a dose of 35 mg/kg, im. All rabbits were blind endothraeal intubeted with neonatal tubes of internal diameter 2,0-2,5 mm. Oxygen was supplied at a rate of 300 ml/min during surgery and 5-10 minute after completion of surgery. Also a retro bulbar block with 0.3ml of 4% Lidocaine HCl was done, by inserting a 2,5 cm, 22 or 20-gouge needle at the lateral canthus of the eye (Fig.3). Rectal temperature, heart and respiratory rate were monitoring during surgery.

Fig.3 Retro bulbar block

After anesthesia, a mild antiseptic povidone-iodine, diluted at 0,2% was used for periocular region and globe asepsia (Gelatt and Gelatt, 1995). Cornea was surgically prepared before SIS grafting by excising all devitalized tissue and irrigated with saline several times. SIS was removed from Neomycin and rinsed with saline sterile solution. For each case SIS was cut with a ophthalmic scissors at the shape we need it. The size was approximately 2 mm larger than the corneal defect. Submucosa graft was placed with the rough surface directly against the defect or against all the cornea if the lesion was severe (Fig.4). The graft was positioned by four simple interrupted sutures, first suture at 12 o'clock position, after a 6-, 3 - and 9 o'clock positions of the corneal defect. After positioning, we fixed firmly the graft to the cornea with other simple interrupted sutures. Grafts were fixed in the eye with 8-0 polyglactin 910 (Vicryl™, Ethicon) or 10-0 nylon (Ethilon™, Ethicon) (Fig.5,6). During the surgery we irrigated regular the eye with sterile saline solution to prevent any drying. After the surgery chloramphenicol 0,5 % (prepared after recipe in drug store) eye drop was topical administered three times per day for 7 days. Animals were evaluated at 3,7,15 until 60 days postoperative.

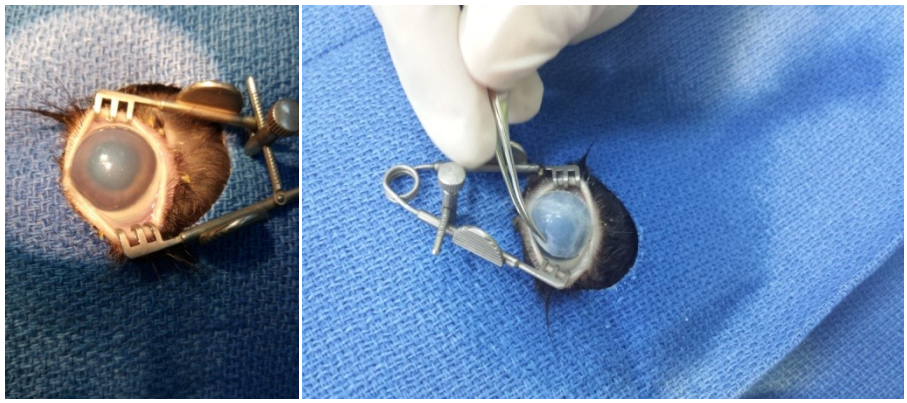

Fig. 4 SIS graft was placed with the rough surface directly against the defect

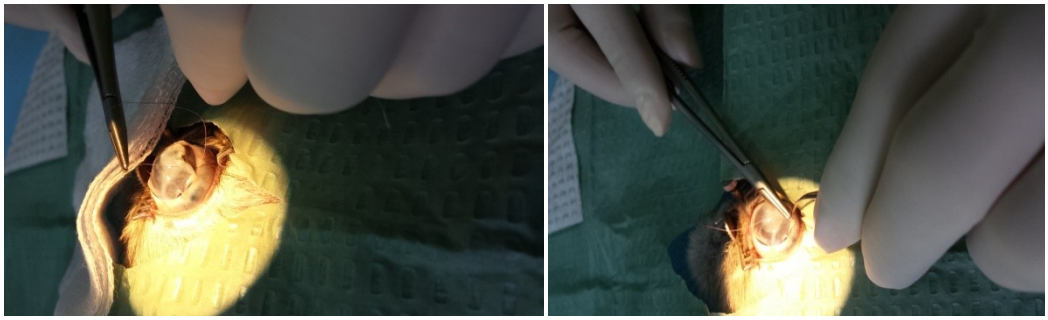

Fig.5 Positioning and fixing the SIS graft

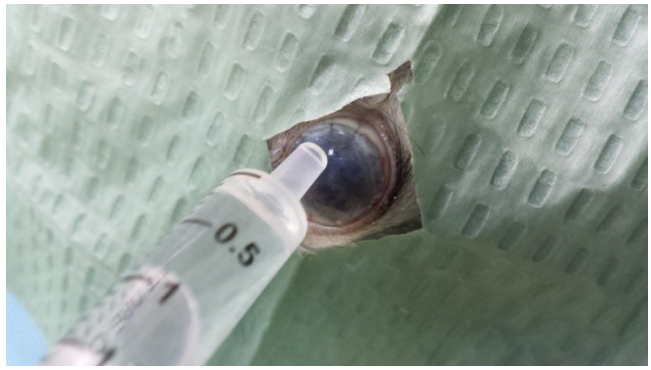

Fig.6 Final aspect of the graft

RESULTS AND DISCUSSIONS

In our study we had five cases, rabbits, different breed and age, six eyes with corneal lesion in which topical treatment failed. With the exception of the case 3, all the subject healed with minimal scarring and neovascularisation (Tab. 1)

Three days postoperatively, all animal presented chemosis and the SIS graft was swollen and opaque. No case showed sign of pain and photophobia. The graft was less opaque and swollen, except case 3 who suffered from o postoperative infection. From the second week after surgery, SIS became transparent and we could observe the graft integration within the cornea. Through the graft the iris and the pupil started being observed after 15-20 days depending on the case. In cases 4 and 5, 7 days after surgery sutures site appeared

swollen, with a mild reaction, but this aspect disappear on the 15 day. We performed a fluoresceine test after 30 days from surgery, and in four cases showed full integration of the SIS within the cornea. All grafts were retained on the surgical site during this study. After 45 days cases 3, 4 and 5 presented a corneal neovascularisation from discret (case 3,4) to mild (case 5).

After the follow up period, 4 eyes (66,6 %) presented a transparent to discrete scar, 1 eye (16,6 %) a mild scar and in 1 eye (16,6 %) a marked scar was present but not obstruct vision.

In four cases chloramphenicol 0,5 % was a good choice for post surgery treatment, no samples of bacterial culture were collected in our study, but no sign of infection was observed.

Tabel 1. Clinical findings and postoperative result on rabbit treated with SIS grafts

Case	*Age*	*Breed*	*OD/OS*	*Corneal Lesion*	*Treatment before surgery*	*Follow up period*	*Outcome*
1	5 months	Angora	OD	Corneal wound-trauma	Kanamycin, ointment 5 days	30 Days	Excellent Transparent scar
2	8 months	Mix breed	OD	Coreal wound	Tobramycin ,drops -7 days	40 Days	Very good Discrete scar
3	1,5 years	Mix breed	OS	Axial corneal deep lesion	Gentamicin ,drops 10 days	60 Days	Bad 3 days after surgery complication Marked scar
4	6 months	Lionhead rabbit	OD	Corneal lesion -trauma	Gentamicin, drops 7 days	45 Days	Very good Discrete scar
5	1,8 years	Mix breed	OD/OS	Alkali burn	Tobramycin +Dexamethasone drops 3 days	60 Days	Good Transparent scar on OD Mild scar on OS

OD-Right eye, OS- Left eye;

Case number 3, presented complication because the owner did not follow postoperative recommendations. Three days after surgery the graft was very swollen and opaque compared to the other cases. Seven days he presented a purulent discharge at the left eye, around the suture site edema it was observed. We cleaned the eye with sterile saline solution and we changed the topical treatment with gentamicin 0,3 % and dexamethasone 0,1 % (Tobradex® , Alcon) eye drops for 10 days. After 5 days the purulent discharge disappeared and suture sites edema decreased. After only 30 days the graft became transparent, healing accrued more difficult than in the other cases. 60 days after surgery, case 3, presented a marked scar and partial integration of the graft, SIS was integrated about 80%.

SIS is an acellular collagen matrix that is initially invaded by fibroblast and the replace by corneal stromal cells (Vanroe et al, 2007). Previous study have reported use of SIS graft in association of conjunctival graft (Lewin, 1999) or a third eyelid flap (Hansen and Guandalini, 1999). Conjuctival graft have the disadvantages of being opaque and obstruct vision and third eyelid flap inhibit topical medication from reaching the cornea and impede the sight of its (Hendrix, 2007). Third eyelid flap it indicated to maintain the graft hydrated ant to proctect the SIS for the blinking movement (Hansen and Guandalini, 1999). In our

cases we performed SIS grafting without third eyelid flap and all the grafts were retained on the surgical site, without signs of dehydration.

Corneal sutures must provide compressive force to create tight seal of the junction between the graft and cornea (Boisjoly et al, 1993). Loose sutures are a couse of graft infection and vascularisation (Panda et al, 2007). We used 8-0 vicryl in 2 cases and 10-0 nylon in 3 cases and simple interrupted sutures technique in all cases. The 2 cases with vicryl presented a mild reaction on the sutures side, but the absorbable material have the advantage that we don't have to remove it postoperatively. Use nylon sutures was more benefic than vicryl in our patients, we don't funded any complication provided by suture in that cases.

Contamined donor tissue, infected graft, loose sutures, expose knots, mucus on the sutures (Boisjoly et al, 1993) rejection of the graft or impropriate postoperatively treatment could be the reasons for fail in reconstruction cornea using SIS. The only complication we encountered was a post surgery infection, caused be improper administration of post operatory treatment by the owner.

The used of topical steroids was indicated in one case to reduced edema, otherwise corticosteroids must be cautiously administrated (Hansen and Guandalini, 1999).

Previous studies revealed that SIS grafts can be also successfully used in dogs and cat for corneal reconstruction (Goulle, 2011).

CONCLUSIONS

Sis is translucent and thus offers advantages for corneal surgery. This way of prelevation and storage of fresh SIS is one of the easiest and inexpensive method used in corneal reconstruction surgery.

In summary, this study revealed that fresh SIS cause minimal adverse tissue reaction and allows corneal healing, in 66,6 % eyes with a transparent or a discrete scar.

Nylon suture gave better results than vicryl in our patients.

Post operatory treatment is very import to prevent future complications.

REFERENCES

1. Andrade A., Laus J., Figueirido F. (1999) The use of preserved equine renal capsula to repair lamellar corneal lesions in normal dogs. Veterinary Ophtalmology; 2:79-82;
2. Badylak S., Lantz G., Coffey A., Geddes L .(1989) Small intestinal submucosa as a large diameter vascular graft in the dog. J. Surg. Res. ;47 (1): 74–80;
3. Barros P., Garcia J.A., Laus J., Ferreira A., Salles Gomes T. (1998) The use of xenologous amniotic membrane to repair canine corneal perforation created by penetrating keratectomy. Veterinary Ophtalmology ;1:119–123;
4. Barros P., Safatle A, Godoy C., Souza M, Barros L., Brooks D. (2005) Amniotic membrane transplantation for the reconstruction of the ocular surface in three cases. Veterinary Ophtalmology ;8:189–192;
5. Featherstone H.J., Sansom J. (2000) Intestinal submucosal repair in two cases of feline ulcerative keratitis. Veterinary Record; 146 :136-138;
6. Gelatt, K. N. & Gelatt, J. P. (1995) Handbook of Small Animal Ophthalmic Surgery, Vol 2: Corneal and Intraocular Procedures. New York, NY, USA: Pergamon;
7. Goule F. (2011) Use of porcine small intestinal submucosa for corneal reconstruction in dogs and cats: 106 cases. *JSAP*,53:34-43.
8. Hansen P., Guandalini A. (1999) A retrospective study of 30 cases of frozen lamellar corneal graft in dogs and cats. Vet Ophthalmol.;2(4):233-241;

9. Helène M. Boisjoly ,Tourigny R, Bazin R., Patricia A. Laughrea, Ide Dubè, Ghislaine, Chamberland, Julie Bernier, Roy R. (1993) Risk Factors of Corneal Graft Failure. Volume 100, Issue 11, November, Pages 1728–1735;
10. Hendrix D.(2007). Canine conjunctiva and nictitating membrane. In: Veterinary Ophthalmology. 4th ed. K.N. Gelatt (ed.) Ames: Blackwell Publishing. 675-680.
11. Kim J., Tseng S. (1995) Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. Cornea ;14:473-84;
12. Lei Shi, Ronfard V. (2013) Biochemical and biomechanical characterization of porcine small intestinal submucosa (SIS): a mini review . Int J Burns Trauma v.3(4) : 173–179.
13. Lewin G. (1999) Repair of a full thickness cornescleral defect in a German shepherd dog using porcine small intestinal submucosa. Journal of Small Animl Practice ;40:340-342;
14. Panda A., Vanathi M., Kumar A., Dash Y., Priya S. (2007). Corneal graft rejection Surv Ophthalmol. Jul-Aug;52(4):375-96;
15. Vanore M., Chahory S., Payen G. & Clerc B. (2007) Surgical repair of deep melting ulcers with porcine small intestinal submucosa (SIS) graft in dogs and cats. Veterinary Ophthalmology ;10:93–99

CLINICAL ASPECTS IN CANINE ATOPIC DERMATITIS

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Abstract:*Canine atopic dermatitits is an allergic condition with a strong genetic component that manifests through a hypersensitivity type I reaction in the acute phase and type IV in the chronic phase and an increased production of allergen specific IgE.*
The aim of this paper is to highlight the main clinical aspects that can be observed in canine atopic dermatitits and that are to be considered when making a differential diagnosis.
35 dogs were diagnosed with atopic dermatitis and recorded at the Medical Clinic from the Faculty of Veterinary Medicine from Iasi, presenting with generalized pruritus, localized alopecia, erithema, papulas, crusts and lesions secondary to scratching. Microscopic exam of skin smears was made to exclude parasitic and mycotic dermatoses. The main clinical symptom observed in all cases was generalized pruritus that preceeded any other clinical sign, followed by erithema and alopecia localized in the flexural regions, on the head and the abdomen. Canine atopic dermatitis is a dermatological allergic condition with clinical symptoms that can be associated with almost any other allergic reaction.

Keywords: *canine atopic dermatitis, allergic reactions, clinical aspects, etiology.*

Canine atopic dermatitis is an allergic, inflammatory and pruritic condition triggered by a cascade of hypersensitivity reactions type I and IV towards a large range of allergens, especially airborne ones. The IgE hyperproduction that may be found during serological determinations is considered to be the starting point of the clinical signs but atopy evolves thanks to a sum of factors that interact and determine one another leading to a complex pathogenesis. Among these factors the main ones are: genetic background, breed, skin barrier defects, different types of allergens that when being present all at once lead to the summation effect and surpassing the threshold point (Marsella, 2001). The allergens involved in provoking flares are not just airborne but also food, fleas, bacterial or mycotic (Guaguere et al., 2004; Solcan, 2011).

Season, age and body site are also important when considering the type or severity of lesions and the evolution pattern of the disease.

The affected dogs belong or are crossbreeds of breeds known to be predisposed (Jaeger, 2010).Typical signs include pruritus (mostly generalized), erythema, alopecia and papules. The secondary lesions are excoriations and erosions that follow scratching (they are frequently associated with superficial pyoderma), hyperpigmentation and parakeratosis (Scott et al., 2001).

MATERIALS AND METHODS

Between October 2012 – December 2013, 35 dogs were registered with atopic dermatitis and recorded at the Medical Clinic from the Faculty of Veterinary Medicine from Iasi, presenting with generalized pruritus, localized alopecia, erythema, papules, crusts and lesions secondary to scratching. Differential diagnostic was carried out based on anamnesis, clinical signs, microscopical examination of skin scapings and response to an elimination diet.

RESULTS AND DISCUSIONS

The aim of this paper is to highlight the main clinical aspects that can be observed in canine atopic dermatitis and that are to be considered when making a differential diagnosis.

The main clinical symptom observed in all cases was generalized pruritus that preceded any other clinical sign, followed by erythema and alopecia localized in the flexural regions, on the head and the abdomen (Table 1).

Table 1

Types of lesions and their distribution in dogs with atopic dermatitis in this study

Localization	Nr.	Lesions	Nr.
Interdigital	10 (28.5%)	Erythema	35 (100%)
Dorsolombar	20 (57%)	Alopecia	33 (94.3%)
Abdominal	25 (71.4%)	Papules	17 (48.5%)
Inguinal	27 (77%)	Pustules	5 (14.2%)
Axilae	30 (85.7%)	Crusts	20 (57%)
Pinnae	15 (42.8%)	Scales	23 (65.7%)
Flexural	25 (71.4%)	Hyperpigmentation	8 (22.8%)
Inner-thighs	27 (77%)	Hyperkeratosis	10 (28.5%)
Periorbital	5 (14.2%)	Excoriations	12 (34.2%)
Face	5 (14.2%)	Erosions	1 (2.8%)
Pectoral	15 (42.8%)	Reddish tint of the coat	15 (42.8%)
Lat. thorax	10 (28.5%)		
Perineal	10 (28.5%)		
Cervical	15 (42.8%)		

Most dogs had the onset of the disease under 3 years (Table 2). Also, most of them lived both indoors and outdoors (short walks mostly) and received mixt food (commercial and leftovers from the owner's table) (Table 3).

Table 2

Seasonality and age of onset

Sex		Seasonality	Age of onset (years)				
F	M		< 1	< 2	<3	3-6	> 6
25 (60.9%)	16 (39%)	36 (87.8%)	10 (24.3%)	22 (53.6%)	26 (63.4%)	4 (9.7%)	11 (26.8%)

Table 3

Environment and allergens

Environment		Food			External disinfestation	
outdoor	indoor	commercial	commercial hypoallergenic	mixt	constant	insuficient
14 (34.1%)	27 (65.8%)	19 (46.3%)	9 (21.9%)	13 (32%)	18 (43.9%)	23 (56%)

During clinical examination we identified primary and secondary lesions on body sites specific for atopy:

- diffuse and localised alopecia (Fig. 1, 2), erythema (Fig. 3), papules (Fig. 4, 5), pustules (Fig. 6), edish taint of the coat due to chronic irritation and intensive licking (Fig. 7, 8), escoriations and erosions (Fig. 9, 10).

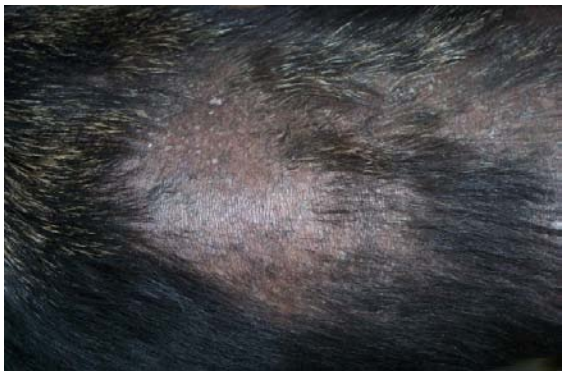
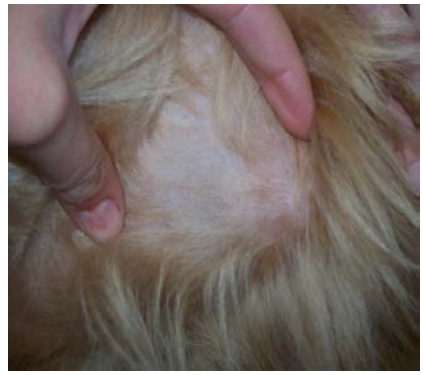



Fig. 1, 2 - Diffuse alopecia, respectively circumscribed alopecia in dogs with atopic dermatitis

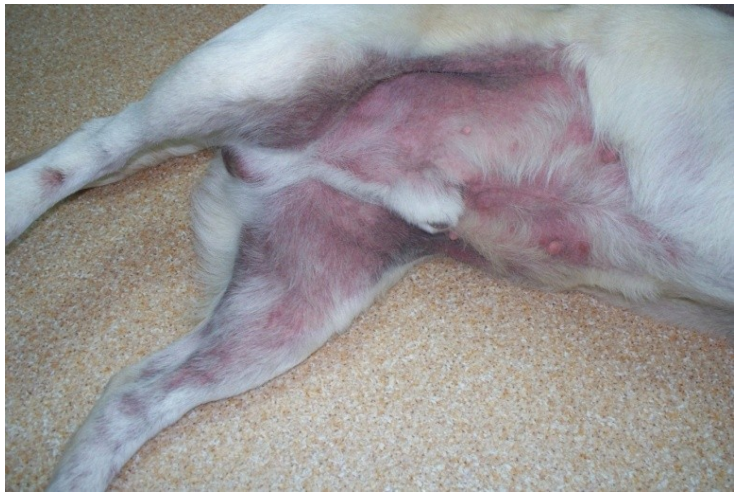


Fig. 3 – Generalized erythema in a patient

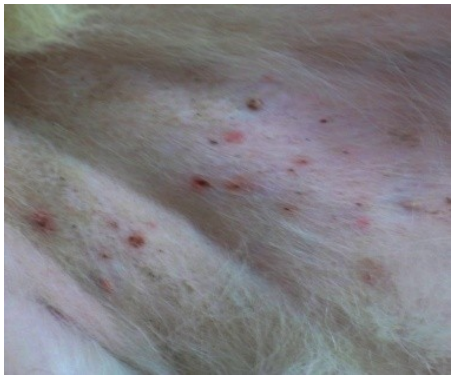
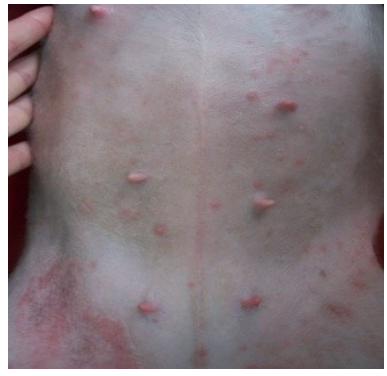



Fig. 4, 5 – Papules and erythema on the abdomen

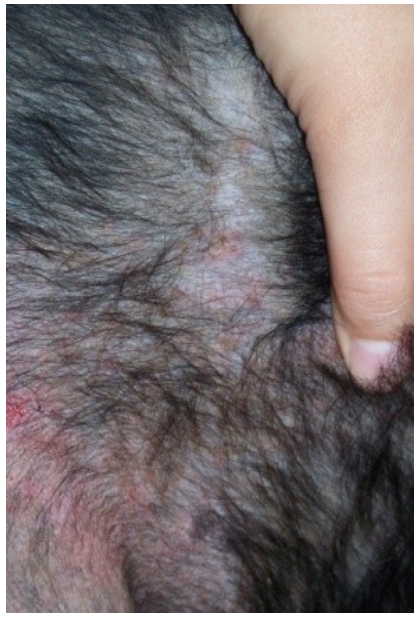
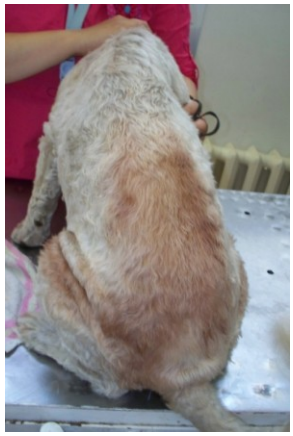


Fig. 6 – Pustules and crusts in a German Shepherd



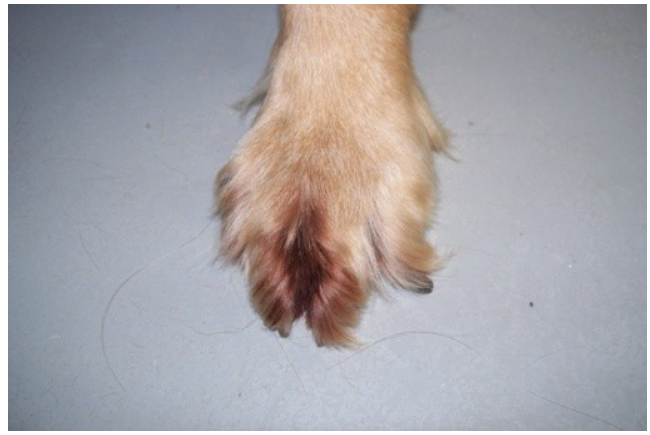
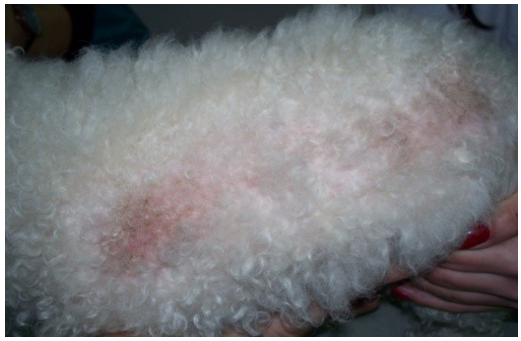


Fig. 7, 8 – Dark coloration of the fur due to intensive licking



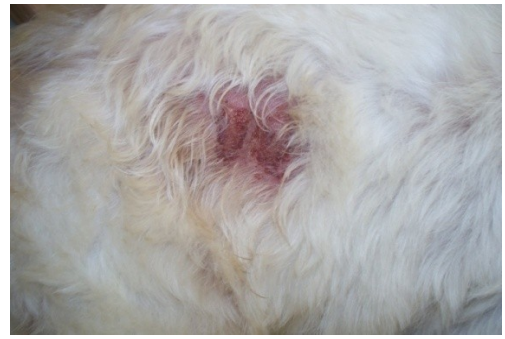


Fig. 9, 10 – Erosions in two patients

As a general aspect we found dogs with atopic dermatitis that exhibited in 3 patterns:

- very discreet clinical signs or just pruritus and associated conditions;
- only primary lesions;
- primary and/but mostly secondary lesions;

Also, depending on the pattern presented, a generalized aspect (Fig. 11, 12, 13) may be seen or only some body sites may be involved (Fig. 14, 15, 16, 17, 18, 19).

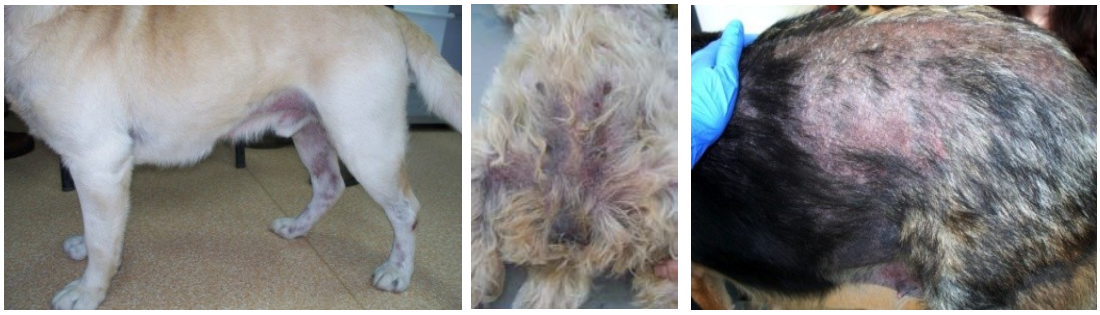


Fig. 11, 12, 13 – Generalized aspects in canine atopic dermatitis

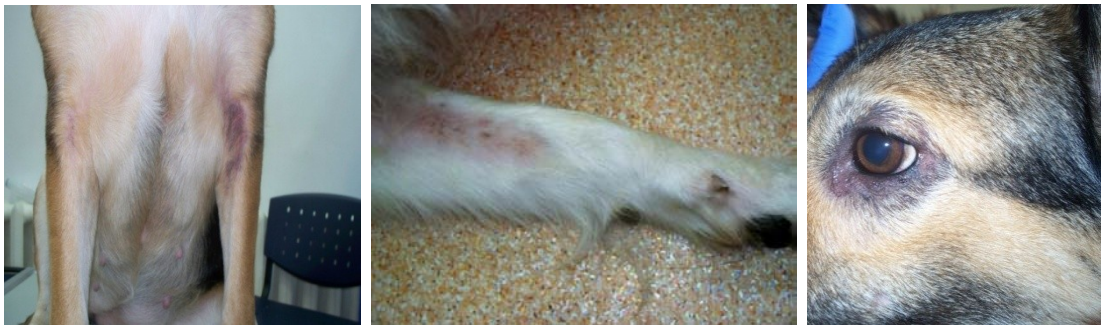


Fig. 14, 15, 16 - Localized aspects in canine atopic dermatitis

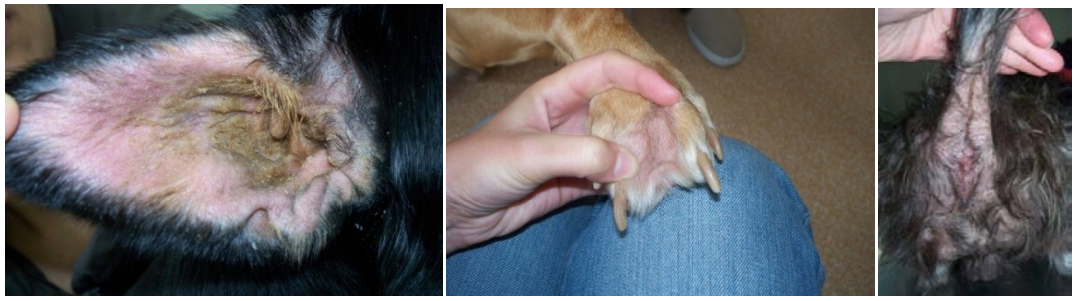


Fig. 17, 18, 19 - Localized aspects in canine atopic dermatitis

Our diagnosis of atopic dermatitis was established on history, clinical examination, skin scrapings, dietary trial and therapeutical response, in accordance with literature specifications (Olivry, 2010).

Clinical examination aimed to find the presence of primary or secondary lesions in specific body sites and also specific diagnostic criteria (Favrot et al., 2010).

Most of our patients had the debut of the disease before reaching 3 years of age and manifested either simply with pruritus of the face, feet and axilae (Bizikova, 2015) or also with recurrent skin or ear infections (Olivry et al., 2010). We found no patient with pododermatitis and regarding the paws we found only erythema, pruritus, alopecia and reddish tint of the fur.

The most affected areas were the axilae, the flexural regions, the abdomen and the inguinal region.

We found external otitis in 40% of our patient and 5 of them manifested it as a first and only symptom. Also, conjunctivitis was found in 5 dogs and cheilitis in 2 (usually when food allergy was involved). It is important to keep in mind that these symptoms may evolve on their own before any skin erythema or pruritus (Scott et al., 2001).

The associated conditions that may evolve at the same time as atopy are food and flea allergy (other external parasites may be found as well but less often) and we found them in more than 30% of our patients. Food allergy evolving concurrent with atopy is now known to be actually food induced atopic dermatitis (FIAD) (Bizikova, 2015). We suspected FIAD in dogs that exhibited perineal alopecia, erythema and pruritus but also digestive symptoms such as soft fecals, diarrhea, flatulence, occasional vomiting and cheilitis (Griffin et al., 2001).

In atopic dogs that have been intensively treated with corticoids, Cushing syndrome may be diagnosed but we did not find any patient with this illness. In cases like this pruritus is an important orientation factor when performing a differential diagnosis: it's presence is high in allergic dermatitis but absent in endocrine disorders and varies in parasitic dermatitis.

Flea allergy was seen in 25% of the patients included in this study and was responsible for the flares that lead to the consult. After eliminating the actual parasites and their allergenic fecals from the skin and coat, the patients still exhibited with symptoms concurrent with atopy, which was in fact the final diagnostic. This proves that not only airborne allergens may trigger acute episodes of atopy but many other as well: food, parasitic, bacterial and mycotic allergens (Olivry et al., 2001).

CONCLUSIONS

1. Atopic dermatitis is an allergic condition with clinical manifestations that mimic almost any other dermatitis. Other similar illnesses may evolve at same time with atopy determinig a complex clinical pattern.
2. Diagnosing canine atopic dermatitis as a primary or only condition in a patient needs the exclusion of parasitic, endocrine and other allergic dermatitis as well as internal illnesses.

Acknowledgment

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REFERENCES

1. Bizikova P., santoro D., Marsella R., Nuttal T., Eisenschenk M.N.C., Puchen-Haston C.M. (2015) - Review: Clinical and histological manifestations of canine atopic dermatitis. ESVD and ACVD, Veterinary Dermatology, 26, 79-e24;
2. Favrot C., Steffan J., Seewald W., Picco F. (2010) – A prospective study on the clinical features of canine atopic dermatitis and its diagnosis. Journal Compilation ESVD and ACVD, Veterinary Dermatology, 21, 23-31;
3. Griffin C.E., DeBoer D.J. - The ACVD task force on canine atopic dermatitis (XIV): clinical manifestations of canine atopic dermatitits . Elsevier, Veterinary Immunology and Immunopathology, 81(2001), 255-269 ;
4. Guaguere E., Hubert Th., Muller A., Prelaud P. – Dermatologie du chien. Masson-AFFVAC, Paris, 2004 ;

5. Jaeger K., Linek M., Power H. T., Bettenay S. V., Zabel S., Rosychuk R.A.W., Mueller R. S. (2010) – Breed and site predispositions of dogs with atopic dermatitis: a comparison of five locations in three continents. Journal Compilation ESVD and ACVD, Veterinary Dermatology, 21, 119-123;
6. Marsella Rosanna, Sousa A. Candace (2001) - The ACVD task force on canine atopic dermatitis (XIII): threshold phenomenon and summation of effects. Elsevier, Veterinary Immunology and Immunopathology, 81, 251-253;
7. Olivry T., Hill B. P.- The ACVD task force on canine atopic dermatitis (IX): the controversy surrounding the route of allergen challenge in canine atopic dermatitis. Elsevier, Veterinary Immunology and Immunopathology, 81(2001), 219-225;
8. Olivry T., DeBoer D.J., Favrot C., Jackson H.A., Mueller R.S., Nuttal T., Prelaud P. (2010) - treatment of canine atopic dermatitis: 2010 clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. ESVD and ACVD, Veterinary Dermatology, 21, 233-248;
9. Scott W. D., Miller H. W., Griffin E. C. (2001) – Muller and Kirk's small animal dermatology. Saunders;
10. Solcan Gh., Vulpe V. (2011) – Dermatologie în Medicina internă a animalelor, coord. Falcă C., Vol II, Ed. Eurostampa, Timişoara , 351-528;

WINTER PARASITIC CO-INFECTIONS IN COMMON CARP (*CYPRINUS CARPIO*) FROM A POLYCULTURE SYSTEM

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Abstract
This study is based on laboratory diagnosis of parasitic diseases of cyprinids (Cyprinus carpio) from a poly-culture rising system from Iasi County, for a period of six months. This study is an interpretation of parasitic co-infections, registered in winter and spring, (from December 2014 to May 2015) in earth pond-raised cyprinid, from a semi-intensive polyculture system. We collected a total of 55 cyprinids, hat were transported in the laboratory and acclimatized in glass water tanks with a capacity of 120 l (maximum 15 fishes), in order to detect parasitic stages that resist in winter time. Water quality parameters ware monitored with Aquanal-Fishwater Lab from Sigma-Aldrich. Fishes were daily examined for clinical signs. Parasitological diagnosis was made by microscopic examination of the gills and skin scraping, from dying fishes. Our investigations, leaded to the identification of one protozoan - Ichthyophtirius multifiliis, two digenetic trematodes - Gyrodactylus spp., Dactylogyrus spp. and one copepod Lernea cyprinacea, with different intensity depending on the water temperature. Lernea cyprinacea was found in winter (January and February) at an average intensity of 13 parasites per fish and immediately after collection. We identified a parasitic co-infections between Gyrodactylus spp., Dactylogyrus spp. and Lernea cyprinacaea, which indicates inappropriate hygienic condition of the water.
Key words: cyprinid, parasitic diseases, poly-culture rising system

INTRODUCTION

Due to the fact that in 2010, 47% of the fish used in alimentation was resulted from aquaculture, this activity is one of the most important domain in food producing biotechnology, Pathological evolution in fish (bacterial, viral or parasitic diseases), cane determine the decrease in fish quality, slow development and high mortality. The economic losses resulted by their disease evolution can reach even 100% when these are not identified in time and adequate measures applied (FAO, 2012).

Pathological occurrences can have catastrophic effects in aquaculture. For example, an episode of *Ichthyophtirius multifiliis* that affects fingerling can produce a rate of mortality of 75-100% in maximum two weeks if the water temperature favors the development of the parasite. Once the disease is installed, fighting and reducing the losses is almost impossible (Noga, 2010). Virals and bacterials are spread by feces, biological fluids, equipment, containers and tools but can also transferred by ichthyophagous birds or hematophagous parasites. Contamination occurs horizontally: through water, adult infected fish or the corps and sediments. In vertical contamination, the fish eggs get contaminated bacterian or viral (Savu, 2011). For the parasites, the infected elements are mobile and they are swimming freely in water and carried by this. This study is an interpretation of parasitic co-infections, registered since winter until spring, in earth pond-raised cyprinid, from a semi-intensive poly-culture system. Acclimatization was made in order to detect parasitic stages that resist in winter time. Parasitic disease evolution in fish depends on the water temperature and by increasing this parameter we evaluate the presence of parasitic resistance form. Parasitic diseases restrict the development of aquaculture industry by causing important losses for farmers (Bondad-Reantoso *et al*., 2005).

MATERIALS AND METHODS

This research was performed from November 2014 – to May 2015 on cyprinids from a poly-culture fish farm in Iași County. The biological material used was represented by the juvenile common carp (*Cyprinus carpio* Linnaeus 1758*)* with average weight of 92 grams (between 64g and 120g). Collection was made, monthly depending on the health status of the fish stock, in a total of 55 fish, representing 11% of the total number of clinically examined fish. After collection, dermal scrapes were performed, and the fishes were transferred in the laboratory and acclimatized in glass tanks (120 l) with 70% de-chlorinated water and 30% water from growth tank. Feeding was made ones a day, with pelleted feed („Crap 250" from Evialis) according to producer recommendations (1% of body weight).

The clinical examination of the fish was performed at collection, and daily for those acclimated. Cyprinids with clinical sings of disease were subjected to parasitological diagnosis that was made by microscopic examination of the gills and skin scraping.

Monitoring of the water physical-chemical parameters was made every 2-3 days, using *Aquanal-Fishwater Lab from Sigma-Aldrich*, and if necessary, their adjustment was made by replacing water in a total volume of 70%.

The degree of the parasitic infestation has been measured as the number of parasites per examined fish. The parasitic species ware identified based on the morphologic characteristics (Jones *et al*., 2005; Noga 2010.).

RESULTS AND DISCUSSION

After clinical examination of the fishes from the growth tank, no significant sings were observed. The first sings of respiratory damage were seen after 14 days of acclimatization and consisted in: agitation and dyspnea characterized by excessive respiratory movement, aerophagia. After microscopic investigations we diagnosed the following parasitic diseases: one protozoosis – ichthyophthiriasis (*Ichthyophthirius multifiliis* – fig.1*,2*), twomonogenetic trematodosis – dactylogirosis (*Dactylogyrus spp*. *fig. 4,*) gyrodactilosis (*Gyrodactylus spp. Fig. 5*), and lerneasis – an infestation with the copepod *Lernea cyprinacea – (Fig. 6)*.

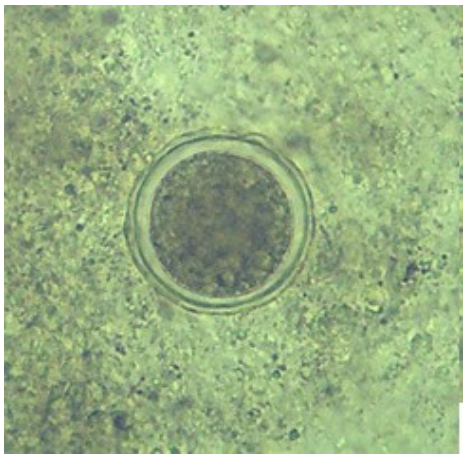

Fig. 1. *Ichthyophthirius multifiliis -tomont*

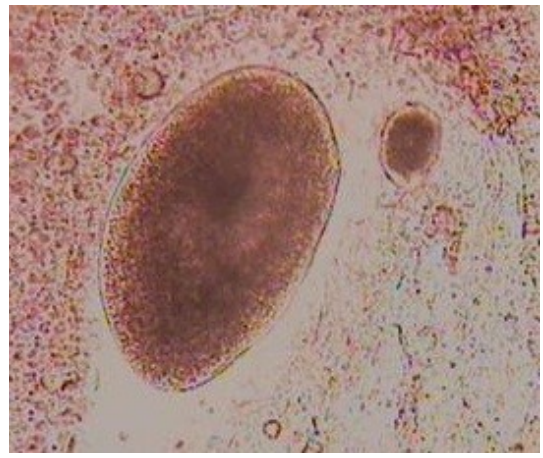

Fig. 2 *Ichthyophthirius multifiliis – trophont*

Out of the four parasite species, the highest intensity was observed for the ciliated protozoan *Ichthyophthirius multifiliis.* If in December we identified 20 trophonts/fish, but in the spring months we observed that in their number increases, reaching to be 10 times higher (120 trophonts/fish) in May 2015.

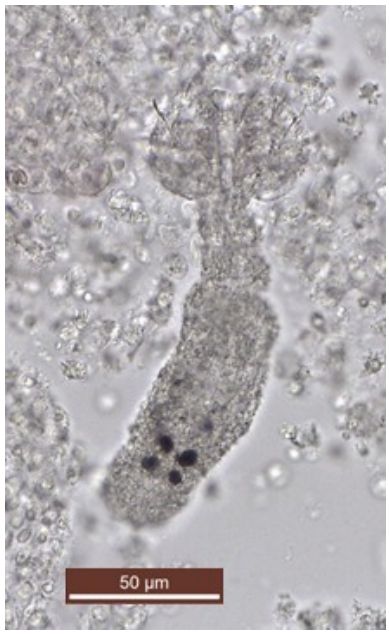

Fig. 4. *Dactylogyrus spp*: presence of ocular spots

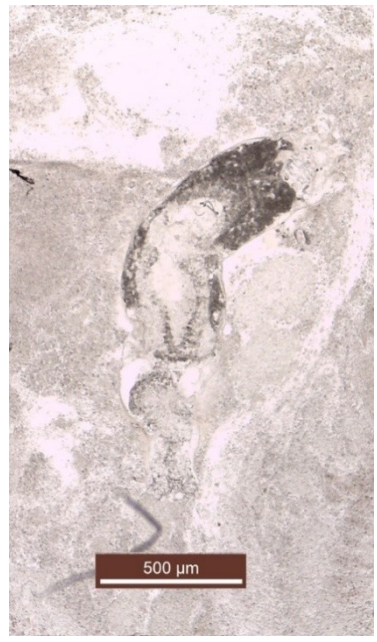

Fig. 5. *Gyrodactylus spp*: presence of embrionar hooks

Identification of the two trematodes ware made based on the presence of the ocular spots for *Dactylogyrus spp. (Fig.4)*, and the embryonic hooks for *Gyrodactylus spp. (fig.5)*(Euzet, 1998).

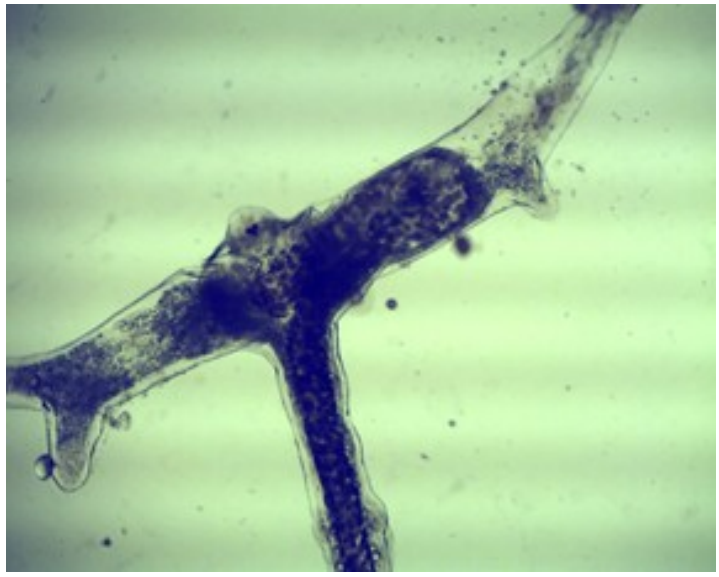

Fig. 6. *Lernea cyprinacea* x 40

After the parasitological examination of the cyprinid fishes, we revealed the presence of parasitic co-infection, characterized by simultaneous development of 3 parasitic entities: *Gyrodactylus spp, Dactylogyrus spp and Lernea cyprinacea (*fig.*7)*. The presence of the two monogenetic trematodes is not surprising and occurs often in polyculture farms, considering that 95% of these are ectoparasitic species (Euzet and Combez, 1998). This is also confirmed by us due to the fact that 90% of the examined fish were infested with these two species.

Because, during the winter, the identification of the protozoan *Ichthyophthirius multifiliis* was done after a period of acclimatization when the water temperature rised from 6°C to 23°C, we cannot include him in the co-infection phenomenon.

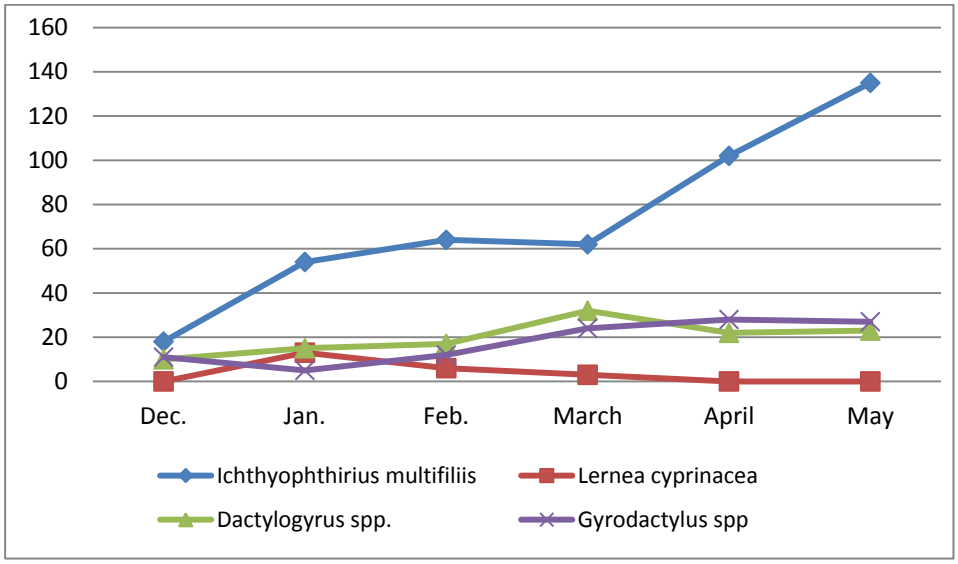

Fig. 7. Winter parasitic co-infection in common crap (*Cyprinus carpio)*

Ichthyophthirius multifiliis is known as a high pathogenic protozoan, that can cause up to 100% mortality of the fish stock so, the identification of the trophonts (adults) and theronts (invasive elements) in the smears from the dermal scrapes is sufficient for disease diagnostic and for quick treatment, before high mortality appearance. Juvenile fishes are the most susceptible to sickness, for which death occurs in 2-3 days in case of infection with 50-100 tomites (Noga, 2010). The capacity to adapt itself to thermal variations, of the water pH, salinity and species is very known, this is the reason why it is difficult to control and eradicate it, in polyculture farms.

The ciliated protozoan *Ichthyophthirius multifiliis* develops on a thermal plate between 3 and 27 °C, and the life cycle shortens with the increasing water temperature. This fact is observed in our research by increasing 10 times the number of parasites/fish observed in May. This increase is followed by the shortening the incubation time to 5 days, in fishes that were acclimatized to 23°C.

If in winter the identification of *Ichthyophthirius multifiliis* stages (trophont and tomont) of was possible after at 14 days of acclimatization, in the spring (March-May), this parasite was revealed in the dermal scrapes that were performed, immediately after fish collection. The identification in winter time of the ciliate *Ichthyophthirius multifiliis*, in acclimatized fishes, confirms the presence of „tomonts", the resistance elements, in water.

Dactylogyrosis and gyrodactylosis were identified during the entire research period, both in the dermal and gill scrapes performed immediately after fish collection and in those performed after acclimatization. These trematodosis were identified at the highest intensity in spring time, and we registered in March 2015 a number of 32 parasites/fish for *Dactylogyrus spp* and in April, 28 parasites/fish for *Gyrodactylus spp*. It is well known that temperature is a key factor for the development of monogean trematodes (Hirazawa *et al*., 2010), and that was confirmed in our samples.

The identification of the copepod *Lernea cyprinacea* was performed in winter time during the necropsy examination after fish collection. The maximum intensity of the infestation was observed in January and it was of 13 parasites/fish, with the tendency of decrease with increasing the water temperature. Therefore, in March the intensity of infestation decreased to 3 parasites/fish. In the next months this parasite cane not be identified, even in laboratory conditions.

CONCLUSIONS

1. Parasitological diagnosis made in winter and spring time, offers the possibility of estimation of fishes health status during summer.
2. The gradual collection of the live fish within 6 months, between winter and spring, established the presence of 4 species of parasites (*Ichthyophthirius multifiliis*, *Gyrodactylus spp., Dactylogyrus spp*., *Lernea cyprinacea*) which survive during the winter.
3. The identification in winter of the ciliate *Ichthyophthirius multifiliis*, on fish that were acclimatized in the laboratory confirms the presence of the resistance elements named „tomonts" in the water. Once with rise of the water temperature, the tomonts break and the invasive forms of the species are released and are named theronts with the first signs of the disease occuring.
4. The parasitic co-infections between *Gyrodactylus spp., Dactylogyrus spp*. and *Lernea cyprinacea*, indicates: inappropriate hygienic condition of the earth ponds, a perfect environment for parasites development; and that the specific prevention and control measures, are very difficult and sometimes inefficient this type of raising system.

REFERENCES:

1. Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, Jr., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. and Shariff, M., 2005, *Diseases and health management in Asian aquaculture*. Veterinary Parasitology, vol. 132, no. 3-4, p. 249-272.
2. Eiras, J.C., Takemoto, R.M. And Pavanelli, G.C., 2006. *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes*. Maringá: Eduem. 199 p.
3. Euzet, L. and Combes, C., 1998. *The selection of habitats among the monogenea*. International Journal for Parasitology, vol. 28, no. 10, p. 1645-1652.
4. FAO, 2011, *Diagnostics, Prevention and Therapy of Fish Diseases and Intoxication*.
5. Hirazawa, N., Takano, R., Hagiwara, H., Noguchi, M. and Narita, M., 2010. *The influence of different water temperatures on Neobenedenia girellae (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack Seriola dumerili (Carangidae) and the histopathological effect of this parasite on fish skin.* Aquaculture, vol. 299, no. 1-4, p. 2-7.
6. Jones, A., Bray, RA. and Gibson, DI. (Eds), 2005*. Keys to the Trematoda*. Vol. 2. Wallingford: CAB International. 745 p.
7. Savu M, Niculae M., Cadar D., Kiss T., Krupaci F., Andru C., Bordeanu A., 2011, *Environmental Microbiota Related to Bacterial Carriage in Cultivated Trout (Oncorhynchus mykiss and Salvenilus*

fontinalis), Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine, Vol.1 no. 68
8. Noga E., 2010, *Fish Disease: Diagnosis and Treatment*2nd edition , Wiley-Blackwell, San Francisco, California,

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PARASITOSIS WITH ATYPICAL OCCURRENCE IN BROOK TROUT SALVELINUS FONTINALIS M.

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Abstract
This paper is a case study that describes an episode of ichthyophtiriosis in brook trout (Salvelinus fontinalis M.*) from a fish farm in Neamt County. Diagnosis was made based on clinical signs, microscopic examination of skin and gills scraping and wet squash-smear preparation of gills. Using light microscopy, parasites were identified in 100% of examined trout, at an intensity of 13 trophonts/fish. The emergence of this disease was a surprise to us because the water which supplies the raceway tank comes from an underground source and is considered free of parasitic entities. Anamnesic information shows that the source of invasive elements (tomtits of Ichthyophthirius multifiliis) was represented by three cyprinid fishes (Alburnus alburnus L.) introduced as live fish feed.*

Keywords**:** *Ichthyophthirius multifiliis, trout*, *protozoa*.

INTRODUCTION

Ichthyophtiriosis, named "Ich" or "white spot disease", is a protozoan disease caused by the ciliate *Ichthyophthirius multifiliis.* This disease is highly contagious, spreads rapidly with dramatic evolution especially in overcrowded tanks. *Ichthyophthirius multifiliis* is the most known protozoan found on fish, has no host specificity being able to infect marine or freshwater fishes. This parasite cannot survive without host, but if left untreated, this disease may result in 100% mortality.

Usualy "Ich" is transmitted into a pond by unsterile fishing nets, carrier fish, or by water source represented by a natural river or stream. Adults measure 0.5 to 1.5 mm in size, is uniformly ciliated, have 2-4 micro-nucleus and one horseshoe-shaped macro-nucleus that can be seen in older individuals. Adults leaves the infected fish, develop into tomont that, on the bottom of the tank, transforms into thin-walled cyst. Within the cyst, after many division results over 2,000 small tomits, and these are the invasive elements of the parasite. When the tomtits are released they elongate, become theronts, swim to a hostand penetrate the epithelium using strong swimming and a penetrating gland that produce hyaluronidases.

After penetration they feed with epithelium tissue while being protected under the fish's mucus, from chemical treatment. Primary immune response against *Ichthyophthirius multifiliis* is to increase the production of mucus and is given by IgT. Following intra-peritoneal immunization with live streams researchers observed increased immune response by hyper production of mucus (von Gersdorff Jørgensen, 2008). It is known the action of antibodies in triggering complement factor but does not know the action of complement against "Ich" (Alishahi and Buchmann 2006).

Because only the theront and tomont stages are sensitive to bath treatments, it is important to known *Ichthyophthirius multifiliis* life cycle, so we can interrupt it. Life cycle of

this ciliate is conditioned by water temperature, at 5°C it needs 20 days to be completed and only 3-4 days at 20°C. Also it can resist at pH between 6 and 10 and at dissolved oxygen concentration of 0,6-0,8 mg /l (Olsen*et al.,* 2011). Al this characteristic makes this ciliatosis very difficult to treat.

MATERIAL AND METHODS

This paper is a study case of *ichthyophtiriosis* in brook trout from a small fish farm in Neamt County. *Ichthiophtiriosis* appeared in the summer (august 2015) in 24 month old brook trout *(Salvelinus fontinalis* M*)*. Fishes were introduced 3 months prior to the first clinical sign of disease. Feeding was done with granulated dray feed, after producer recommendation and live feed.

In the farm brook trout is bred concrete tanks (fig.1) with a capacity of 500 fishes/ tank, but populated with only 100 fishes/tank. Water is supplied from an underground source through an electric pump that ensures a flow in/flow out of 13l/minute. Also, water UV sterilizations applied thru a system that recirculates the water from the medium level of the tanks.

Aeration is obtained by pumping atmospheric air in to the tanks, and the temperature is maintained as low as possible by recirculating the water.

Fig. 1 Brook trout raising in concrete tanks

Diagnosis was made in the farm, based on clinical signs. Confirmation was made by microscopic examination of skin and gills scraping and wet squash-smear preparation of the gills. Monitoring water physic-chemical parameters was made with Aquanal-Fishwater Lab from Sigma-Aldrich, and by using accredited standard method for quantitative examination.

All para-clinical examinations were made at National Sanitary Veterinary and Food Safety Laboratory Iasi.

RESULT AND DISCUSSION

Clinical observation of trout leaded to the identification of the classical signs: presence of the respiratory disorder characterized by: extreme movement of the operculum, aerophagia, swimming on the surface of the water and isolation of the sick fished.

Anatomo-pathological examination, revealed lesion like: presence of white spots with dermic localization, mucus hyper secretion and gill necrosis (Fig. 2). Mortality rage registered after 6-7 days of clinical evolution was of 40%.

Etiological diagnosis was established by optical microscopy, when parasites development stages ware observed: trophont (fig.3), tomits and tomonts. Parasites were identified in 100% of examined trout, at an intensity of 13 trophonts/fish. This small value is due to the fact that, the paraclinical examination was made after transportation at refrigeration condition (4-8°C for 3 hours), knowing that after 30 minutes this ciliates (trophont) abandon the host and transforms into resistance stages (tomonts) (Noga, 2010).

Fig. 3. Mucus hyper-secretion with dermal localisation

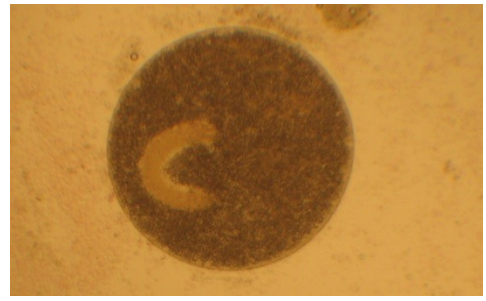

Fig. 4. Trophont of of*Ichthyophtirius multifiliis*

Analysing physico-chemical parameters of the water from the breeding tanks revealed the fallowing data: pH = 8,13; NO2 =0,043 mg/l; NO3 = 0,58 mg/l; NH4 =0,28 mg/l. These parameters were in normal range for brook trout raising. Parasite adaptability at physical-chemical variation of the water, especially temperature, is very high and pathological occurrences can have catastrophic effects in aquaculture (Alishahi and Buchmann, 2006). For example, an episode of *Ichthyophtirius multifiliis* that affects fingerling can produce a rate of mortality of 75-100% in maximum two weeks if the water temperature favours the development of the parasite.Once the disease is installed, fighting and reducing the losses is almost impossible (Woo and Bruno, 2006; Miron L, 1999).

General preventive measures aimed at: preventing fish exposure to parasites, swift identification of the disease and when clinical manifestations appear, drug treatment of infected fish and immunization is required. Physical treatments intended to destroy free swimming therontes, interrupting the life cycle and thus preventing reinfection. This was done by continuously circulating the water and by UV treatments (De-Hai Xu *et al.,* 2011). In this case by using UV treatments of the water that flows in the tanks, ichthiophtiriosis could not be prevented because of the water source is considered to be free of parasites.

The treatment in ihtioftiriosis is required by the identification of a single *Ichthyophtirius multifiliis* trophont and consists in breaking the parasites life cycle by environmental manipulation and by modifying the physico-chemical parameters of water to prevent the development of the parasitic stages (Shin *et al*, 2009). Also drug therapy should not be excluded especially for heavy infestations, by short or long term baths with formalin, depending on the degree of infestation, on the chosen concentration and not least on the farm structural possibilities (Noga, 2010). Use of ultraviolet (UV) sterilization to kill theronts of *Ichthyophthirius multifiliis* is recommended at a dose for theronts of 100,000 μWsec/cm^2 (Young, 2011). In our case, the water disinfection was made from the middle of the tank, in thick layer and the UV sterilization was ineffective.

CONCLUSIONS

1. The emergence of this disease was a surprise to us because the water which supplies the concrete tanks, comes from an underground source, considered to be free of parasitic entities.
2. Anamnestic information shows that the source of invasive elements (tomites of *Ichthyophthirius multifiliis*) was represented by cyprinid fishes (*Alburnus alburnus* L.) introduced as live feed.
3. Because the drug therapy is so difficult and the farm have a small number of fishes, we recommended the depopulation and disinfection methods to be applied.

REFERENCES

1. Alishahi M., Buchmann K., 2006 – *Temperature-dependent protection against Ichthyophthirius multifiliis following immunization of rainbow trout using live theronts*, Diseases of Aquatic Organisms, vol. 72, p. 269-273

2. De-Hai Xu, Phillip H. Klesius, Eric Peatman, Zhanjiang Liu, 2011 - *Susceptibility of channel catfish, blue catfish and channel blue catfish hybrid to Ichthyophthirius multifiliis,* Aqua. Vol. 311 p. 25–30

3. Heinecke Rasmus D., Kurt Buchmann, 2009 - *Control of Ich. using a combination of water filtration and sodium per carbonate: Dose-response studies*, Aquaculture, vol. 288, p. 32–35

4. Miron Liviu, 1999, *Tratamente antiparazitare la Salmonidele crescute intensiv pe lacurile Montane din România*, Lacurile de acumulare din Romănia, Vol. 2, p. 107-116

5. Olsen M,, Kania Per W., Heinecke R. D., Skjoedt K., Rasmussen K. J., Buchmann K., 2011, *Cellular and humoral factors involved in the response of rainbow trout gills to Ichthyophthirius infections:* Molecular and immunohistochemical studies, Fish & Shellfish Immun.30, p.859-869

6. Shinn A.P., Picon-Camacho, Bawden R., Taylor N.G.H., 2009 - *Mechanical control of Ichthyophthirius multifiliis Fouquet, 1876 (Ciliophora) in a rainbow trout hatchery*, Aquacultural Engineering, vol.41, p. 152–157

7. Von Gersdorff Jørgensen Louise, Egemen Nemli, Rasmus D. Heinecke Martin K. Raida, Kurt Buchmann, 2008, *Immune-relevant genes expressed in rainbow trout following immunisation with a live vaccine against Ichthyophthirius multifiliis*, Diseases of Aquatic Organisms, vol. 80, p. 189 – 197

8. Woo P. T. K., D. W. Bruno, 2006 - *Fish diseases and disorder,* vol. 1, *Protozoan and Metazoan Infection,* 2nd edition

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THE EFFECTIVENESS OF PHYSIOTHERAPY ASSOCIATED WITH NAIDS IN SPONDILODISCITIS OF DOG- CASE REPORT

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Abstract:*Veterinary Physiotherapy is an important component in preventing and treating the locomotors pathology, improving fitness and recovery postoperative in pets. The aim of our study was to evaluate the effect of physiotherapy combined with NSAIDs and recovery period in joint diseases in dogs, aimed at improving the patient's life.*
In the Medical Clinic of the Faculty of Veterinary Medicine was presented a small sized crossbreddog, 14 years old, with paralysis of the hindquarters, intense pain and altered general health status. Clinical and radiological examinations revealed an acute inflammatory process aseptically with cerebral palsy hind limb, associated with intense pain on palpation of the spine. It initiated a protocol of 30 physiotherapy sessions, each session using techniques like electro, passive / active exercises and therapeutic massage. Simultaneously meloxicam (Loxicom) was given as an analgesic and anti-inflammatory 0.1 mg / kg orally14 days and Cosequine tablets, 1 / day for 10 days, to restore and degeneratedcartilage.
Physiotherapy techniques associated with the use of NSAIDs have greatly improved patient status at every successive evaluation.

Key Words:*dog, paralysis of the hindquarters, physical therapy, NSAIDs, rehabilitation*

INTRODUCTION

Veterinary Physiotherapy is a new area, which emerged in our country and is in the process of development and it's successfully used in restoring: motor function, muscle atrophy, improve mobility of joints, pain and preventing permanent dysfunction-irreversible.Physical therapy is recommended to be done only by specialists. After full clinical and laboratory examination; when a diagnosis was established and a certain area was pinpointed as the extent of damage. Only after a careful investigation,involving a radiological and biochemical examination even possibly an eco-cardiologic scan the treatment can be decided. The treatment used in veterinaryphysiotherapy are: massage, passive movements to tone the muscles, cooling or hot water (known to mankind since ancient times), electro muscle, laser therapy, ultrasound therapy, therapeutic physical exercises, hydrotherapy, acupuncture, electro acupuncture (*Baxter and McDonough, 2007*).

Veterinary physiotherapy, may be used in animals of all ages, both for young animals, for harmonious growth and for adult animals, to help improve; fitness as well as to old animals, with specific disorders according to age (*Sutton, 2004*). Also, veterinary physiotherapy play an important role in the management of postoperative orthopaedic surgery, hastening the process of healing and recovery of animal pathology and muscle management, joint and nerve osteo-traumatic, degenerative or inherited.

Maximum efficiency of physiotherapy can be obtained when determining specific techniques from the disease diagnosed in parallel with adjuvant drug therapy (*Lindley and*

Watson, 2010). In our study we used physiotherapy, associated with anti-inflammatory drugs (tablets and topic gels) indicated for the symptomatic treatment of painful osteoarthritis, rheumatoid arthritis, and also symptomatic treatment of nutritional supplements for joint diseases.

MATERIAL AND METHODS

Our case study was a crossbred dog, male, aging 14 years and weighting 6.5 kg, diagnosticated with Cushing syndrome and degenerative spondilodiscitis, selected from the clinical files. This case shows the hindquarters paresis with high sensitivity whentouching the affected area and an obvious alteration of his general status. Clinical examinations, radiological (fig. 1) and the aseptic haematological check confirm a chronic inflammation to his spine level. At neurological examination for posterior limbs, reactivity has been diminished. Patellar reflex could not be better evaluated as a result of muscle spasticity. Perianal reflex was present, intense sensibility perception noted, and proprioceptive delayed.

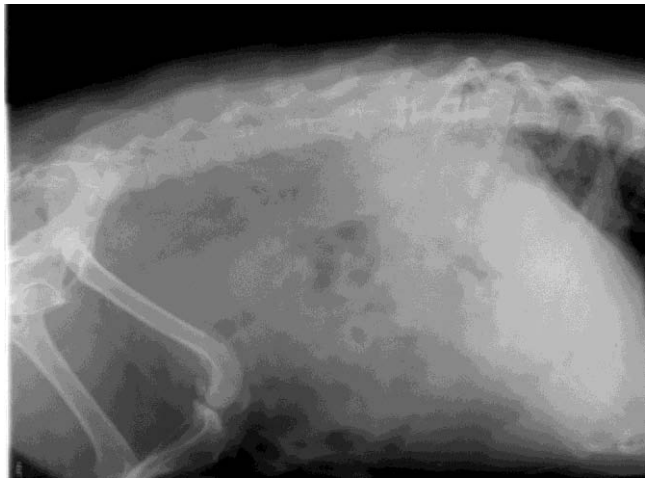


Fig.1. Rx lombo sacral spine – tight spaces L1-L6
Degenerative space L7- S1

As working material for improving the lives of patients has used the machine Intelect®Vet, special balls for physiotherapy and steady platform. Intelect®Vet is a revolutionary product for veterinarians who provide physical therapy in animals. The device is equipped with two independent channels for electrotherapy, containing four forms of interferential current- Premodulated, Russian, High Volt - and an ultrasound probe with a frequency of 1 and 3 Mhz used in the rehabilitation of patients with orthopaedic or neurologicproblems. This way, the device offers four methods of therapy in a single system, namely: electrical stimulation, ultrasound stimulation, and ultrasound combination STIM and laser therapy.

As medication we used specific anti-inflammatory gels for the therapeutic massage, Cosequine tablets, 1 / day for 10 days and meloxicam cp x 1 mg, 0.1mg / kg for 14 days.

To rehabilitate patients in physiotherapy 30 sessions were performed using electrostimulation techniques, massage, passive range of movement and active range of movement (exercise).Regarding electrostimulation in the first 10 sessions a program for reduction of muscle spasms and pain relief was used, followed by 20 sessions of stretching for muscle toning.Stretching and extent of muscle and joints provided an amazing benefit forentire body, reducing muscle tension, improving blood and lymphatic circulation and joint mobility. Also, contributes to a better rest and relaxation. Massage is a technique whereby pressure is exerted on skin tissue, subcutaneous and muscle. The pressure over suture has multiple effects on tissue movement and blood circulation and lymph.

RESULTS AND DISCUSSION

The benefits of physical therapy for animals have been widely accepted for many years in veterinary medicine, but, however, the clinical practice of physical therapy for animals is a relatively new field in our country. Each technique used in physical therapy of animals has many different advantages and not all techniques are useful for every musculo-osteo-articular problems.

In our study a sequentially improvement was observed, with each session of physiotherapy combined with NSAIDs and thus improve the overall condition of the patient's joint mobility (Figure 2) .The recovery period was about 3 months, but with visible and encouraging results, both for patient and for the owner.

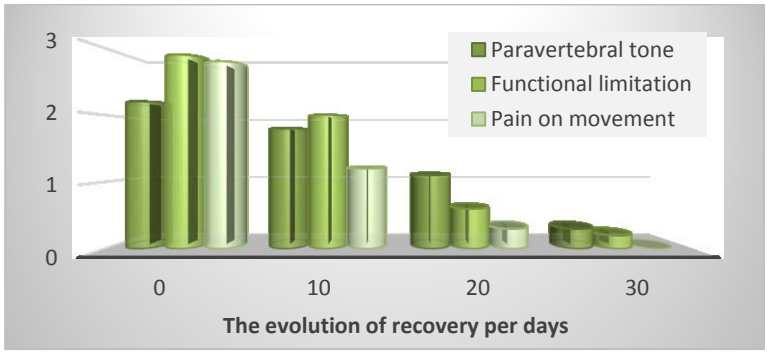
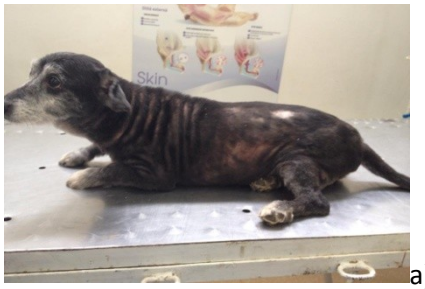
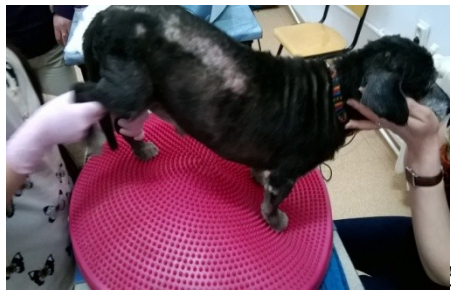


Fig.2. Evolution of Patient Status during physiotherapy

Using various physiotherapy techniques, we found that they are very effective on the body and that has no side effects and does not involve any risks to patient. Thus, the electro stimulation, which causes muscle contraction artificially by pulses, helped us to manage muscle spasms and increase the muscle tone. There for by applying passive range of motion, stimulated and rebuilt the affected limb joint mobility and exercises induced by active movements helped the patient to regain proprioception.

Therapeutic massage was performed using anti-inflammatory gels and contributed obviously to stimulate blood circulation, alleviating pain with meloxicam and improved joint mobility (fig. 3: a, b, c).



a)

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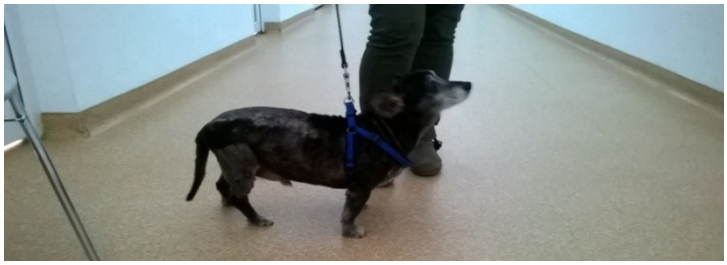
c)

Fig. 3.Results of physiotherapy applied: a) the initial paraparesis of patient; b) balance exercises platforms;c) final recuperation of the patient

After the patient was able to maintain its normal quadripodal position, imposed a series of maneuvers including applying pressure to the hind limbs, fingers pressing the dorsal surface of the basin.

Passive range of motion (PRM) was conducted through joint flexion and extension, which resulted in significant increase joint movement of the patient in our study. Passive movement is the degree of mobility of joints, but produced no muscle contraction, but through an external forces that contribute to the mobilization. Thus, there is a pressure sutures soft spots that help to maintain a normal state of cartilage, muscles, tendons and ligaments *(Bockstahler et al., 2004; Manning et al., 1997)*. Procedure was applied after the animal was relaxed. The massage consists of a slight flexion of joints, repeated for each joint of the affected limb, without causing pain. Flexion and extension of the joints were made rhythmically in a manner that mimics the normal gait.This is achieved gradually and is beneficial for neuro muscular rehabilitation *(McGowan, 2007).* The time of work was adapted according to the degree of disability of the animal. For patients in decubitus or inable to stand up, PRM should be initiated as quickly as possible to prevent adhesions between the soft tissues and bones and tissues to enhance extensibility *(Downing, 2011).*The PRM does not prevent muscle atrophy, but increase muscle tonus *(Bockstahler, 2004)*and can contribute to improving the production of synovial fluid, the diffusion of nutrients, reduce pain and soothe the animal *(Hardy, 1998)*.

The range of active movements can be performed using toys, balls or balance platforms, starting with small movements and then gradually increasingthe amplitude. In our case, the patient was placed on the exercise ball to improve proprioception and joint

stability.Small movments of the ball were induced to create an unstable surface. Additionally, consolidation was applied to walking on sloped.The combination of physiotherapy techniques and NSAIDs showed significant results, since both methods specifically acts to improve joint mobility (*Grimmer et al., 2002).*

The therapeutic action of massage on muscle tissue concern in: circulatory effects, significantly reducing of muscular pain and relaxing the animal, particularly important in preparing patients for the recovery program (*Sutton*, 2004). *Massage* involves acting on the body with pressure structured, unstructured, stationary, or moving-voltage, motion, vibration, manual or using mechanical parts. Target tissues may include muscles, tendons, ligaments, skin, joints, or other connective tissue type, and also lymphatic vessels or organs of gastro intestinal system. Therapeutic massage is part of the medical and recovery procedures and is a good treatment for motor disorders. It also stimulates blood circulation, has a painkiller effect, improves joint mobility and local metabolism. And, not least, therapeutic massage brings considerable benefits in diseases such as disc herniation, cervical spondylosis, arthritis, tendinitis etc.

CONCLUSION

Physiotherapy, extended during the 30th session and using multiple techniques associated with drug therapy meloxicam and Cosequine® improved patient's condition by reducing pain, and improving functional mobility.

REFERENCES

1. Baxter G.D., McDonough S.M., 2007 – *Principles of electrotherapy in veterinary physiotherapy*. In: Catherine McGowan, Lesley Goff, Narelle Stubbs. Animal Physiotherapy – Assessment, treatment and rehabilitation of animals. Blackwell Publishing.
2. Bockstahler B., Levine D., Millis D., 2004 – Essential facts of physiotherapy in dogs and cats. Babenhausen, Germany, BE Vet Verlag.
3. McGowan C. M., Goff Lesley, Stubbs Narelle, 2007 - *Animal Physiotherapy: Assessment, Treatment and Rehabilitation of Animals*. Blackwell Pub.
4. Downing R., 2011 – *The role of physical medicine and rehabilitation for patients in palliative and hospice care*. Vet Clin Small Anim 41: 591-608.
5. Grimmer K, Kumar S, Gilbert A, et al. 2002. *Non-steroidal antiinflammatory drugs (NSAIDs): physiotherapists' use, knowledge and attitudes*. Aust J Physiother, 48:82–91.
6. Hardy M., Woodall W., 1998 – *Therapeutic effects of heat, cold and stretch on connective tissue*. J Hand Ther 11:148-156;
7. Lindley S., Watson P., 2010 - *BSAVA Manual of Canine and Feline Rehabilitation, Supportive Care and Palliative Care*, BSAVA Publications, Cheltenham.
8. Manning A.M., Ellis D.R., Rush J., 1997 – *Phiscal therapy for the critically ill veterinary patient. Part II. The musculoskeletal system.* Comp Cont Educ Pract Vet 19:803-807.
9. Sutton A., 2004 – Massage. In: Millis D.L., Levine D., Taylor R.A., editors: *Canine rehabilitation and physical therapy*. St Lous (MO): Saunders Elsevier, p 303-309.
10. Zbângu A., Solcan G., 2014 – *The impact of the anti inflammatory treatment on dogs with intervertebral disc disease based on Olby recovery score*. Lucr. st. Med. Vet., Timisoara vol XLVII (4), pp 156-160

THE USE OF POLYTETRAFLUOROETHYLENE (PTFE) , IN VASCULAR ANASTOMOSES IN PIGS

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Abstract:*The aim of this study was to evaluate the efficiency and the biointegration capacity of polytetrafluoroethylene (PTFE) and to see the response of the organism to the graft. The research was performed in the Surgery Clinic of the Faculty of Veterinary Medicine from Cluj-Napoca, over a period of 3 months, from march 10 th until may 10th, 2015, on a total of five pigs, weighing between 50-60 kg, Large White breed. The biomaterial used was polytetrafluoroethylene (PTFE) and the approached surgical technique consisted of end-to-end anastomosis achieve between the biomaterial and and the aorta using non-resorbable sutures such as Prolene, 4-0. Animals were assessed for 30 days, by daily clinical examination. Post surgery patients offered a favorable clinical response without systemic reactions to denote signs of graft rejection. PTFE can be used with good results, representing a viable solution in vascular lesions that compromise the morphofunctional integrity of the vessel.*

Keyword*: anastomosis, pig, PTFE*

INTRODUCTION

In veterinary medicine the vascular prosthesis are lees approached and studied, even though in human medicine this types of prosthesis are well known and have been used with success for a long time (Ueberrueck T. *et al.,* 2005).

This study is aimed to highlight the anastomosis technique and to evaluate the performance of the prosthetic graft over a period of 30 days.

MATERIALS AND METHODS

The research was performed in the Surgery Clinic of the Faculty of Veterinary Medicine from Cluj-Napoca, over a period of 3 months, from march 10 th until may 10th, 2015, on a total of five pigs, weighing between 50-60 kg, Large White breed. . The biomaterial used was polytetrafluoroethylene (PTFE) (Fig.1.) and the approached surgical technique consisted of end-to-end anastomosis achieve between the biomaterial and the aorta using non-resorbable sutures such as Prolene, 4-0 (Fig.2.)

Fig.1.- Polytetrafluoroethylene (PTFE)

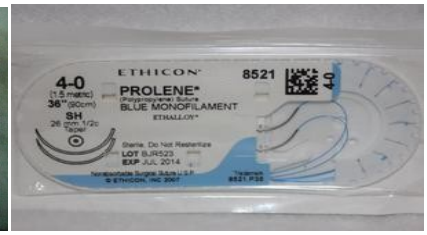


Fig.2.- Prolene 4-0

For the surgical procedure we also need appropriate instruments, from the conventional ones to specific instruments used in vascular surgery. The animals used in this

study were subjected to a 24 hour diet, and 30 minutes prior to surgery they received atropine 0.2 ml s.c followed by diazepam 2 mg / kg, i.v and ketamine 2 mg/kg, i.v (Fig. 3.)

After intubation, the anesthesia was achieved by gaseous narcosis with Isoflurane (Fig.4).

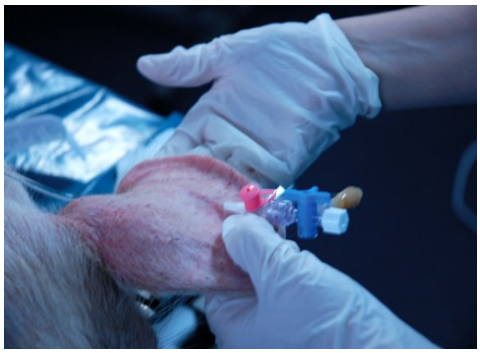


Fig.3- Peripheral venous cannulation

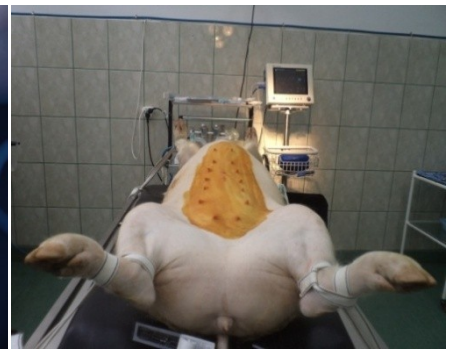


Fig.4- Animal restraint and intubation

After performing the asepsis and antisepsis procedure and applying local anesthesia by infiltration with 2% Procaine solution, laparotomy is performed on the midline (Fig. 5.), the incision starting from the subxiphoid region to the prepubian region.

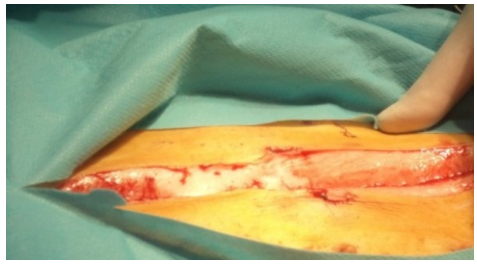


Fig.5-Midline incision

The next step is to identify (Fig. 6.), isolate and secure the aorta (Fig. 7), than the aorta is clamped using the Satinski forceps (Fig. 8.,a), after these procedures we do a full section of the aorta (Fig. 8.,b).

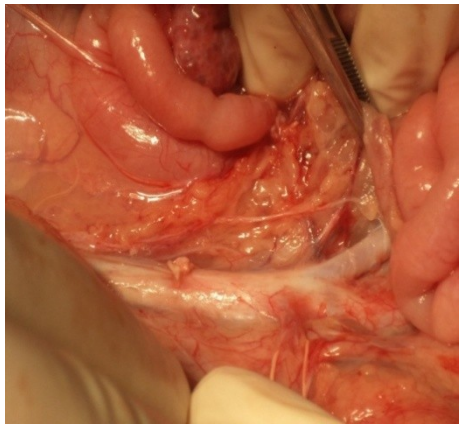


Fig. 6. Identification of the aorta

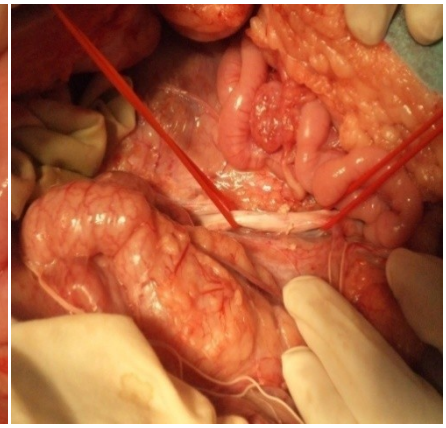


Fig. 7. Fixation of the aorta

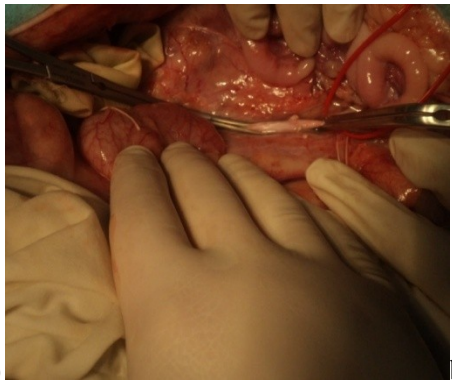
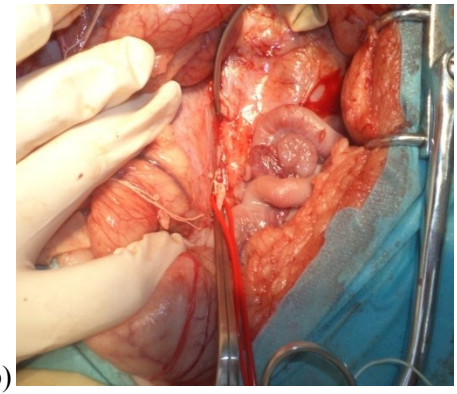
a)  b) 

Fig. 8. (a) Clamping and (b) full section of the aorta

Vascular prosthesis were pre coagulated and both ends adjusted. A end-to-end anastomosis was performed between the artery and the graft.

The ends are approximated and two stay sutures are placed at equidistant points usually at the corners. The sutures are held to steady the vessel and to rotate the vessel if required while the anastomosis is being performed around the circumference (Fig.9.). The direction of the needle is always from the outside to the inside of the graft and than inside to outside of the artery (Linton, *et al.,*1955).

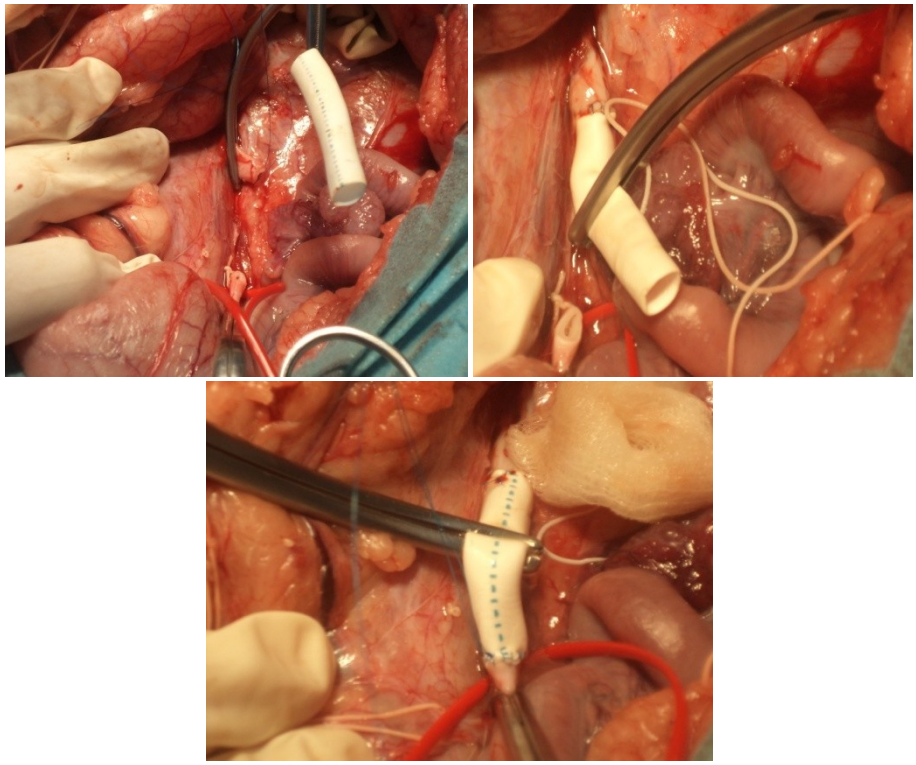


Fig.9. End-to-end anastomisis

After performing the two anastomosis (proximal and distal) and after releasing the Satinski forceps, blood circulation is restored (Fig. 10.), and this is evidenced by the presence of pulse waveforms.

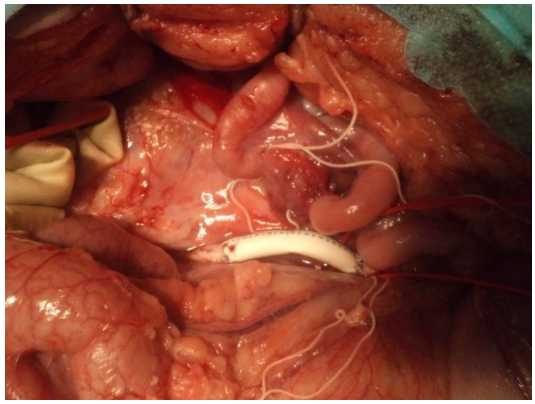


Fig.10- Restoring the blood circulation

The last steps consist of retroperitoneal suture (Fig, 11.), closure of the abdominal wall in continuous suture and simple interrupted stitch of the subconjunctival tissue and skin (Fig. 12. a,b).

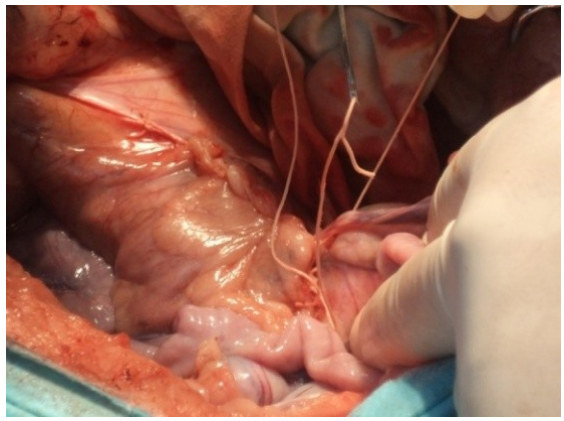


Fig.11- Retroperitoneal suture

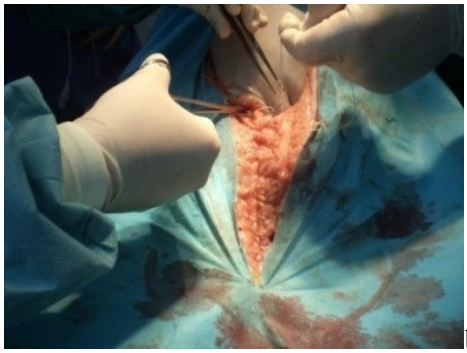
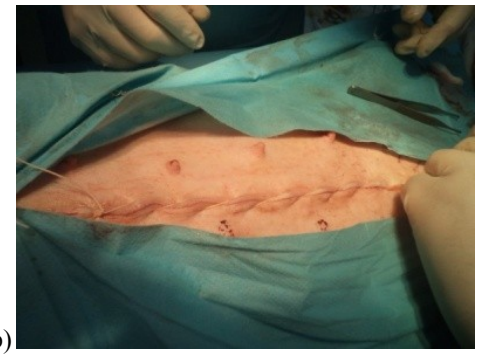
a)  b) 

Fig. 12. (a,b) Closure of the abdominal cavity

RESULTS AND DISCUSSION

The aortic anastomosis using the synthetic prosthesis PTFE has been performed with good results on swine, the technique ensuring adequate blood flow. The anastomosis was performed without suture line bleeding.

Physiological constants were monitored both during surgery and in the days preceding it. Thus, during the operation was a slight tachycardia noticed, the internal

temperature and respiratory rate remained within physiological limits. The animals were heparinized daily, 30 I.U/KG.

Postoperatory the first 3-4 days there was an edema observed at the hind limbs that resolve in a few days by an appropriate treatment.

CONCLUSION

The use of PTFE graft, already used in human medicine, can be used with good results on animals. No side effects, intolerance or implant site reactions were found. The favorable evolution of the patient depends on the ability of the surgeon to perform the surgical procedure as fast as possible, to avoid severe ischemia.

REFERENCES

1. Linton, R. R. (1955). Some practical considerations in the surgery of blood vessel grafts. Surgery: 38:817
2. Ueberrueck T., L. Meyer, R. Zippel, G. Nestler, T. Wahlers and I. Gastinger (2005) Healing characteristics of a new silver-coated, gelatine impregnated vascular prosthesis in the porcine model, Zentralbl Chir 130(1):71–76

SHORT-TERM VERSUS LONG-TERM EXTENDERS FOR BOAR SEMEN: DIFFERENCES IN PRESERVING THE KINETIC PARAMETERS WITHIN SEVEN DAYS OF STORAGE

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Abstract:*Almost all of the semen production units in swine industry continue to use the storage in liquid form, at 17°C for several days as the method of preservation and for this purpose, a multitude of extenders are available on market. Of them, some guarantee the quality of semen only a couple of days (short-term extenders) while others claim to maintain the biological value of the sperm up to one week or even longer (long-term extenders).*

This study aimed to identify the differences (if any) as regards the kinetic parameters within seven days of storage between a short-term and a long-term extender for boar semen.

Five ejaculates from five healthy and sexually mature Pietrain boars were collected by means of manual method and diluted with both BTS® (short-term) and DiluPorc™ (long-term). The extended semen was examined in the same day of dilution as well as after one, two, three, four, five and seven days of storage, by means of SpermVision 3.7. The following parameters were determined: total motility (TMot), progressive motility (PMot), straight line velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF).

Both extenders proved to be effective within the first seven days of preservation. However, significant differences ($p<0.05$), were revealed in terms of sperm velocity in the day of dilution, as well as in Days 2, 3, 4 and 7, with superior values in case of DiluPorc™. No significant differences were found for the other kinetic parameters.

In conclusion, DiluPorc™ seems to provide slightly better results in case of longer storage of semen, but we think that both the examined extenders are suitable for semen preservation up to seven days without major differences in terms of kinetic parameters. Although BTS® guarantees the quality of sperm only for three days, in our study it maintained all the ejaculates within good values for a much longer period.

Keywords: *boar semen, extender, kinetic parameters, storage*

INTRODUCTION

The preservation in liquid form, at 17°C for several days is still the most used method of semen preservation in boar, maintaining higher sperm fertility compared with freezing method (*Hu et al, 2015*).

Although sperm freezing represents a more desirable method, offering many advantages in example the facilitation of the distribution of agriculturally desirable genes, the help in controlling the transmission of certain pathogens, thereby protecting the health status of the herd, the minimization of the effects of a sudden outbreak of a contagious illness or a natural disaster (*Bailey et al, 2008*), unfortunately boar sperm are more sensitive to hypothermic shock, suffering more pronounced biochemical and functional disorders compared to other species, which leads to reduced motility, viability and transport in female genital tract, thus affecting the fertilizing potential (*Silva et al., 2015*). This constrains the

frozen-thawed semen to be used usually only in case of transferring valuable genetic material between swine breeding centers (*Roca et al., 2011*) and maintains the preservation at room temperature (15-20°C) as the standard method in case of boar semen.

Therefore, almost all of the semen production units in swine industry continue to use the storage in liquid form, at 17°C for several days as the method of preservation and for this purpose, a wide range of commercial extenders have been developed. Of them, some guarantee the quality of semen only a couple of days (short-term extenders) while others claim to maintain the biological value of the sperm up to one week or even longer (long-term extenders). Although most inseminations are performed within three days after collection, a practice that would make unnecessary the use of long-term extenders, however, some authors suggest that using this type of extenders is beneficial, as they are more efficient in preserving sperm quality and fertility (*Pinart Elisabeth et al, 2015*).

Among other tests, measurement of changes in motility over the storage time is an important tool in evaluating the ability of a particular extender to preserve sperm quality (*Martin-Hidalgo et al, 2013*). Motility is still one of the most evaluated indicators of sperm quality, especially in AI centers, as it is well known that a high value of this parameter is essential for the fertilization process (*Runceanu et al, 2007*).

This study aimed to identify the differences (if any) as regards the kinetic parameters within seven days of storage between a short-term and a long-term extender for boar semen.

MATERIAL AND METHODS

Five ejaculates from five healthy and sexually mature Pietrain boars were collected by means of manual method (*Bogdan, 1999; Ciornei, 2012*) within a commercial unit from Germany specialized in the production of boar semen. After general examination, all the ejaculates were divided in two aliquots and diluted in two ways: with a short-term extender - BTS® (Minitube, Tiefenbach, Germany) and with a long-term extender - DiluPorc™ (Sinus Biochemistry & Electrophoresis GmbH, Heidelberg, Germany) following the specifications of each producer. The extended semen was examined in the same day of dilution (Day 0) as well as after one (Day 1), two (Day 2), three (Day 3), four (Day 4), five (Day 5) and seven (Day 7) days of storage, by means of SpermVision version 3.7 (Minitube of America - MOFA®, Verona, WI, USA) connected to a Zeiss Axio Scope.A1 (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) optical microscope equipped with a plate warmed at 38°C. The following parameters were determined: total motility (TMot), progressive motility (PMot), straight line velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF). Settings were adjusted as follows: for total mobility parameter were taken into account sperm showing any movement, but for progressive mobility parameter have been considered only moving sperm presenting an average speed (VAP) of minimum 25 μm/s and a coefficient of straightness (STR) of at least 0.3.

The data stored in the computer were processed using the software IBM SPSS® Statistics version 21 (IBM® Corporation, Chicago, IL, USA). Results are presented as mean values and standard deviation (SD). Differences were considered statistically significant

when p <0.05. To highlight the significant differences between the two methods, Independent t-test analysis was applied, taking into account the presumption of equal variation.

RESULTS AND DISCUSSIONS

Both extenders presented good values after seven days of preservation, in terms of kinetic parameters (table 1). The values of progressive motility remained over 60%, which is the threshold value for use in artificial insemination, as recommended by some authors (*Flowers, 1997*).

Table 1

The values of seminal parameters for each type of extender, as determined after seven days of storage

		Mot	Mot	AP	CL	SL	TR	IN	OB	LH	CF
hort	ean	5.43[a]	6.31[a]	6.43[a]	20.76[a]	3.37[a]	.76[a]	.36[a]	.46[a]	.44[a]	2.06[a]
erm	*D*	.50	.24	.21	0.17	.24	.07	.06	.04	.87	.46
ong	ean	4.37[a]	6.88[a]	6.89[b]	32.69[a]	4.20[b]	.80[a]	.41[a]	.50[a]	.58[a]	3.17[a]
erm	*D*	.33	.74	.74	3.05	.66	.08	.07	.05	.90	.23

Within same column, different superscripts indicate significant differences between extenders (p<0.05)

Interestingly, no significant difference was observed between the two extenders in terms of total and progressive motility (p<0.05). In fact, only two of the kinetic parameters that we determined showed significant differences, namely the average path velocity and the straight line velocity (table 2). Both of these two parameters showed higher values when the long-term extender was used, which means it may enhance the metabolism of the spermatozoa, helping them to move faster.

Table 2

Independent t-test analysis of the results offered by the two extenders, after seven days of storage (presumption of equal variations)

	Levene's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
TMot	2.933	0.125	0.991	8	**0.351**
PMot	0.009	0.928	-0.222	8	**0.830**
VAP	0.254	0.628	-2.551	8	**0.034**
VCL	0.085	0.778	-0.871	8	**0.409**

VSL	0.766	0.407	-4.951	8	**0.001**
STR	0.026	0.876	-0.849	8	**0.421**
LIN	0.016	0.903	-1.212	8	**0.260**
WOB	0.020	0.891	-1.296	8	**0.231**
ALH	0.075	0.791	-0.256	8	**0.805**
BCF	0.105	0.755	-0.520	8	**0.617**

All the other kinetic parameters presented only slight differences after seven days of storage, without statistical significance (table 2), suggesting that the two extenders tend to offer the spermatozoa similar conditions.

The variation of motility according to the type of extender

For a better observation of the influence of the extender on semen motility, the values determined in each day of preservation are presented, starting with the day of dilution and ending with the seventh day of storage (figures 1 and 2).

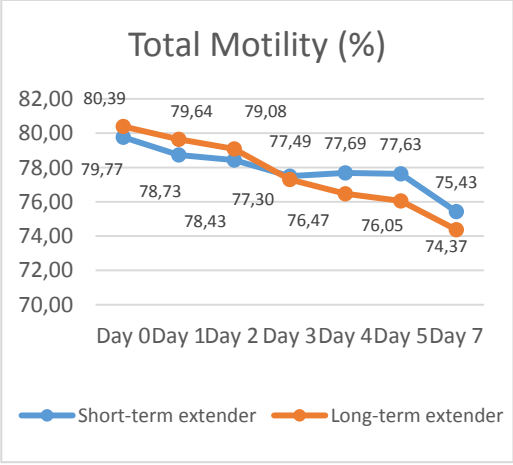

Figure 1. The variation of total motility during preservation, according to the type of extender

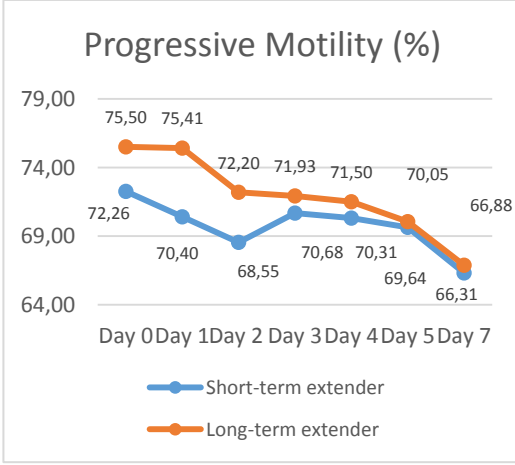

Figure 2. The variation of progressive motility during preservation, according to the type of extender

In our study, total motility defines the proportion of the spermatozoa showing any type of movements, while progressive motility defines the only the spermatozoa with progressive, forwarding movements (average speed of minimum 25 µm/s and a coefficient of straightness of at least 0.3).

The total motility showed minimal differences between the two extenders, below 2% in all of the days. As for progressive motility, there was a visible difference in the first three days, but after that the values tended to equalize, being almost identical in the seventh day. This aspect was not expected, as the short-term extenders guarantee good values of motility only for three days. Even though the long-term extenders claim to contain some substances that protect the seminal cells better during long storage time, in our experiment this effect was not visible in terms of motility.

The variation of sperm velocity according to the type of extender

The velocity of the spermatozoa within the sample is reflected by three parameters: average path velocity, curvilinear velocity and straight line velocity. Their effect on the quality of semen is not yet completely known. For example, while some authors (*Holt et al, 1997; Park, 2013*) report a negative correlation between VAP and parturition rate, others (*Didion, 2008*) state that a positive, but weak correlation exists between these coefficients. Higher values for these parameters mean a faster movement of the sperm, which could help in reaching the fertilization site in a shorter time, but could also indicate a faster consumption of energy resources.

Although the long-term extender offered higher values for all of these three parameters in any of the seven days of study (figure 3, 4 and 5), in our study, curvilinear velocity showed no significant difference ($p>0.05$) between the two extenders in any of the days (figure 3). However, some significant differences were revealed in terms of VAP in days 0, 2, 4 and 7 ($p<0.05$) as well as in terms of VSL in days 0, 2, 3, 4, and 7 ($p<0.05$), with higher values offered by the long-term extender.

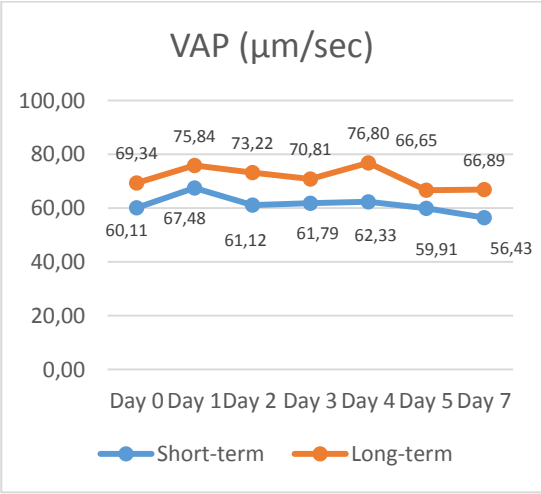

Figure 3. The variation of average path velocity during preservation, according to the type of extender

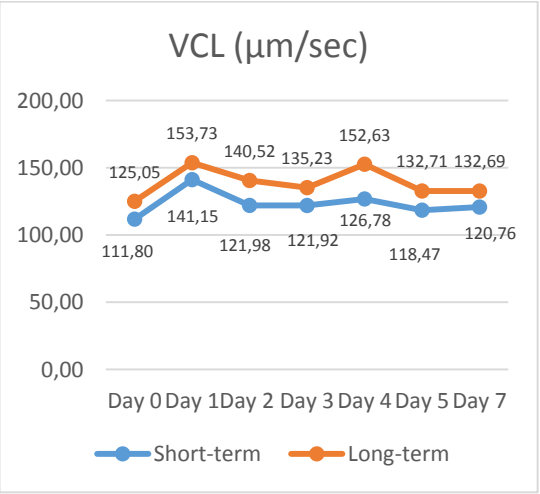

Figure 4. The variation of curvilinear velocity during preservation, according to the type of extender

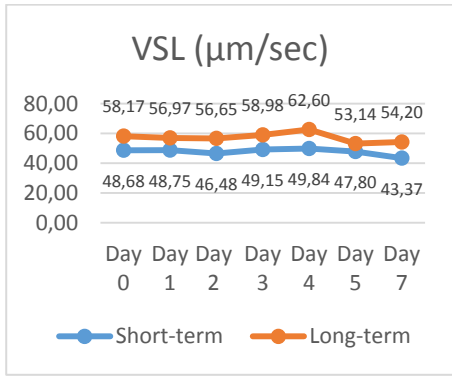

Figure 5. The variation of straight line velocity during preservation, according to the type of extender

These results suggest that the long-term extender used in our experiment may enhance the sperm metabolism more than the short-term one, but not on a regular basis.

The effect of extender type on sperm movement direction

There are several indicators determined by CASA which reflect the direction of the sperm and the accuracy of their movement, namely: the coefficient of straightness (STR) which represents the ratio between VSL and VAP; the coefficient of linearity (LIN) which means the ratio between VSL and VCL; the wobble coefficient which is the ratio between VAP and VCL; amplitude of lateral head displacement (ALH) which represents the mean width of sperm head oscillation; the beat cross frequency (BCF) which is the frequency of the sperm head crossing the sperm average path. Briefly, when STR, LIN, WOB are higher, the sperm movement is more regular, while higher values of ALH and BCF indicate a more corrugated path, which suggest a less efficient movement.

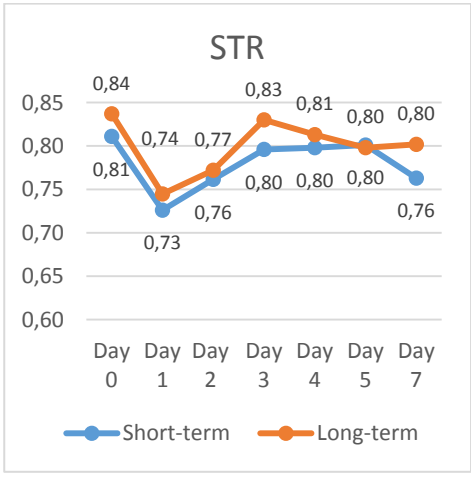

Figure 6. The variation of straightness coefficient during preservation, according to the type of extender

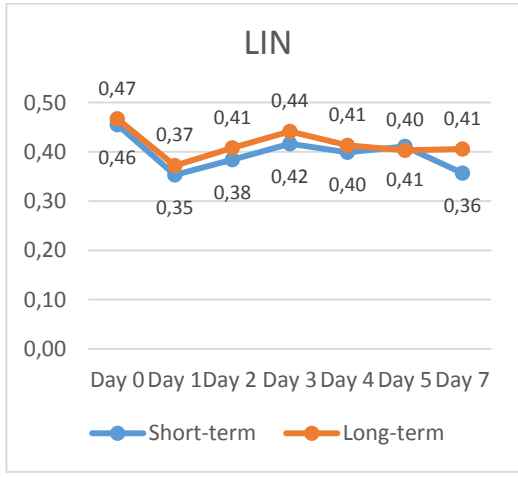

Figure 7. The variation of linearity coefficient during preservation, according to the type of extender

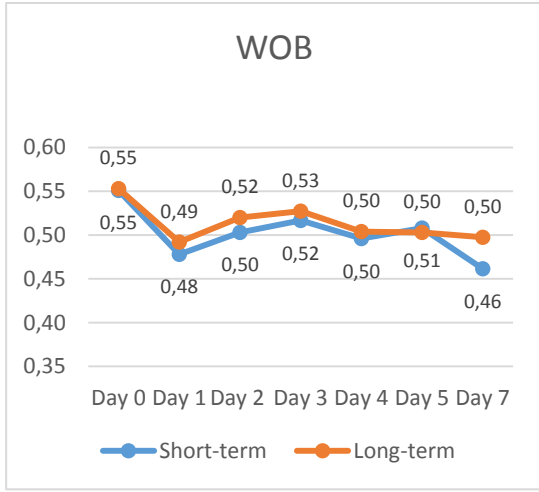

Figure 8. The variation of wobble coefficient during preservation, according to the type of extender

The STR, LIN and WOB coefficients showed clear differences between the days of study (figures 6, 7 and 8). However, no significant differences were recorded between the two extenders ($p>0.05$) in any of the days. Both extenders showed the higher values in the day of dilution, then a drop in the day after, followed by an increase until the third day after dilution and a smooth decrease until the last day of study.

As for ALH, the higher values for both extenders were recorded in Day 2 after dilution (figure 9), with low or moderate differences between the days of study. Again, the statistical analysis revealed no significant difference between the two extenders, not even in the Day 2 after dilution, when the difference was the highest ($p=0.27$).

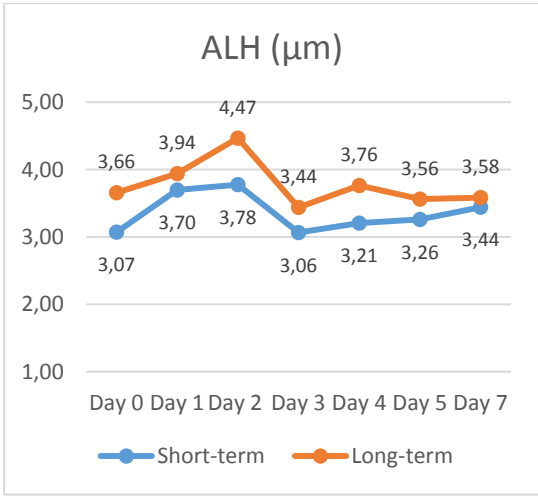

Figure 9. The variation of amplitude of lateral head displacement during preservation, according to the type of extender

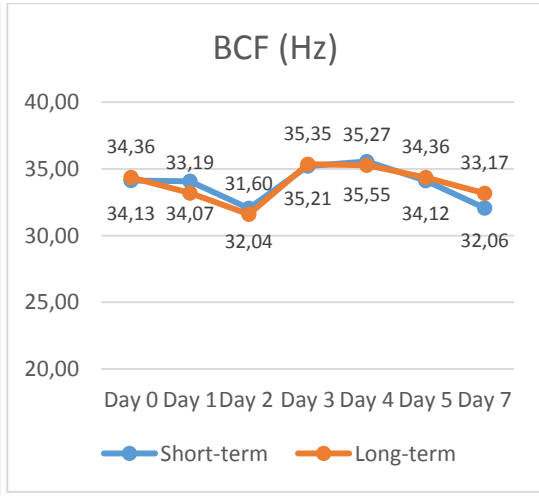

Figure 10. The variation of beat cross frequency during preservation, according to the type of extender

ALH shows the average distance that the sperm head travels in transversal direction from its average path. A higher value for this parameter reflects a longer distance covered by the spermatozoon in a wrong direction and thus a less efficient consumption of energy.

Minimal differences between the two extenders were revealed in terms of BCF, with no statistical significance ($p>0.05$). Also, the differences between the days of study were relatively low, except the increase between Days 2 and 3 after dilution (figure 10). BCF shows the frequency of sperm head intersecting the average path, and the lower values recorded in Day 2 can be correlated with the higher values of ALH in the same day, as the sperm head traveled a longer distance in order to get back to its average path, thus requiring a longer time.

CONCLUSIONS

In conclusion, the long-term extender used in this study (DiluPorc™) seems to provide slightly better results in case of longer storage of semen, but we think that both the examined extenders are suitable for semen preservation up to seven days without major differences in terms of kinetic parameters. The only significant differences revealed by statistical analysis were in terms of sperm velocity, but not in all of the days. All the other parameters showed no significant differences, but it must be said that the long-term extender offered slightly higher values almost every time.

Also, although BTS® guarantees the quality of sperm only for three days, in our study it maintained all the ejaculates within good values for a much longer period, in terms of kinetic parameters.

ACKNOWLEDGEMENTS

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The authors want to thank Besamungsverein Neustadt an der Aisch, Germany, for their support during the study.

REFERENCES

1. Bailey JL, Lessard C, Jacques J, Brèque C, Dobrinski I, Zeng W, Galantino-Homer HL (2008). Cryopreservation of boar semen and its future importance to the industry. Theriogenology 70, 1251–1259.
2. Bogdan AT (1999). Tratat de reproducţie şi însămânţări artificiale la suine. Editura Tehnică Agricolă, Bucureşti.
3. Ciornei ŞG (2012). Materialul seminal de vier şi biotehnologii de reproducţie la suine. Editura "Ion Ionescu de la Brad".
4. Didion B.A. (2008). Computer-assisted semen analysis and its utility for profiling boar semen samples Theriogenology, , 70 (8), Pages 1374–1376
5. Flowers W.L. (1997). Management of boars for efficient semen production. J Reprod Fertil Suppl 52, 67–78.
6. Holt C, Holt W.V., Moore H.D.M., Reed H.C.B., Curnoc R.M**. (**1997). Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. J Androl. 18: 312-323

7. Hu S, Zhang X, Han C, Wei S, Xie D, Du R, Hu J (2015). Effects of Three Different Diluents on Quality of Boar Semen Stored at 17°C. Journal of Northeast Agricultural University. Vol. 22 (2). 36-46
8. Martin-Hidalgo D, Baron FJ, Robina A, Bragado MJ, Hurtado de Liera A, Garcia-Marin LJ and Gil MC. (2013). Inter- and Intra-breed comparative study of sperm motility and viability in Iberian and Duroc boar semen during long-term storage in MR-A and XCell extenders. Anim. Reprod. Sci. 139: 109-114
9. Park S. (2013). Effects of sow, boar, and semen traits on sow reproduction. Doctoral thesis, University of Nebraska-Lincoln.
10. Pinart Elisabeth, Yeste Marc, Prieto-Martínez Noelia, Reixach Josep, Bonet Sergi (2015). Sperm quality and fertility of boar seminal doses after 2 days of storage: Does the type of extender really matter? Theriogenology 83 (9), 1428-1437.
11. Roca J, Parrilla I, Rodriguez-Martinez H, Gil MA, Cuello C, Vazquez JM, Martinez EA, (2011). Approaches towards efficient use of boar semen in the pig industry. Reprod. Domest.Anim. 46 (Suppl. 2), 79–83
12. Runceanu L, Cotea C, Drugociu D, Roşca P (2007). Reproducţie, obstetrică şi ginecologie veterinară. Ed. “Ion Ionescu de la Brad”, Iaşi, pp 164-171.
13. Silva CG, Cunha ER, Blume GR, Malaquias JV, Báo SN, Martins CF (2015). Cryopreservation of boar sperm comparing different cryoprotectants associated in media based on powdered coconut water, lactose and trehalose. Cryobiology, 70: 90-94

DISLOCATION AND CONSERVATIVE RELOCATION OF THE HIP JOINT - AN EXPERIMENTAL STUDY OF THE RABBITS

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Abstract:*The aim of this study was the identification of the radiographic changes that occur in the acetabulum after induction of experimental hip dislocation in the young rabbits and after conservative hip relocation (closed reduction) at different time intervals. The left hips of the 30 rabbits were dorsal dislocated (Langenskiold et al., 1962 - method) under general anaesthesia. The right hip was untouched and used as a control in all cases. The conservative hip relocation (closed reduction) and Ehmer sling immobilization was performed at different time intervals (1, 7, and 14 days). The growth of the hips was followed radiographically. Trauma of the triradiate cartilage and blood presence in the coxofemoral joint, affects negativlly acetabular development of the young rabbits. Premature physeal closure of the triradiate cartilage is followed by dimensional incongruity between the acetabulum and femoral head - acetabular dysplasia and femoral head dislocation. Early reduction after traumatic hip dislocation may not prevent dysplasia and consecutive dislocation.*

Keywords: *hip dislocation, conservative relocation, rabbit*

Cranio-dorsal coxofemoral luxation is a common orthopedic condition causing lameness in rabbits that may be caused through iatrogenic, congenital, or traumatic means (Coleman et al. -2015). Dysplasia of the acetabulum or subluxation - dislocation hip joint in the rabbits was experimental reproduced in the young rabbits trough experimental fusion of the triradiate epiphysial growth plate (ilio-ischial, ilio-pubic, and ilio-ischio-pubic) of the acetabulum (Hallel and Salvati – 1977). Current treatment methods for coxofemoral luxation in rabbit patients typically begin with conservative management (Coleman et al. -2015).

Both in humans and rabbits is proven the association between traumatic disruption of the acetabular triradiate cartilage and acetabular dysplasia and luxation of the hip (Blair and Hanson, 1979; Liporace et al., 2003). Trauma to the triradiate cartilage or its blood supply can adversely affect acetabular development due to premature physeal closure (McDonnell et al., 2007).

This investigation was aimed the identification of the radiographic changes that occur in the acetabulum after induction of experimental hip dislocation in the young rabbits and after conservative hip relocation (closed reduction) at different time intervals.

MATERIALS AND METHODS

Thirty rabbits all aged three weeks, mixed breed, both sexes, were arbitrarily divided in three equal groups. The left hips of rabbits were dorsal dislocated under general anaesthesia. The right hip was untouched and used as a control in all cases.

The protocol for general anaesthesia included premedication with xylazine (2 mg/kg b.w., i.m.) and ketamine (5 mg/kg b.w., i.m.) followed by induction with propofol (3 mg/kg b.w., i.v.). General anaesthesia was maintained by mask with 2% isoflurane in oxygen.

For hip dislocation was used the method of Langenskiold et al. – 1962. The hip was flexed and adducted and was dislocated by pressure applied at long axis of the femur. Only slight pressure was sufficed to rupture the capsule and tear the round (teres) ligament of femoral capitis.

After induction of experimental hip dislocation the conservative hip relocation (closed reduction) was performed at different time intervals: in group 1 after one day, in group 2 after seven days, and in group 3 after fourteen days. For a period of ten days after the reduction, the left leg was immobilized in an Ehmer sling.

Radiographic evaluation was carried out by conventional radiography using this Siremobil Compact L (Siemens) device and radiological facility type Multix Swing (Siemens). Image processing was done via the computerized radiography (CR) CR Vista Direct View (Caresteam) and AQS Vet Standalone software (Arzt + Praxis GmbH).

The X-ray evaluation was performed preoperative and at 7 days intervals for twelve weeks. The radiographic examination was performed in the ventro-dorsal position under anaesthesia. It was followed: stability of the femoral head relocation, the femoral head and acetabulum bone density, the growth of new tissue on the acetabulum and the dorsal length of acetabular rim compared with the healthy bone tissue of the right hips.

All statistical analyses were performed using Microsoft Windows version 6.3 a computer software statistic program. The resulting data were expressed as mean ± standard deviation (±SD). The differences between groups were analyzed with a non-parametric test - Wilcoxon Signed Ranks test (IBM SPSS Statistic 2.0). For values of $p \leq 0.05$ considering significant differences and for $p < 0.001$ highly significant.

RESULTS AND DISCUSSION

In the preoperative radiographic evaluation were identified the femoral and acetabulum growth plates in all thirty young rabbits (three weeks) – fig 1.

In twenty-seven rabbits left hip dislocation was successful – fig. 2, and in three rabbits was failed because the proximal femoral fracture has occurred – fig. 3.

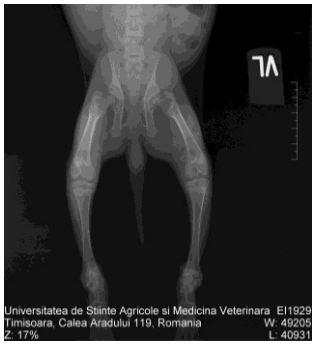

Fig. 1. Preoperative radiographic evaluation

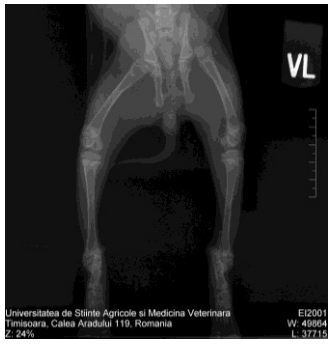

Fig. 2. Left hip dislocation

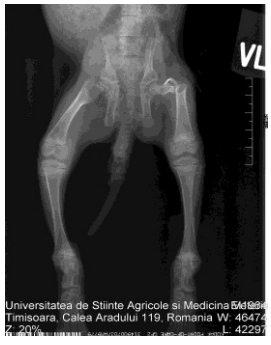

Fig. 3. Femoral fracture

Hip relocation by closed reduction was managed in 100% of rabbits in the group 1 (10 of 10), in 75% in the group 2 (6 of 8) – fig. 4, and in 44% of rabbits in the group 3 (4 of 9) – fig. 5. Unsuccessful of the hip relocation in the groups 2 and 3, respectively after seven and ten days of the dislocation – fig. 6, were due to acetabulum overload with inflamed and hypertrophic tissue of joint capsule and ligaments.

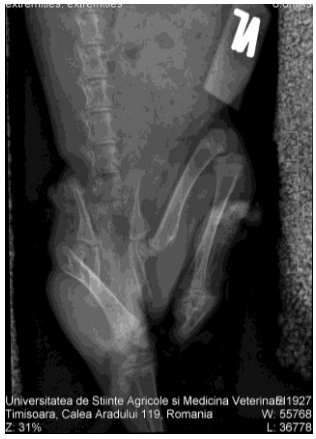

Fig. 4. Left hip relocation after closed reduction and immobilized in an Ehmer sling – group 2.

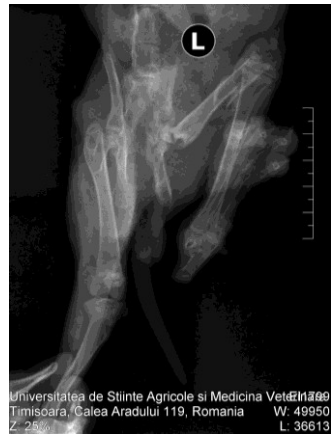

Fig. 5. Left hip relocation after closed reduction and immobilized in an Ehmer sling – group 3.

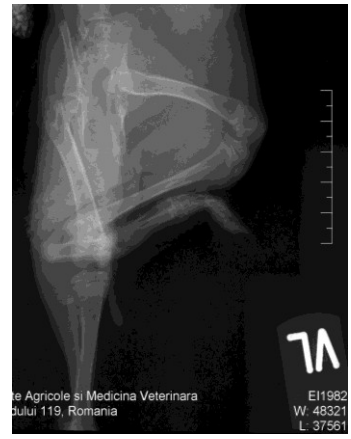

Fig. 6. Unsuccessful of the hip relocation – group 3

At radiographic evaluations at 4 weeks after dislocation-relocation in six out of ten rabbits belonging to group 1 – fig 7 were recorded subluxation of the left femoral head. In the group 2 and 3 subluxation were recorded in all rabbits (six rabbits of group 2, and four rabbits of group 3). The acetabular cavity appeared slightly loaded, with triradiate epiphysial growth plate's partial or total closed and specific alteration in density and outline of the femoral head was observed.

The acetabular region is distinctly thicker on the left side than on the unluxated right side. Varying degrees of avascular necrosis of the femoral head was registered in all three groups of immature rabbits - fig 8. The changes are obvious also in the subsequent radiographic evaluations.

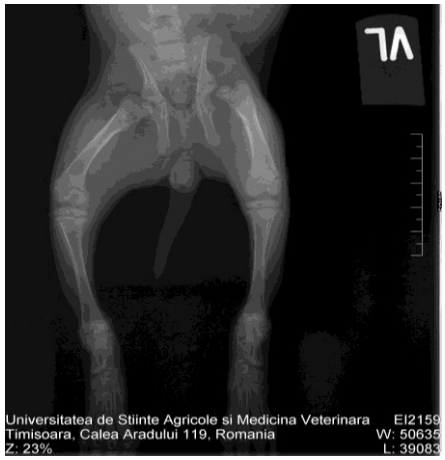

Fig. 7. Left hip subluxation after 4 weeks – group 1

Fig. 8. Avascular necrosis of the left femoral head – group 3

On radiographic evaluations made at 6 and 8 weeks, were recorded acetabular dysplasia associated with dislocation of the left femoral head – fig. 9, in all, twenty, subjects. Pronounced acetabular dysplasia and dislocation of the femoral head and associated changes (hypertrophic - osteoarthritic and/or osteolytic) in the femoral head, neck and trochanteric region was evident at assessments made at 10 and 12 weeks – fig. 10 and 11. Similar findings are reported by Solni and Ritsila – 1984 after a wedge-resection of the acetabular roof.

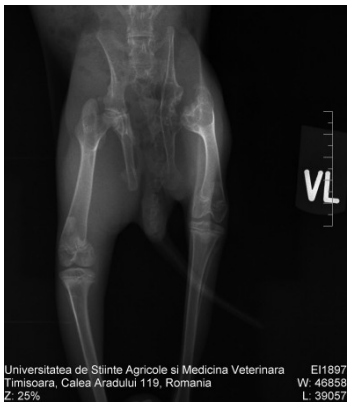

Fig. 9. Acetabular dysplasia and dislocation of the left femoral head

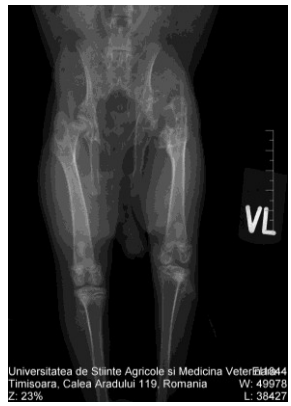

Fig. 10. Deformation of the femoral head, neck and trochanteric region

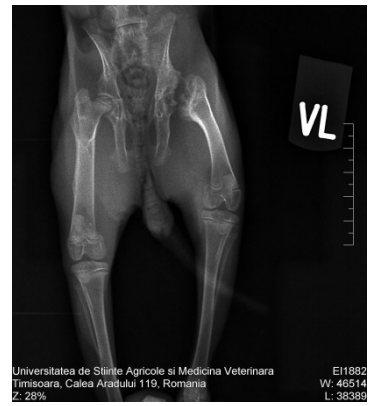

Fig. 11. Avascular necrosis and osteolysis lesions of the left hip

Comparative measurements of the dorsal length of acetabular rim of the left hip versus right hip are presented in table 1.

Table 1

The dorsal length (mm) of acetabular rim
(Comparison between right and left limbs)

Time (weeks)	**n**	**0**		**2**		**4**		**6**		**8**		**10**		**12**	
Limbs **R**ight/**L**eft		R	L	R	L	R	L	R	L	R	L	R	L	R	L
Group 1	10	9.2	9.2	9.5	8.6	9.6	8.0	10.6	8.0	10.6	8.0	10.6	8.2	10.6	8.2
Group 2	6	9.0	9.0	9.5	8.0	9.5	7.5	9,6	6.3	9.6	6.0	10.2	6.0	10.2	6.0
Group 3	4	8.5	8.5	8.7	8.0	9.0	7.6	9.1	7.0	9.5	6.7	9.25	5.3	10.0	5.5
X	**20**	**8.9**	**8.9**	**9.2**	**8.2**	**9.3**	**7.7**	**9.8**	**7.1**	**9.9**	**6.9**	**10.1**	**6.4**	**10.2**	**6.5**
± SD		1.9	1.7	1.7	1.6	2.0	1.6	1.9	1.5	1.9	1.9	1.7	2.3	1.5	2.3
p				0.018		0.005		0.033		0.005		0.012		0.015	

Notable differences between the size of right and left hip acetabulum appear at 8-10 weeks after dislocation-relocation of left hip – table 1 and fig. 12.

Traumatic dislocation of the hip affects acetabular development due to premature physeal closure and caused dislocation of the left femoral head at 6-10 weeks after trauma in all young rabbits included in this study. Similar data are reported by Hallel and Salvati – 1977 after experimental fusion of the triradiate epiphysial growth plate, Solni and Ritsila – 1984 after a wedge-resection of the acetabular roof in young rabbits and in human medicine by Blair and Hanson - 1979, McDonnell et al - 2007, Liporace et al -2003, after trauma.

Early reduction after traumatic hip dislocation may not prevent dysplasia and consecutive dislocation as in study's of Raab et al – 1988 they reported data after hip dislocation produced by extension of the knee joint.

Trauma to the triradiate cartilage and blood presence in the coxofemoral joint, have affected acetabular development by premature physeal closure. Consecutive dimensional incongruity between the left acetabulum and femoral head may be seen in all subjects (20 rabbits) included in this study - fig. 12. Another trauma (femoral fracture – fig. 13) that does not involve the coxofemoral joint and not affect the triradiate epiphyseal growth plate, is not followed by acetabular dysplasia and femoral head dislocation.

Fig. 12. Left hip subluxation after 6 weeks – group 1 dorsal length of acetabular rim of the left hip (VL) and right hip - white dotted line

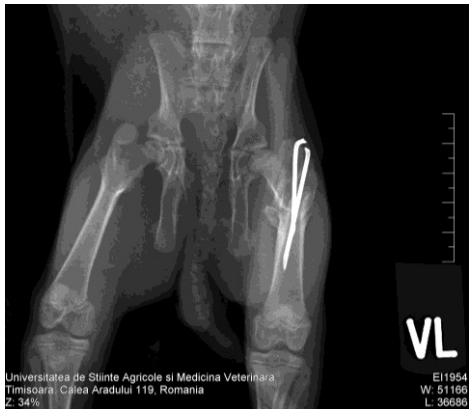

Fig. 13. Femoral fracture healing – unsuccessful dislocation of rabbit belonging to group 2, x-ray at 6 weeks.

CONCLUSIONS

Trauma to the triradiate cartilage and blood presence in the coxofemoral joint, negatively affects acetabular development of the young rabbits.

Premature physeal closure of the triradiate cartilage is followed by the dimensional incongruity between the acetabulum and femoral head - acetabular dysplasia and femoral head dislocation.

Early reduction after traumatic hip dislocation may not prevent dysplasia and consecutive dislocation.

REFERENCES

1. Coleman AK, Palmer HR, Johnston SM - Femoral head and neck ostectomy for surgical treatment of acute craniodorsal coxofemoral luxation in rabbits. *Journal of Exotic Pet Medicine,* DOI: http://dx.doi.org/10.1053/j.jepm.2015.04.06
2. Hallel T, Salvati SA – premature closure of the triradiate cartilage: a case report an animal experiment. Clin. Orthop., 1977, 124:278-281.
3. Blair W, Hanson C. Traumatic closure of the triradiate cartilage: report of a case. J Bone Joint Surg Am. 1979, 61(1):144-5.
4. Liporace FA, Ong B, Mohaideen A, Ong A, Koval KJ. Development and injury of the triradiate cartilage with its effects on acetabular development: review of the literature. J Trauma., 2003, 54(6):1245-9.
5. McDonnell M, Schachter AK, Phillips DP, Liporace FA. Acetabular fracture through the triradiate cartilage after low-energy trauma. J Orthop Trauma., 2007, 21(7):495-8.

6. Langenskiold A, Sarpio O, Michelsson JE. Experimental dislocation of the hip in the rabbit. J Bone Joint Surg., 1962, 44(1):209-215
7. Solni J, Ritsila V. Experimentally produced growth disturbance of acetabulum in young rabbits. Acta Orthop Scand., 1984, 55:14-17.
8. Raab P, Löhr J, Krauspe R. Remodeling of the acetabulum after experimental hip joint dislocation--an animal experiment study of the rabbit. Z Orthop Ihre Grenzgeb., 1998,136(6): 519-524.

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THE FIRST HEMODIAFILTRATION PROCEDURE IN ROMANIA, USING THE HDF KIT CHILD, AT DOG WITH MULTIPLE ORGAN DYSFUNCTION (MODS) DEVELOPED AS A RESULT OF SEVERE SEPSIS, ORIGIN POINT ENTEROTOMIA, MADE IN BOWEL OBSTRUCTION WITH FECAL IMPACTION
CASE REPORT

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Abstract:*The originality and novelty of the clinical case, derives from we succeeded the first time in Romania , achieving curative treatment sessions extracorporeal blood, type hemodiafiltration (HDF), in order to purify the blood of a dog found in severe sepsis, toxic substances derived from aerobic metabolism/anaerobic_and that normally were excreted_through renal function, hepatic, pulmonary. Following the severe sepsis developed, as a complication of enterotomia, needed to unblock a blocked bowel fecal impaction, developed of dysfunctions and then multiple organ failure, has necessitated putting into practice blood purification procedures, along with other curative means. The Kit HDF Child, allowed us operation HDF session, in the conditions of total volume of extracorporeal blood , in small amount. Hemodiafiltration unlike the hemodialysis, and has demonstrated superior efficacy by the action of active physical principle of convection together with passive diffusion.*

Key words*: Dog, MODS, Hemodiaflitration*

INTRODUCTION

So far, in veterinary medicine in Romania, using extracorporeal blood purification techniques are just about hemodialysis technique, main indication- acute or chronic kidney failure**.** If we refer only to addressing chronic renal failure in human medicine, the process of hemodialysis (HD), as blood purification technique began to be replaced by technical hemodiafiltration (HDF) because of multiple therapeutic advantages results from the combination of two physical-chemical processes, namely diffusion (HD) and convection (HF. Moreover, haemodiafiltration (HDF) with hemofiltre 0.8 1.4 or 1.9 m2 of membrane pores of 30μm and dialysis dose calculated at 30 ml / kg / h is not only therapeutic valences in the filtration of small molecule renal prosthesis (urea, creatinine, microelements such as K) but also in the treatment of molecules of average weight category which fall cytokines proinflammatory and anti-inflammatory responsible cascade uncontrollable mess immune system if the septic state general progress to severe sepsis, septic shock and failure multiple organ.

MATERIAL AND METHOD

Dog, 9 years old, male, 35 kg which required surgery represented by laparotomy with enterotomie for removing of fecal impaction, following an coprostaze, located above the colon. Confirming the existence of a mass in the colon which prevents intestinal transit was made based on radiological examination with contrast.

The decision to intervene invasive for removing fecal impaction by enterotomie was taken after unsuccessful attempts to mobilize and eliminate fecal impaction by purgatives or enemas high. After evaluating the patient's anesthetic risk and framing so I was to work on an complex anesthetic protocol with narconeuroleptanalgezie and endotracheal intubation.

Achieving laparotomy on the white line, the isolation portion of the colon which contained faecal impaction outside the abdominal cavity and isolation using sterile fields. Enterotomia incision It followed a healthy portion of the colon and remove faecal impaction with double suture of the intestinal wall, followed by intensive lavage with 0.9% NaCl. Around the wound enteral applied to a product in gel form, very adherent to the intestinal serosal biodegradable within 7 days, with the purpose of the barrier antiaderentiala, Hyalobarier gel, containing inter alia, hyaluronic acid. Closing the abdominal cavity was performed by double suture the abdominal wall. The antibiotic protection was performed by general antibiotics for 5 days.

At 3 days after surgery, patient's general condition has deteriorated of clinical point of view, syndrome of fever, lethargy emphasized, hyperthermia 40C, sensitivity diffuse abdominal, mucous apparent red vineyard-violet, tachypnea-30 moves / minute, tachycardia, 160 beats / minute, threadlike pulse, hypotension, oliguria, low urine output, vomituritie, halitosis mouth, jaundice then discreetly and after evident.

The biochemical exam has revealed an increase in indexes values of renal (urea cretinina) indicating renal dysfunction and then failure, and worsening liver function with hyperbilirubinemia accompanied by increased serum transaminases, gradual degradation of blood coagulation encephalosis liver accompanied by state drowsiness.

Cardiovascular function is also affected by hypotension, threadlike pulse, tachycardia and arrhythmia. Respiratory dysfunction is also present with PO2 altered, deep breathing, thoraco-abdominal slight violet tinge.

The haematologic and biochemical examination confirmed renal and hepatic suffering. The hematological examination revealed an increase of 15.8 RDW (red cell distribution width) from 11.9 to 14.5% versus 15.8, and thrombocytosis (476 - compared to normal = 143-400 K / μL).

Nr. Crt	Analysis of the:	Results	Normal values
1	Glucoza	93,70	62-100 mg/dl
2	Uree	141,10	24-54 mg/dl
3	ALAT	42,90	<40 U/L
4	ASAT	42,60	<30 U/L
5	Creatinina	2,89	0,45-1,58 mg/dl
6	GGT	8,30	<10 U/L
7	PAL	146,6	30-140 U/L
8	Na	135,5	135-155 mmol/l
9	K	3,44	3,5-5,5 mmol/l

10	A-Amilaza	1234,5	< 900 U/L
11	Lipaza	40,3	8-42 U/L
12	Proteine tot	7,4	4,8-6,4 g/dl
13	Albumine	2,82	1,77-3,14 g/dl
14	B-Globuline	0,65	0,55-1,34

Table 1. The biochemical examination before the procedure HDF

Following of the clinical picture well expressed by a generalized peritonitis and biochemical examinations and monitoring tool that indicate multiple organ failure (kidney, liver, heart, respiratory) as a result of severe sepsis installation, it was decided the following protocol: Respiratory support, Cardiovascular support, antibiotherapy, intervention explorer and therapeutic purposes, extracorporeal blood purification in order to sustain the renal failure and to decrease circulating levels of inflammatory mediators, TNFα, respectively interleukins IL -1 and IL-6.

Regarding the extracorporeal blood purification technique was passed to the inventory of material and technique necessary to achieve hemodiafiltration (HDF) central venous catheter - catheter respectively hemodiafiltration short duration, with the following characteristics: thickness 07 Fr (2.33 mm), biluminal long by 13 cm. The kit that includes central venous catheter consists of: Guide of 0.81 mm x 50 cm Dilator hidrofilc 2.33 mm, 3.00 mm dilated, 18G injection needle 1.25 mm x 7 cm, syringe 5 cc scalpel sterile field

Fig.1 The kit that includes central venous catheter

The device treatment blood extracorporeal continuous HF 440 - Infomed with the following features of possibilities therapeutics: SCUF, HD / HDF / HF, TPE, HP, CPFA, with 4 pumps (blood Ultra filtered, two substitutes), weights substitutive and ultrafiltered , heparin syringe, display, detector and blood gas bubble trap), blood pressure sensors, pre-filter, venous ultra filtered) heater.

Fig. 2. Continues extracorporeal blood purification device HF440

The kit child HDF has the following characteristics: Hemofiltre 0.8 m2, arteriovenous lines, line replacement, ultra filtration collection bags, bag heater. The reason that I use a special kit is -Child- total extracorporeal blood volume is low (75 ml) according to the patient's weight. (20-40 kg = kit child).

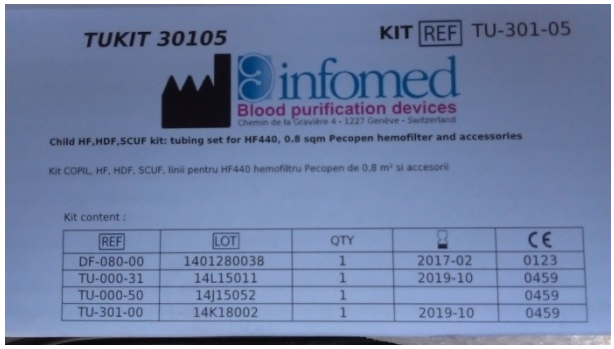

Fig.3 The Kit HDF – Child

DGK 200 replacement solution, bicameral solution in bags of 5000mL. The two-chamber bag with 4500 ml + 500 ml solution: Na + = 140; K + = 2.0; Ca2 + = 1.75; Mg2 + = 0.5; Cl = 111.5; Lactate = 3.0; Glucose = 1.0; HCO3- = 32.0 mmol / l.

The heparin sodium, bottle of 25,000 i.u. - anticoagulation protocol used in the extracorporeal circuit. Taurolok - closing solution to preventing catheter colonization with pathogenic germs. High risks on malfunctions CVC is due catheter-associated infections. These infections can be triggered by microbial colonization of the catheter and from here microorganisms can spread into the bloodstream.

Fig.4 Taurolok - catheter closing solution

The protocol abatement technique of blood using the technique HDF assuming fulfillment of the following steps: analysis of complex biochemical blood counts, determining the status of coagulation by analyzing APTT's and INR, protocol development of anticoagulation in order to have a hypocoagulability blood that will allow circulation extracorporeal Blood without jeopardizing patient safety, setting an intravenous catheter and tranquilization of the patient, determining a central venous catheter - right jugular vein and closing the catheter until the coupling circuit extracorporeal, putting into practice an array of techniques for monitoring cardiovascular function, respiratory and metabolic, installing circuit extracorporeal and priming of the circuit, the coupling circuit extracorporeal patient, implementation of the anticoagulation protocol, patient monitoring and coagulation of vascular by APTT and INR, filling in HDF, running manner continuous session HDF and not as in case of HD intermittent. The protocol return of blood to the end of the session HDF patient and extracorporeal circuit decoupling patients with aseptic procedures, respecting the closure catheter and anticoagulation of catheter.

The complex analysis of biochemical blood counts, coagulation status determination by analyzing APTT and INR's was: normal 72-102 sec. Average 50 x 2 = 100 sec APTT average value around which we had to hypocoagulability placed to achieve effective extracorporeal circuit.

Anticoagulation protocol development in order to have a blood hypocoagulability that will allow extracorporeal blood circulation without jeopardizing patient safety was:

Aim pursued for us was to maintain a APTT more than 2 times, two and a half times than normal, with the intention to decrease the coagulability blood below the risk clogging hemofiltre and at the same time not cause uncontrollable bleeding or the wound surgery 3 days after surgical intervention or gastric or other intracavitary or extracavitare sites. For this purpose we used sodium heparin (unfractionated heparin) in 25,000 unit vials in dilution 500 IU in 2 ml of NaCl 0.9%. Basically a 50 ml syringe is 12,500 IU vial is half of the 25,000 IU. Anticoagulation protocol included two timelines: one during priming site and consisted of injecting a syringe pump using 10,000 IU or 20 ml heparin diluted / 1200 ml of substitution DGK 200 (K2). Replacement solution is a poly-ionic composition Ca, K, Na, Cl and bicarbonate in a two-chamber bag. One room contains the acid component and the second contains an alkaline bicarbonate Na, the two intermingled even before the meeting hemodiafiltration. The goal of priming is to fill lines replacement solution, to remove air from the lines to make a good lavage of hemofiltre. From the resulting debris will fall hemofiltre of manufacturing and composing microfilamantele semipermeable membrane will expand, become expanded. Heparin priming will attach to microfilaments semipermeable mebrane and prevent clogging of the blood and thus the impossibility of extracorporeal blood circuit.

Fitting an peripheral intravenous catheter was done for the management of substances neurotropic purpose depression and to intervene in case of hypotension effective way. I used iv Midazolam and Medetomidine in i.v.

Fixing a central venous catheter - right jugular vein catheter closure until the extracorporeal circuit connection. This step is essential in achieving the necessary and essential blood flow carrying blood purification therapy. Blood flow / min required is estimated by us to 100-120ml/min.

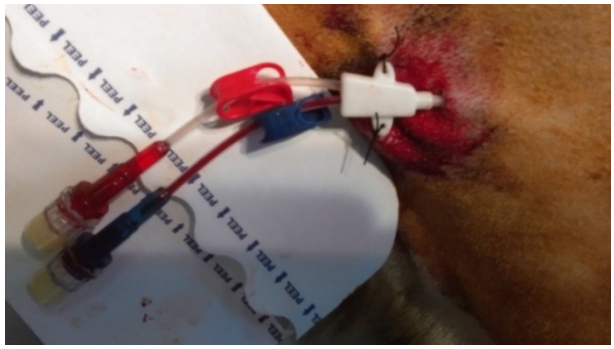

Fig.5. The fixing of a catheter - short term on the jugular – right

Putting into practice an array of techniques for monitoring cardiovascular function, respiratory and metabolic functions. I opted for PO2 monitoring, temperature and heart rate.

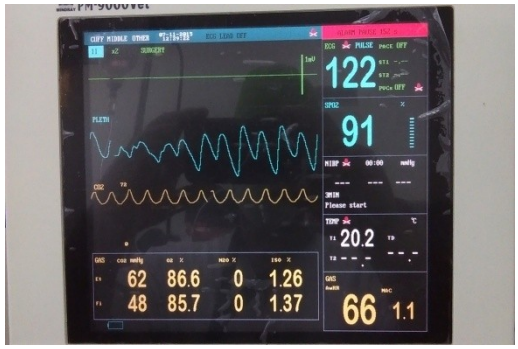

Fig. 6. the technical Panel Patient Monitoring

Installing the HDF Kit HF440 device requires the following steps: Chek list blood purification device to identify any technical problems before unsealing HDF kit. This is done technician menu entering the device, the device and the central venous catheter at a distance of about 50 cm from the patient, so that the scales be free and do not support things around. Wheel locking device. Power to a Power Source 220V power, switching on and then applying kit lines on pumps, installing sensors and hemofiltre, applying pressure sensors, bags collection ultrafiltration and solutions replacement, load syringe pump with a syringe heparin diluted with 1200 ml priming initiating replacement solution, coupling lines arteriovenous patient / device and initiating treatment. This step requires strict antisepsis measures catheter and coupling unit to pacent to avoid complications of catheter-related infections. I used chlorhexidine as antiseptic catheter plugs and using sterile field of the window.

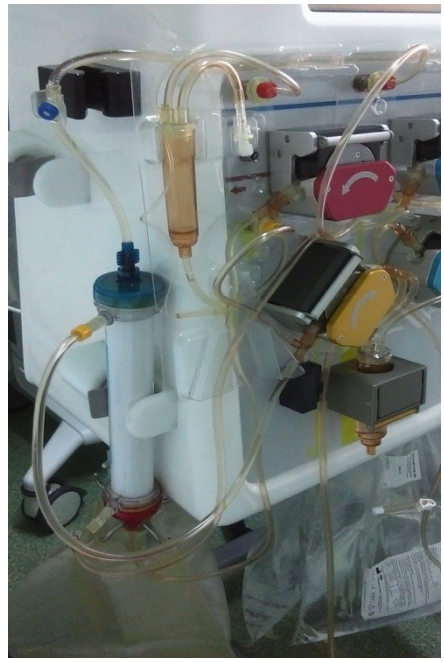

Fig. 7 Priming extracorporeal circuit

Putting into practice the protocol anticoagulation departed from principle of ensuring minimal anticoagulation extracorporeal blood treatment session to enable hemodiafiltration. This principle is ensuring APTT with a 2X higher than normal. In a 50 ml syringe heparin sodium 25,000 IU I used a found in a 5.0 ml vial. I used 2.5 ml, ie 12,500 i.u. in 47.5 mL of normal saline for a total of 50 ml by heparin diluted with 250 IU / ml of heparinized saline. I used a continuous dose of 2 ml / h.

Fig. 8 The syringe pump with syringe loaded with a diluted heparine

The monitoring of patient and vascular coagulation by APTT and INR, filling of the sheet in HDF. Protocol requires monitoring of APTT's and INR 4 in 4 hours. We opted for APTT monitoring and INR's every 2 hours. Conducting the session HDF continuous manner and not as in cases of intermittent HD assumed blood flow / minute for 80 ml and duration exceeding 4 hours. The dialysis solution flow rate was 2000 ml / h and ultrafiltration of 1200 ml / h. It tried keeping the dose of dialysis indication to 30ml / kg / h, to have a high efficacy of the of purification of blood. HDF session duration was 20 hours.

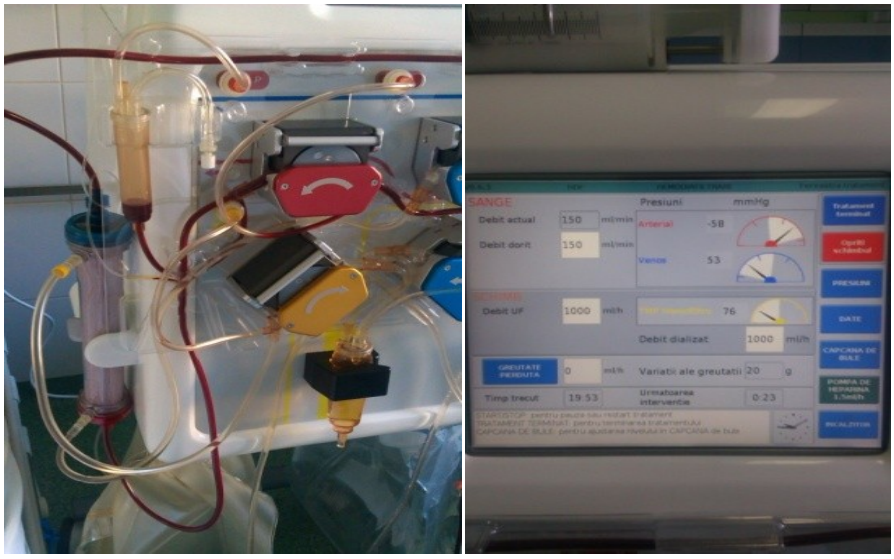

Fig. 9 Conducting HDF and main screen HF440

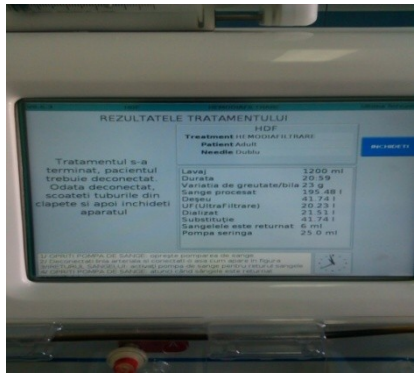

Fig.10 Window at end of treatment

The protocol return of blood to the end of the session HDF patient and extracorporeal circuit disconnection patients with aseptic procedures respecting the closure catheter and catheter anticoagulation.

RESULTS AND DISCUSSIONS

These therapeutic procedures were repeated in three sessions, each with a few hours of operation of the device. Following these three procedures HDF, who worked over 3 days (with interruptions for several hours between sessions) accompanied by measures aseptic surgical peritoneal lavage, antibiotics, specific measures intensive care (vasopressors, oxygen administration, solutions electrolytes and nutrients) to the resumption managed dysfunction kidney diuresis, low body temperature, exiting condition of sepsis and severe depression. Blood urea decreased after each session with average values of 20 mg / dL and creatinine was brought after the first session in the normal range, ie below 1.50 mg / dl.

The purpose of using this technique is warranted and lowering levels cytokines proinflammatory IL-1 and IL-6 - involved in the cascade process septic general, molecules with medium molecular weight and can leave the vascular bed through the pores membrane of hemofiltre under hydrostatic pressure applied to the column of blood. Of course with medium molecular weight molecules will cross the semipermeable membrane pores and

small sized molecules respectively urea, creatinine and electrolytes much of ions K, Na. In the case described, the role HDF is to replace the function excretory kidney affected first phase dysfunction and then failure and shorten the tops of cone the levels of cytokines IL-1 and IL-6, thus restoring the correct answer immune and thus gaining time to fight antibiotics. On the other hand small blood flows / minute which is being allowed a slow extrarenal treatment continues without hemodynamically destabilizing been so patient in a critical situation. It managed so stable hemodynamics, avoiding sudden changes in electrolyte and acid-base balance as well as the elimination of low and medium molecular weight molecules. There is a slow treatment that allows a balancing osmotic vascular-interstitial and cellular compartments. We used extracorporeal circuit kits are recommended for children so that patients between 20 and 40 kg due to low blood just under extracorporeal - 75 ml respectively. Undoubtedly there are some elements that discourage medical team in putting into practice such a technique, complex. The first of them is related to knowledge of art, knowledge anticoagulation extracorporeal circuit, a dialysis catheter insertion technique - short term - of a size sufficient to ensure a high flow of blood (100-120-150 ml / min), long waits therapy which requires the existence of a team and not least the high cost of the procedure - reasons for delay in deciding HDF therapy.

CONCLUSIONS

This procedure - HDF - blood treatment applied by continuous manner, at least in intention to keep it 12-24 hours replaces for efficient manner, excretory function of the kidneys, and purge in addition low molecular weight molecules and average molecular weight molecules - restoring the correct response of the immune situations sepsis. On the other hand no patient hemodynamically destabilizing and osmotic balance, slow manner, vascular-interstitial cell compartments.

Hemodiafiltration using a kit child is an effective therapeutic solution for addressing IRA secondary septic situations. HDF stabilizes acid-base balance in situations of metabolic acidosis.

REFERENCES

1. James F.Winchester si col, 2004, Sorbents in Acute Renale Failure and End – Stage RenalDisease: Middle Molecule and Cytokine Removal 22:73-74
2. Thomas Rimmele, Critical Care, 2011, Clinical Review: Blood purifications for sepsis
3. M.Page, si col, 2007- cas clinique - Utilisation de la Hemodiafiltration au cours du traitement d'choc septic. Annales Francaises d'Anesthesie et de Reanimation

EMERGENCE OF CANINE HEPATOZOONOSIS IN WESTERN ROMANIA SUPPORTS THE GEOGRAPHICAL EXPANSION OF THE DISEASE

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Abstract:*Hepatozoonosis is an arthropod-borne disease caused by Apicomplexan protozoa from the family Hepatozoidae, genus Hepatozoon described in amphibians, reptiles, birds, marsupials and mammals. Transmission of Hepatozoon spp. in dogs occurs by ingestion of ticks that contains mature oocysts. The aim of this study was to investigate the prevalence of H. canis in dogs from the west and south-west Romania by using non-molecular and molecular techniques and the relationship between infestation and some epidemiological factors. During study 260 symptomatic and asymptomatic dogs from eleven west and south-western Romania counties were investigated by Diff Quik stain and PCR for presence of Hepatozoon spp. Molecular surveillance of blood samples from dogs in the western and south-western Romania, showed a 9.3% prevalence of canine hepatozoonosis. No statistical differences were observed between prevalence reported in age, gender, race, habitat and provenance. Following amplification of 18S rDNA gene sequence Hepatozoon canis species was identified. The results demonstrate the expansion of this disease transmitted by vectors in non-endemic regions and is first screening in the canine population in Romania.*

Keywords: *Hepatozoon canis, dogs, prevalence, Romania.*

INTRODUCTION

Hepatozoonosis is an arthropod-borne disease caused by over 300 different species of protozoa of the family *Hepatozoidae*, suborder *Adeleorina*, *Hepatozoon* genus described in amphibians, reptiles, birds, marsupials and mammals. Of these, more than 120 species are found in reptiles and about 50 have been reported in mammals (Baneth, 2001, 2011, Vincent Johnson, 2014).

The genus name is due to the merogonic development of type strain *Hepatozoon muris* in the rat liver. The species from amphibians, reptiles and birds are red blood cells parasites, while in mammals *Hepatozoon* spp. gamonts are found mainly in leukocytes. There is a variety of haematophagous arthropod vectors that serve as the definitive host for different species of *Hepatozoon*. These hosts include ticks, mites, mosquitoes, sand flies, tsetse flies, fleas, lice and reduviidae (Baneth 2006, Macintire and Vincent-Johnson, 2006).

Transmission of *Hepatozoon* spp. occurs by ingestion of the definitive host, an invertebrate that contains mature oocysts, by the intermediate host, a vertebrate. There have been identified two species, in domestic dogs as intermediate host, namely *Hepatozoon canis* and *Hepatozoon americanum* (Baneth 2001, 2004, 2011, Vincent-Johnson, 2014 Ivanov and Tsachev, 2008).

The general lack of information on the spread and prevalence of canine hepatozoonosis in Europe, the lack of molecular studies to determine the species and the lack

of information on *Hepatozoon* infection in the canine population of Romania led to the present study on 260 dogs, apparently asymptomatic and symptomatic, from different counties of Romania.

The purpose of this study was to investigate the prevalence of *H. canis* in dogs from the west and south-west Romania by using non-molecular and molecular techniques and the relationship between infestation and some epidemiological factors.

MATERIALS AND METHODS

Area and animals studied

During 2011-2015, in order to identify of some pathogens with blood localizations, including etiological agents from *Hepatozoon* genus, a total of 260 symptomatic and asymptomatic dogs were investigated.

The research was conducted at the Parasitology and parasitic diseases clinic of Faculty of Veterinary Medicine Timisoara. Samples representing of dogs blood from various localities in western and south western Romania were collected.

The study was conducted in both rural and urban areas, in eleven west and south-western Romania counties (Arad, Bihor, Caras-Severin, Dolj, Hunedoara, Gorj, Mehedinti, Olt, Satu-Mare, Timis, Valcea) with the support of veterinarians from private veterinary clinics and owners.

Dogs were cases of University Veterinary Clinics, private clinics, shelters or dog households. Animal's age ranged from 2 months to 16 years and there were several purebreds and crossbreds. Symptomatic animals had at least one clinical sign characteristic of a morbid entities, the most common clinical signs recorded were fever, hemoglobinuria, jaundice, dyspnea, arthritis, lameness, resistance to antibiotics related to previous contact with a tick, anorexia progressive weakening.

Working methods

After general examination of each animal whole blood sample were collected in sterile vacutainer with anticoagulant EDTA by puncture of the cephalic vein. In the day of collection or at a later date the samples were processed by classical techniques or molecular biology to highlight the presence of blood parasites concerned. The smears were stained by Diff-Quik method and then microscopically examined.

The first stage in the molecular analysis was the isolation of genomic parasitic DNA from the blood sample analyzed. This extraction was performed using PureLink®Genomic DNA Mini Kit kit (INVITROGEN®). The purified DNA product obtained was kept in a freezer at - 20° C until further processed.

The extracted DNA was subjected to polymerase chain reaction (PCR) of the 18S rRNA gene fragment (about 666 base pairs section) using specific primer set HepF (forward) (5'-ATACATGAGCAAAATCTCAAC-3') and HepR (reverse) (5'-CTTATTATTCCATGCTGCAG-3') and amplification conditions described by INOKUMA et al., 2002.

Positive and negative controls were also included in the reactions. In addition, to confirm the results of PCR 9 PCR products of *H. canis*, randomly selected, were purified and sequenced (Macrogen Europe®, Amsterdam, The Netherlands) using the same primers.

Control of the amplicons was performed by electrophoresis in a system horizontal submerged electrophoresis in 1.5% agarose gel at 120 V and 90 mA for 60 minutes.

After migrating samples in agarose gel, migrated DNA fragments in gel image was captured using a UV photodocumentation system (Molecular Imager® Gel Documentation System DocTM XR + Bio Rad®). Acquisition was performed using image analysis program Quantity One ver. 4.6.5., and using the computer program to calculate the amount of USI Vilber Lourmat amplified fragments.

Statistical analysis of the results was performed using GraphPad Software QuickCalcs to evaluate possible differences between epidemiological data of the dogs in the study. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

After examining blood samples (Table 1) different results depending on the method used were obtained. Thus, examination of stained Diff-Quik smears method revealed gamonts in 10 of the 260 dogs examined, which is a prevalence of 3.8%.

Table 1

Sinoptic of positive samples resulting from epidemiological investigation of *Hepatozoon canis* infection in 260 dogs studied, in the western and south-western Romania

	Hepatozoon canis	
Epidemiological data	***Diff-Quik* (%)**	***PCR* (%)**
Age		
≤ 2 years (*n*=70)	2 (2,9)	4 (5,7)
> 2 to 6 years (*n*=110)	3 (2,8)	8 (7,3)
≥ 6 years (n= 80)	5 (6,3)	12 (15,0)
Gender		
Female (*n*=149)	6 (4,0)	14 (9,4)
Male (*n*=111)	4 (3,6)	10 (9,0)
Breed		
Pure (*n*=210)	6 (2,9)	16 (7,6)
Mixed (*n*=50)	4 (8,0)	8 (16,0)
Habitat		
Urban (*n*=190)	6 (3,2)	15 (7,9)
Rural (*n*=70)	4 (5,7)	9 (12,9)
Owner		
With (*n*=230)	5 (2.2)	18 (7,8)
Without (*n*=30)	5 (16,7)	6 (20,0)
Total	**10/260 (3,8%)**	**24/260 (9,3%)**

n – dogs examined; % - prevalence obtained

Diff-Quik method is sensitive and specific, gamonts (fig. 1) are observed based on the morphological appearance in leukocytes in mammals, and the disadvantage of this method is that only a small percentage of infection can be detected by this method.

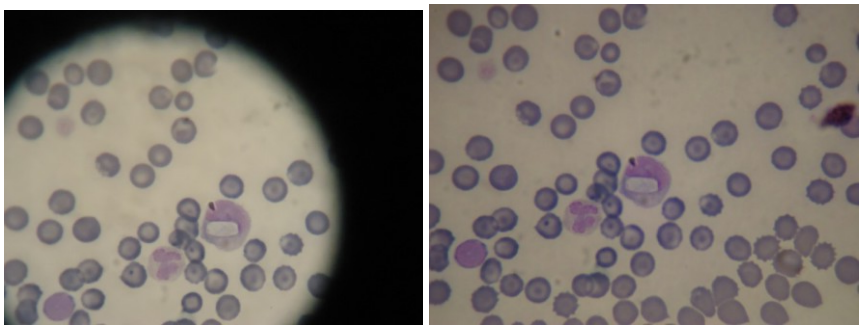

Fig. 1. *Hepatozoon* spp. (original)

Molecular biology analysis of 260 blood samples from dogs originating from 11 counties of western and south-western Romania revealed the presence of *Hepatozoon* spp etiologic agents. Molecular screening of the 260 blood samples from dogs in the western and south-western Romania, registered a prevalence of 9.3% (24/260) for canine hepatozoonosis. Thus, based on amplification of the 18S rDNA sequence of the gene *Hepatozoon canis* species was identified.The products of migration in 1.5% agarose gel of the PCR revealed consistent thickness and apparent bright strips at about 666 bp (Fig. 2)

Sequencing was done successfully for all the selected samples (n = 9), and confirmed the results of the conventional PCR. Genetic sequences of 18S rRNA isolated from *H. canis* in dogs, were identical to each other, and indicated the presence of a single genotype in our country. The presence of this parasite in dogs is not surprising, given that tick *R. sanguineus* is wide distributed in the studied area (Imre et al., 2012, 2015).

During the sampling medical history and data about every animal we obtained and also additional data on the origin (232 dogs from urban areas and 28 dogs from rural areas), breed (50 mongrels and 210 pure-bred), age (≤2 years 70 dogs, 146 dogs from 2 to ≤ 6; 44 dogs aged over 6 years), gender (149 females and 111 males) (Table 1).

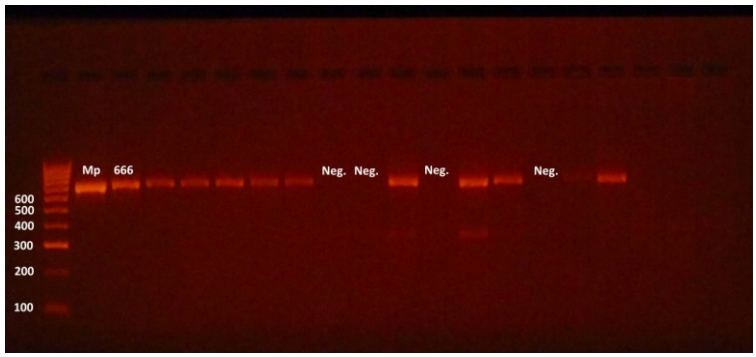

Fig. 2. Migration of PCR products in molecular diagnosis of *Hepatozoon canis* (Mp-positive control; Neg. – negative samples, 666 – positive samples) (original)

It was observed that the prevalence increased with age and were affected both females and males (9.4% - and 9.0 F - M), both pure breed dogs (7.6%) and cross breed (16%). The infestation was present in dogs in urban areas (7.9%) and those in rural areas (12.9%) in those with owners (7.8%) and without owner (20%). No statistical differences were observed between prevalence reported in age, gender, race, habitat and provenance.

The prevalence of hepatozoonosis has different values in the world. In the United States, Grenada, Yabsley et al., 2008, have identified a prevalence of 7%, by PCR in the dogs included in the study. In the south-eastern USA, LI et al. (20) examined blood samples of 614 dogs using the same technique and have found a prevalence of 27.2% for *H. americanum*, 2.3% *H. canis* and a value of 2.3% for mixed infections (*H. americanum* + *H. canis*). In the southern US, Allen et al., 2008, identified a prevalence of *H. americanum* of 1.83% and 98.8% for *H. canis*.

By examining blood smears, Mundim et al., 2008, established in dogs of Minas Gerais, Brazil, a seroprevalence of 77.39% for *H. canis*, while O'DWYER et al., 2001, by the same technique, reported a 39.2% seroprevalence in dogs in Rio de Janeiro, Brazil. In 2008, Metzer et al., 2008, examined samples from wild cats from different zoos by PCR and identified a prevalence of 17.24% to 3.44% *H. canis* and *H. americanum*. Also in Brazil, Lima de Miranda et al., 2011, found in a tick (*Rhipicephalus microplus*) from a dog *H. canis* oocysts.

Mylonakis et al., 2005, tested by ELISA serological method, 69 samples from dogs in Greece and obtained 65.2% seropositivity for *H. canis*.

On the Aegean coast of Turkey, Karagenc et al. 2006, studied samples from 349 dogs and obtained a prevalence of 10.6% through blood smears method, 36.8% by indirect immunofluorescence and 25.8% by PCR (for *H. canis*).

In France, Criado-Fomelio et al., 2009, examined by PCR serological samples to identify hepatozoonosis in dogs and cats. The authors identified a prevalence of 0.9% and 1.7% for *H. canis*.

In Croatia, Vojta et al. 2009, have obtained a prevalence of 11.6% in dogs.

CONCLUSIONS

Molecular surveillance of blood samples from dogs in the western and south-western Romania, showed a 9.3% prevalence of canine hepatozoonosis.

Following amplification of 18S rDNA gene sequence *Hepatozoon canis* species was identified. The results demonstrate the expansion of this disease transmitted by vectors in non-endemic regions and is first screening in the canine population in Romania.

ACKNOWLEDGEMENTS

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REFFERENCES

1. ALLEN, K. E., LI, Y., KALTENBOECK, B., JOHNSON, E. M., REICHARD, M. V., PANCIERA, R. J., LITTLE, S. E. (2008) - *Diversity of Hepatozoon species in naturally infected dogs in the southern United States*. Vet. Parasitol., 154: 220–225.
2. BANETH G. (2004) - ***Canine hepatozoonosis-Two different diseases.*** *29th World Small Animal Veterinary Association World Congress Proceedings; Rhodes, Greece* 2004.
3. BANETH G., (2006) - CHAPTER 74 Hepatozoonosis in GREENE CE. Infectious Diseases of the Dog and Cat, 3rd ed.Elsevier, St. Louis, Missouri, USA, 1387 pp. ISBN 1-4160-3600-8,
4. BANETH, G. (2001) - *Hepatozoonosis, canine. In Encyclopedia of Arthropod-transmitted Infections of Man and Domesticated Animals*, Ed M.W. Service. Oxon, CAB International. pp 215-220.
5. BANETH, G. (2011) - *Perspectives on canine and feline hepatozoonosis.* Vet. Parasitol., 181: 3-11.
6. CRIADO-FORNELIO, A., BULING, A., PINGRET, J.L., ETIEVANT, M., BOUCRAUT-BARALON, C., ALONGI, A., AGNONE, A., TORINA, A. (2009) - *Hemoprotozoa of domestic animals in France: Prevalence and molecular characterization*. Vet. Parasitol., 159: 73-76.
7. IMRE MIRELA, DUDU ANDREEA, ILIE M S., MORARIU S, IMRE K, AND DĂRĂBUŞ GH. (2015) - *Molecular Survey of Hepatozoon canis in Red Foxes (Vulpes vulpes) from Romania*, Journal of Parasitology 101(4):490-491.
8. IMRE, M., ILIE M. S., MIHALI C. V., OPRESCU I., MORARIU S., HOTEA I., IMRE K., ILIE A., PALCA M., DĂRĂBUŞ GH.. (2012) - *Prevalence of tick species in dogs using classical methods and SEM.* Lucrări. Ştiinţifice Medicină Veterinară 45: 131-136.
9. INOKUMA, H., OKUDA, M., OHNO, K., SHIMODA, K., ONISHI, T., (2002) - *Analysis of the 18S rRNA gene sequence of a Hepatozoon detected in two Japanese dogs*. Vet. Parasitol. 106, 265–271.
10. IVANOV A., TSACHEV I. (2008) - *Hepatozoon canis and hepatozoonosis in the dog*, Trakia Journal of Sciences, 6, 2, 27-35.
11. KARAGENC, T. I., PASA, S., KIRLI, G., HOSGOR, M., BILGIC, H. B., OZON, Y. H., ATASOY, A., EREN, H. (2006) - *A parasitological, molecular and serological survey of Hepatozoon canis infection in dogs around the Aegean coast of Turkey*. Vet. Parasitol., 135:113–119.
12. LIMA DE MIRANDA, R., RIBEIRO DE CASTRO, J., MARTINS OLEGÁRIO, M. M., BELETTI, M. E., MUNDIM, A. V., O'DWYER, LUCIA HELENA, EYAL, O., TALMI-FRANK, D., CURY, MÁRCIA CRISTINA, BANETH, G. (2011) - *Oocysts of Hepatozoon canis in Rhipicephalus (Boophilus) microplus collected from a naturally infected dog*. Vet. Parasitol., 177:392-396.
13. MACINTIRE D. K., VINCENT-JOHNSON NANCY A. CHAPTER 74 Hepatozoonosis in GREENE CE. Infectious Diseases of the Dog and Cat, 3rd ed.Elsevier, St. Louis, Missouri, USA, 2006, 1387 pp. ISBN 1-4160-3600-8,
14. METZGER, BETINA, DOS SANTOS PADUAN, KARINA, RUBINI, A. S., GOMES DE OLIVEIRA, T., PEREIRA, C., O'DWYER, LUCIA HELENA (2008) - *The first report of Hepatozoon spp. (Apicomplexa: Hepatozoidae) in neotropical felids from Brazil.* Vet. Parasitol., 152: 28–33.
15. MUNDIM, A. V., APARECIDA DE MORAIS, I., TAVARES, M., CURY, M. C., MUNDIM, M. J. S. (2008) - *Clinical and hematological signs associated with dogs naturally infected by Hepatozoon spp. and with other hematozoa: A retrospective study in Uberlandia, Minas Gerais, Brazil*. Vet. Parasitol., 153: 3–8.
16. MYLONAKIS, M. E., LEONTIDES, L., GONEN, L., BILLINIS, C., KOUTINAS, A. F., BANETH, G. (2005) - *Anti-Hepatozoon canis serum antibodies and gamonts in naturally-occurring canine monocytic ehrlichiosis.* Vet. Parasitol., 129: 229–233.
17. O'DWYER, LUCIA HELENA, MASSARD, C. L., PEREIRA DE SOUZA, J. C. (2001) - *Hepatozoon canis infection associated with dog ticks of rural areas of Rio de Janeiro State, Brazil.* Vet. Parasitol., 94: 143-150.

18. VINCENT-JOHNSON NANCY (2014) - *Canine and Feline Hepatozoonosis*, Chapter 77, in SYKES JANE E., (2014), *Canine and Feline Infectious Diseases,* Pages 747–759.
19. VOJTA, L., MRLJAK, V., ĆURKOVIĆ, S., ŽIVIČNJAK, T., MARINCULIĆ, A., BECK, R. (2009) - *Molecular epizootiology of canine hepatozoonosis in Croatia.* Int. J. for Parasitol., 39: 1129-1136.
20. YABSLEY, M. J., MCKIBBEN, J., MACPHERSON, C. N., CATTAN, P. F., CHERRY, N. A., HEGARTY, B. C., BREITSCHWERDT, E. B., O'CONNOR, T., CHANDRASHEKAR, R., PATERSON, T., PEREA, M. L., BALL, G., FRIESEN, S., GOEDDE, J., HENDERSON, B., SYLVESTER, W. (2008) - *Prevalence of Ehrlichia canis, Anaplasma platys, Babesia canis vogeli, Hepatozoon canis, Bartonella vinsonii berkhoffii, and Rickettsia spp. in dogs from Grenada.* Vet. Parasitol., 151: 279–285.

MALARIA- THE RISK OF THE RE-EMERGENCE IN ROMANIA

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Abstract:*At present, there is an emergence and re-emergence process of some infectious diseases with vector transmission in the entire world, since they install on very wide territories as a result of adapting pathogenic agents, becoming an integral part of local ecosystems. At the moment of the installation of these diseases on certain territories, they become very difficult to eradicate. The recrudescence of vector diseases (like malaria) is mainly influenced by the changes of climatic factors. Every year, thousands of sick people or carriers of malaria travel to countries free of malaria, reintroducing the risk of the re-emergence of this disease. Based on the information received from the Institute of Tropical Diseases "Victor Babes", we could have an evidence of the cases of malaria diagnosed in Romania in the last 3 years, with detailed information for this years.*

*For diagnosis cases of malaria has been used: rapid diagnostic tests***,** the *microscopic examination of blood films, PCR RT. Correlating the three factors implicated in the transmission of malaria: favourable environmental conditions, presence of the vector and of the human reservoir (with gametocytes), we could highlight the growing risk of the re-emergence of malaria in Romania. The development of tourism between continents, and the annual immigration for work to European countries of thousands of sick people or carriers of Plasmodium coming from malaria endemic areas increase the risk of the re-emergence of this disease in Europe. Therefore, the realisation of programmes of continuous monitoring of the vector populations and of the evolution of climatic factors becomes primordial in Europe, and especially in Romania.*

Keywords: *malaria, re-emergence, gametocytes*

INTRODUCTION

The role of insects as vectors in human and animal pathology was incriminated for hundreds of years, however, scientific knowledge has been registered starting with two great discoveries belonging to Manson and Ross (1897), who established the role of the mosquitoes in the transmission of malaria and filariasis.

The climatic modifications lead to a global warming, favouring the risk of the appearance and development of diseases considered until now tropical diseases (Patz J.A. et al. 2006). Another important factor is the workers' immigration, the economic crisis favouring the passive transmission of new species of culicids from different areas, especially from Italy.

At present, there is an emergence and re-emergence process of some infectious diseases with vector transmission in the entire world, since they install on very wide territories as a result of adapting pathogenic agents, becoming an integral part of local ecosystems. At the moment of the installation of these diseases on certain territories, they become very difficult to eradicate. The recrudescence of vector diseases (like malaria) is mainly influenced by the changes of climatic factors (Bouma M. și colab., 1994; Martens P. și colab., 1999).

Malaria is the disease with the widest distribution on the globe. Millions of people are infected every year in Africa, India, South-East Asia, Middle East, Central and South

America, with more than 41% of the global population under the risk of infestation with malaria (Béguin A., et al. 2011).

Every year, thousands of sick people or carriers of malaria travel to countries free of malaria, reintroducing the risk of the re-emergence of this disease. At present, malaria produces twice more deaths than SIDA, constituting a considerable brake on the socio-economic development of many countries. The necessity of the mapping of the global distribution of malaria is higher, due to its continuous change, and the expenses implicated by its control could produce a huge economic unbalance (Lucas & McMichael, 2005). The establishment of the potential risk to which the population is exposed is important for the allocation of the resources necessary, and also for the evaluation of the global economic impact. There is a mapping of the world distribution of malaria, made by Snow *et al.* in 2002, year when 515 million of clinical episodes of *P. falciparum* were registered. These global estimations are 50% higher than the ones reported by the World Health Organisation (WHO), and 200% higher for the areas outside Africa. The health reports are considered rigorous only for 7 of the 87 endemic countries, and they can be used for their information. Consequently, the need to establish the risk of the re-emergence of malaria in Romania becomes crucial, taking into account the economic deadlock of our country. Malaria is seldom diagnosed in Europe, but it is a medical emergency. In Europe, malaria was eradicated, with the exception of Azerbaijan, Georgia, Kyrgyzstan, Tajikistan and Turkey **(**Helena H. Askling și colab., 2012).

The condition for malaria to become re-emergent in Romania is the presence of the vector belonging to the complex *Anopheles maculipennis* and to the parasite *Plasmodium sp*., in favourable environmental conditions.

MATERIAL AND METHOD

In order to establish methods of supervision and control of malaria in Romania, we considered the last cases of malaria recorded in 2012-2014, to be able to compare the existing system deficiencies in the years 1963-2011 with the present ones. The data were provided by ″Victor Babes″ Institute of Infectious and Tropical Diseases.

We mainly followed the period of the year when the case was diagnosed, the diagnostic method, and the phase of the parasite evolution cycle at the moment of the diagnostic. We also considered the diagnosed *Plasmodium* strain. For the diagnostic we used quick tests, examination with the microscope of slides with thick smear, and we also used techniques of molecular biology, PCR, in the cases where a false negative answer was suspected.

RESULTS

In 2012,17 cases of malaria were diagnosed in Romania, all imported, from which 16 were recorded in persons who wentto malaria-endemic areas in search of work, continuing the course started in 2007, when the occupation of sailor was taken down by workers in very diverse fields (waiter, foreman, or builder). Only one case was female, and all the cases came from Africa.

Of the 17 cases recorded, 2 were diagnosed in the gametogonic phase, which is the period of maximum infectiousness, because now *Anopheles* vectors take *Plasmodium* parasite

and transport it to another host in 9-21 days, according to the*Plasmodium* strain. Also, the cases were diagnosed in April and September, respectively, periods of intense activity of *Anopheles* vector.

Fig. 1. Malaria cases diagnosed in Romania in 2012

One case was positive for *Plasmodium vivax.*The case needs a close observation in the following years, because a relapse might appear at anytime, and it might constitute a source of infection for others.

All the cases recorded were in South-East Romania, areas where the presence of *Anopheles* vectors was signalled, and where the temperatures are favourable to the development of both the vector and the malaria parasite inside its body.

In 2013,31 cases of malaria were recorded, all imported, coming from Africa. The age of the infected persons ranged between 19 and 57, most of them traveling to malaria-endemic areas in search of work, which proves once again that the area for possible work places spread, and the highest concern in what concerns the risk of malariare-emergence in Romania should be focused on the working class, who needs to be educated regarding the tropical diseases.

Fig. 2. Malaria cases diagnosed in Romania in 2013

The cases recorded in 2013 were from all the areas of the country. 12 cases were diagnosed with gametogonic forms, which proves the higher risk of a malaria outbreak, taking into account the existence of the vector, and the favourable climatic factors.

There were 7 cases of infestation with *Plasmodium ovale*, 4 cases with *Plasmodium vivax,* and 2 cases with *Plasmodium malariae*. These cases hould be kept under observation, and the smears should be repeated periodically, or at each fever spurt.

In 2014, 39 cases of malaria were reported, all imported, coming from the African continent area. The age ranged between 20 and 68, most of these people traveling to malaria-endemic areas in search of work.

The cases of malaria were reported in greater number in the South-East area of the country, being related to the poorer economic state of these areas, the population being forced to look for work places in areas which had not presented any interest before. Two cases were represented by tourists, one Greek, and one Israeli, who had visited regions of the African continent before arriving to Romania.

Fig. 3. Malaria cases diagnosed in Romania in 2014

Three cases of infection with *Plasmodium ovale* were recorded, onecase with *Plasmodium vivax,* and one with *Plasmodium malariae*, which could relapse at any time, and it is compulsory to keep them under close observation, testing smears at every fever spurt. Of the cases diagnosed, 6 were in gametogonic phase, representing a high risk for malaria outbreak.

In 2014, an increase of the cases of imported malaria was recorded, most of them traveling to malaria-endemic areas in search of work, proving the continuation of the economic crisis, and people's need to find an income source in farther areas.

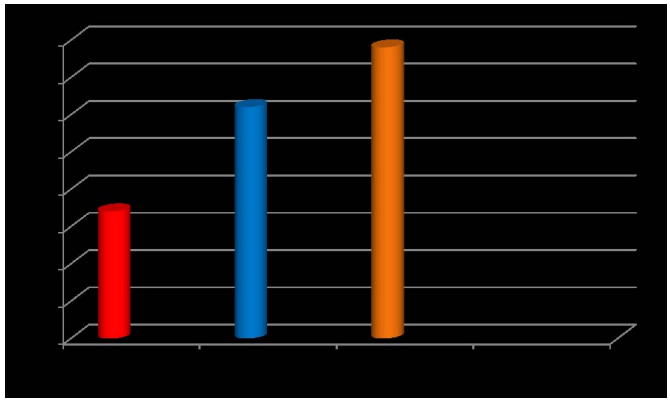

Fig.4. Evolution of malaria cases diagnosed in Romania in the three years of study

DISCUSSIONS

We can see an increase of the cases of malaria from one year to the other, which proves again the lack of information of the population traveling to malaria-endemic areas regarding the methods of prevention and protection against the infestation with *Plasmodium*.

According to the studies we have performed up to present, both climatic factors and the presence of the vector in nature are favourable for the re-emergence of malaria in Romania. The diagnostic of the cases in gametogonic phase in the warm months of the year proves the high risk of an outbreak.

Consequently, it is necessary to take a series of measures, which become a priority in the fight against malaria, and in the maintenance of the status of malaria-unaffected areas, Romania included:

- investments in the preparation of the medical personnel, and specialisation in tropical diseases;
- training medical entomologists, who are completely missing;
- creation of a database to record at a national level all the cases of malaria registered on Romanian territory;
- following-up every case of malaria recorded, with re-examination, especially of the cases diagnosed with *Plasmodium* strains forming hypnozoites in the liver, with risk of relapse;
- equipng the hospitals with diverse drugs specific for the treatment of malaria, according to the areaof infestation;
- communication between tourism companies and hospitals in order to inform the population regarding the protection measures when traveling to a tropical area.

The maintenance of the status of country un affected by malaria becomes both for Romania and for other European countries a mission increasingly serious and difficult.

Therefore, we can see a yearly increase of the cases of imported malaria, proving people's lack of information concerning the risk of contracting the disease, and the measures of preventionand treatment.

The climatic factors play an important role,and our studies show their favourable evolution in the existence of the risk of emergence and re-emergence of diseases on Romanian territory.

BIBLIOGRAFIE

1. Askling Helena, Bruneel F., Burchard G., Castelli F., Chiodini P.L., Grobusch M., Lopez-Vélez R., Paul Margaret, Petersen E., Popescu C., Ramharter M., Schlagenhauf Patricia, 2012- Management of imported malaria in Europe, European Centre for Disease Prevention and Control.
2. Alonso D., Bouma M. J., Pascual M., 2011- Epidemic malaria and warmer temperatures in recent decades in an East African highland. *Proceedings of the Royal Society of London, B*. 278:1661–1669.
3. Bouma M., Sondorp H., Kaay H., 1994- Climate change and periodic epidemic malaria. The Lancet; 343: 1440.
4. Béguin A., Hales Simon, Rocklöv J., Aström C., Louis V.R., Rainer Sauerborn, 2011-"The opposing effects of climate change and socio-economic development on the global distribution of malaria". Global Environmental Change 21,1209–1214.
5. Bradley D.J., 1992- "Malaria: Old Infections, Changing Epidemiology". Health Transition Review. Vol. 02, Supplementary Issue.
6. Lucas R., McMichael A. J., 2005- Association or causation: evaluating links between environment and disease. Bulletin of the World Health Organization, 83(10), 792–795.

7. Martens P., Kovats R., Nijhof S., de Vries P., Livermore M., Bradley D., McMichael A., 1999- "Climate change and future populations at risk of malaria". Global Environ Change; 9:87–99.
8. Muturi E., Burgess P., Novak R., 2008- Malaria vector management: where have we come from and where are we headed? Am Journal Trop Med Hyg 78, 536-537.
9. Patz J.A., Olson Sarah H., 2006- "Malaria risk and temperature: Influences from global climate change and local land use practices". National Academy of Sciences of the United States of America PNAS, vol. 103 no. 15.
10. Snow R.W., Guerra, C.A., Noor A.M., Myint H.Y., Hay S.I., 2005- "The global distribution of clinical episodes of *Plasmodium falciparum* malaria". Nature 434, 214–217.
11. Snow R. W., Marsh K., 2002- T"he consequences of reducing transmission of *Plasmodium falciparum* in Africa". Adv Parasitol; 52:235-64.
12. WHO, 2006- Pesticides and their application for the control of vectors and pests of public health importance, Sixth Edition.
13. World Health Organization, World Malaria Day: save lives though local action 2011. Available at: http://www.who.int/malaria/world_malaria_report_2011/en/.
14. World Health Organization: Severe *falciparum* malaria, Trans Roy Soc Trop Med Hyg 94 (2000) 1–90.

CT-ANGIOGRAPHY OF THE HIND LIMBS IN CAT - ARTERIAL TIME

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Abstract:*Computed tomography is currently the most widely used method of nondestructive and accurate diagnosis in the world that can give a quick response, and could help the specialist intervene in a very short time. In human medicine C.T. examination is a routine, being very popular, was quickly adopted in veterinary medicine also.*

The biological material was represented by a European breed cat of 3 kg. and 4 years old. The patient underwent a clinical examination which checked physiological constants: temperature, pulse and respiration. The contrast used was Visipaque 320. The patient was sedated during the procedure and three exposures were made: sagittal exposure, transverse exposure, dorsal exposure and finally a 3D reconstruction of the vessels hindquarters was performed. The method is particularly useful for identifying of lesions, tumors and metastases, and also to assist in the diagnosis, a tissue-slice CT reveals the presence, size, and spatial location of different formation of modifications of vessels.

Keywords: *CT examination, Visipaque 320, blood vessels, cat*

BACKGROUND

Computed tomography scan, also known as the "computerized axial tomography" (CAT), uses a computer to produce detailed section images of the human body. This way doctors can examine in turn the thin "slices" of the body to identify specific areas of interest. (Oliveira and Ranallo, 2011).

Computer Tomography Scanning of internal organs (liver, pancreas, heart, lung, kidney, brain), bones (spine, bones, hand, legs, pelvic bones), soft tissue (muscles, joints), and blood vessels, provides greater clarity in comparison with ultrasound scans and reveal details on which radiologists can diagnose existing problems at these levels, including different types of cancer.

CT operates on X radiation. When X-rays go through the body, they are absorbed differently, thereby creating a matrix or profile of X-rays with different intensities. This profile is recorded on radiological film, producing an image. If tomographic images, the film is replaced by a banana-shaped detector that measures the radiation profile X. CT Scanner method of investigation has a highly accurate imaging, non-traumatic and very fast, which uses X-rays to obtain detailed images, high resolution and highly precise to highlight the internal organs.With continues advance of spiral CT acquisition the comprehensive information provided by CT can be used to diagnose blood vessels affection through tomographic angiography. Computed tomography (CT) is the recommended first line in investigating strokes. (Coulon and Lewis, 2008; Thrall and Robertson, 2011; Schwarz et al, 2011,)

Angiography is a minimally invasive intervention, non-surgical, painless, which is performed using X-rays and allows the visualization of internal aspects of the blood vessels

of the limbs, internal organs or heart. Angiography uses one of three imaging technologies: re X-rays, computed tomography (CT or CAT) or magnetic resonance imaging (MRI), and require management with contrast agent produce images of major blood vessels throughout the body (Vrzgulova, 1998).

CAT angiography is the administration of a contrast agent into the patient's blood vessel by means of an automatic injector and visualization of blood vessel through computer tomography, which can show some abnormalities such as aneurysms, tumors, abnormal blood vessel occlusion (Shuman et al., 1986).

MATERIAL AND METHODS

The biological material was represented by a 3 kg European breed cat of age 4. The patient underwent a clinical examination: temperature 38.2 ° C, pulse 160, respiration-24, the physiological constant being within normal parameters, the animal is clinically healthy. Forelimb antisepsis was realized by trimming, applying betadine, and a 20G cannula was applied on the cephalic vein for intravenous contrast substances administration.

Sedation was performed using: Xylazine (Bayer) 2% 4 mg / animal i.m., Midazolam (Bayer) 5% i.v., Propofol (Pfizer) 5 mg / animal by intravenous injection if needed. After onset of anesthesia the cat was put on the table in ventro-dorsal recumbency with limbs outstretched and head supported by a cushion. Automatic injector was used for administering the contrast substance intravenously to the patient (fig. 1).

Fig. 1 Automatic injection and CT preparation

The contrast agent used was Visipaque 320 that contains Iodixanol which is an non-ionic X-ray contrast agent, dimeric hexaiodinated and water-soluble. Visipaque was administered by intravenous injection. Vials containing a clear aqueous solution, colorless to pale yellow. Following the injection, iodine bound to organic compounds absorbs radiation from blood vessels / tissues.

RESULT AND DISCUSSION

Before C. T. scanning, radiological investigations are conducted and used to plan the location of CT scan. Manufacturers have different names for this examination (topogram, IP), and can be obtained by performing a dorso-ventral, ventro-dorsal, left or right side scanning. Almost all applications required 2 topogram and are used for planing. At the end of the installation of the patient table must be set to zero on most scanners. With topogram will establish the working sector and source-detector assembly will tilt at an angle that will direct vertebral space (fig. 2).

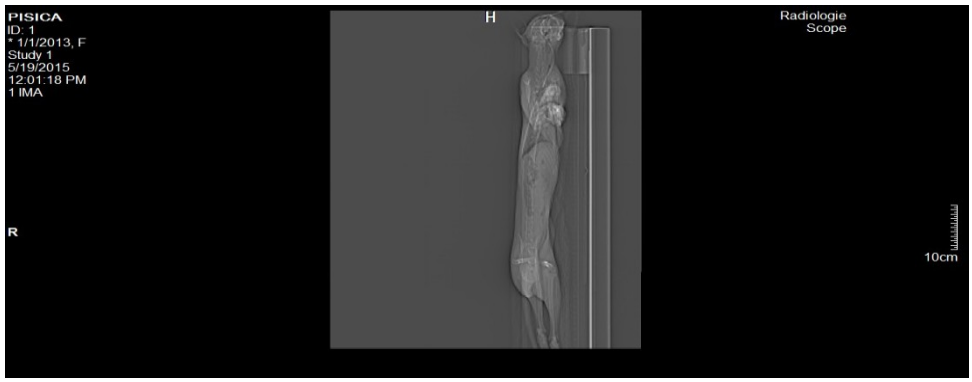

Fig. 2. Topogram in cats, KW: 114 mAs: 173

The patient undergo 3 exposures: sagittal, transverse, and dorsal and 3D reconstruction was performed at the end of the hindlimbs examination. Contrast agent was administered with the help of automatic injector with a flow of 2.0 ml / sec for a period of 17 seconds at a pressure of 120 psi (fig. 3).

Fig. 3 Automatic injection parameters

Sagittal exposure

For sagittal exposure the parameters were kV 110, mAs 35 and the brightness of W:1500 and C: 500 and were highlighted: the heart, thoracic aorta, abdominal aorta, median sacral artery, external iliac artery, femoral artery branch, femoral artery, popliteal and internal pudendal artery (fig.4, fig. 5, fig. 6).

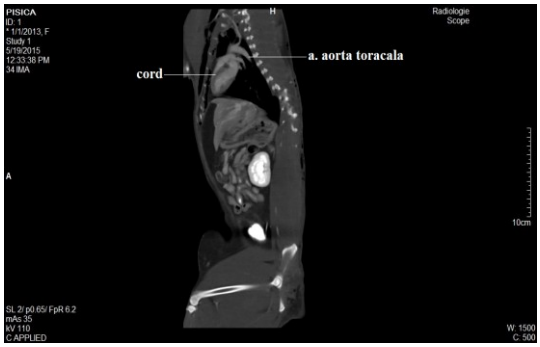

Fig. 4 Sagittal Exposure: The heart and thoracic aorta, exposure parameters: kW- 110 mAs- 35 and brightness of W: 1500 and C: 500

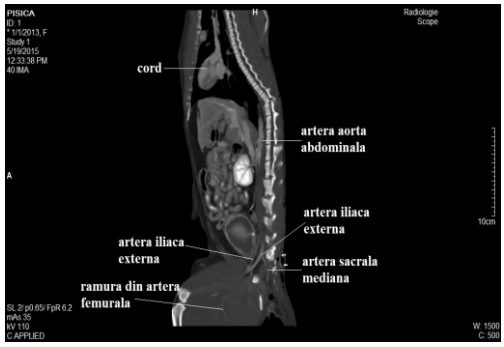

Fig. 5 Sagittal Exposure: heart, abdominal aorta, median sacral artery, external iliac branch of the femoral artery, exposure parameters: kW- 110 mAs- 35 and brightness W: 1500 and C: 500

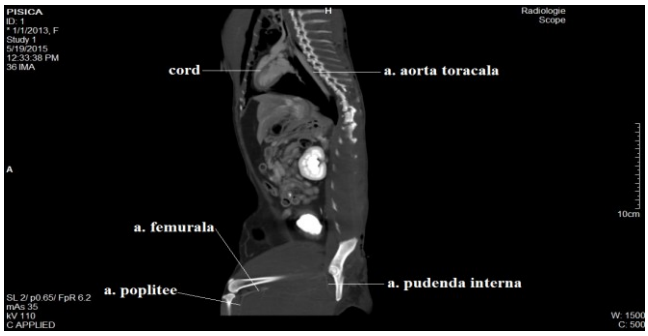

Fig. 6 Sagittal Exposure: heart, thoracic aorta, femoral artery, popliteal artery, internal pudendal artery, exposure parameters: kW- 110, mAs- 35 and brightness of W: 1500 and C: 500

Transversal exposure

In the transversal exposure the used parameters were 110 kV, 16 mAs and the brightness of W: 350 and C: 50 and were highlighted: the heart, aorta. At the parameters of 110 kV and 14 mAs were highlighted: external and internal iliac artery, median sacral artery and femoral artery (fig. 7, fig. 8, fig. 9).

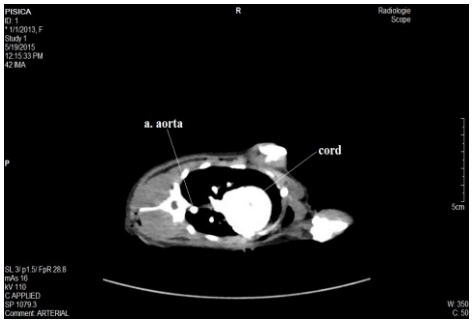

Fig. 7 Transversal exposure: Heart and aorta, parameters: 110 kv, 16 mAs and the brightness of W: 350, C: 50

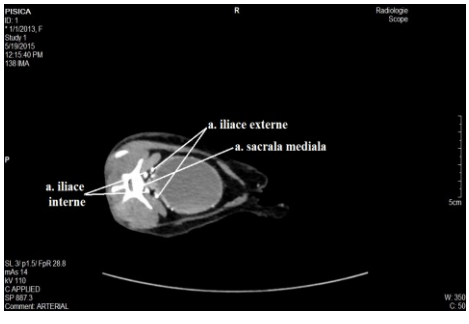

Fig. 8 Transversal exposure: external iliac artery, internal iliac artery, median sacral artery, parameters 110 kV, 14 mAs and the brightness of W: 350, C: 50

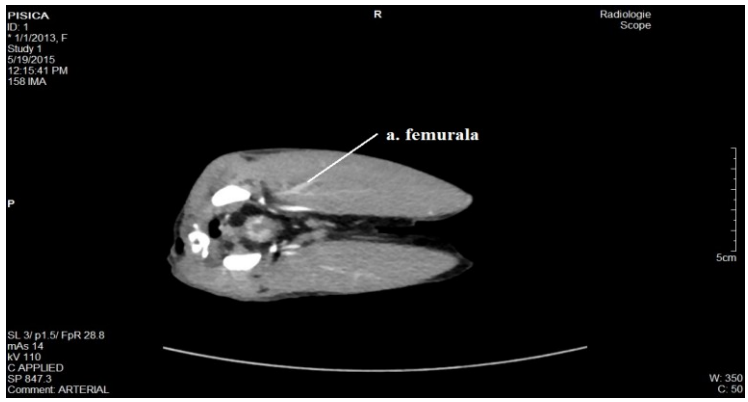

Fig. 9 Transversal exposure: femoral artery, parameters 110 kV, 14 mAs and the brightness of W: 350, C: 50

Dorsal exposure

For dorsal exposure were used 110 kV, 14 mAs and brightness W: 700, C: 80 and were highlighted: heart, aorta, iliac artery (fig. 10, fig. 11, fig. 12).

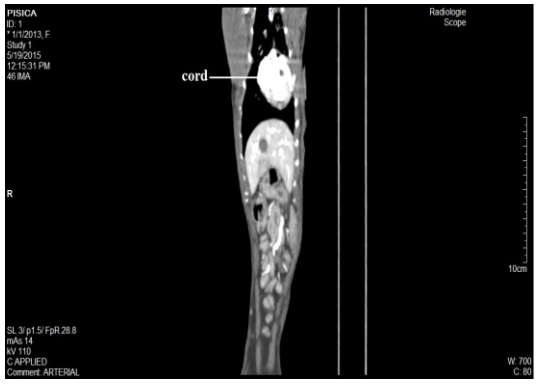

Fig. 10 Dorsal exposure: heart, parameters 110 kV, 14 mAs and brightness W: 700, C: 80

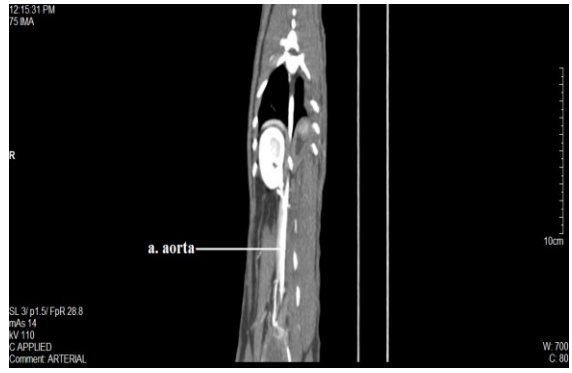

Fig. 11 Dorsal exposure: aorta, parameters 110 kV, 14 mAs and brightness W: 700, C: 80

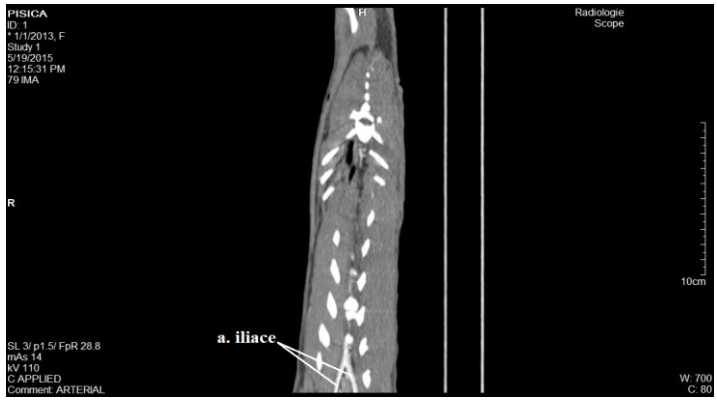

Fig. 12 Dorsal exposure: iliac artery, parameters 110 kV, 14 mAs and brightness W: 700, C: 80

At the end of patient examination was performed a 3D reconstruction of the hindquarters. 3D reconstruction lasted about 30-35 minutes and bones and arteries were highlighted: the aorta, internal iliac, external iliac, femoral saphenous, the tibial cranial artery, caudal tibial artery, femoral artery, popliteal artery, (fig.13).

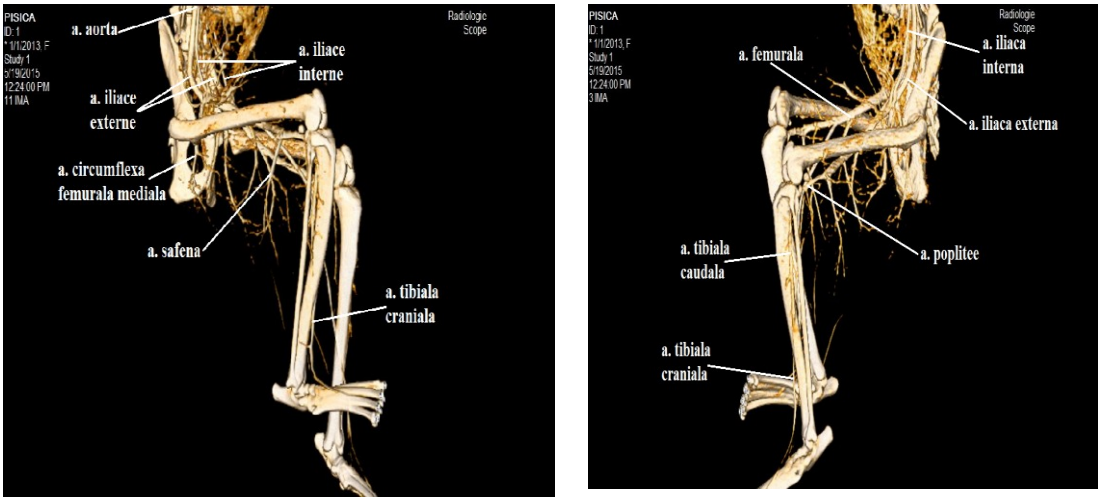

Fig 13 3D reconstruction of the hind limbs vascularization in cat

CONCLUSION

There is a crucial difference between CT scanning and radiography. It is that tomography imaging allows direct purchase and differentiation of soft tissue structures. The method is useful for identifying lesions, tumors and metastases, and also to assist examination of the blood vessels, CT scan revealed the presence, size, and spatial location of a lesion.

Times of the contrast agent administration to make a CT examination of the limbs in cat was calculated between 15 and 20 seconds. The disadvantages of CT scan are the high cost of an examination, and increasing the dose of radiation due to fine sections with high resolution. Computed tomography angiography should be avoided in patients with allergic reaction to contrast agents, in patients with advanced renal disease or severe diabetes.

References

1. Oliveira CR, Ranallo F, 2011, A device for computed tomographicimaging of the thorax of awake cats, USA.
2. Schwarz T, Saunders J & al, 2011, Veterinary computed tomography, Ed Wiley-Blackwell, Oxford
3. Vrzgulova M., 1998**,** Angiology (the cardiovascular and lymphatic systems), Ed. M&Mvydavatel'stvo, Presov
4. Coulon A and Lewis N, 2008, An Atlas of Interpretative Radiographic Anatomy Of the Dog and Cat 2nd Editiom, Ed Wiley-Blackwell, Oxford
5. Thrall DE. and Robertson ID**.,** 2011**,** Atlas of normal radiographic anatomy and anatomic variants in the dog and cat, Ed.Elsevier Inc.
6. Shuman WP , Adam JL , Schoenecker , Tazioli PR and Moss AA**,** 1986, Use of a power injector during dynamic computed tomography

SEVERE PYODERMA IN A CASE OF ADVANCED CANINE HYPOTHYROIDISM

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Abstract:*A nine year old male entire Cocker Spaniel was presented at the Veterinary Teaching Hospital of Iasi, for microbiological investigations regarding severe recurrent pyodermatitis and bilateral external otitis. Dermal examination indicated dry and brittle hair, rat tail, hyperpigmentation and hyperkeratosis on axial and inguinal area, seborrhoea oleosa, myxoedema and tragic facial expression. Clinical and physical examination also revealed a low heart rate of 70 bpm, lethargy, obesity (BSC: 7 of 9) and exercise and cold intolerance. Microbiologic exams revealed staphylococcal pyodermatitis and bacterial otitis with associated flora: Proteus spp, Pseudomonas spp and Staphylococcus pseudintermedius. Also, considering the physical appearance of the dog, specific investigations for hypothyroidism have been recommended. Antibiotic susceptibility tests indicated the following antibiotics suited for all isolated bacterial species: Penicillin, Marbofloxacin and Neomycin. Serum biochemistry confirmed the suspicion of hypothyroidism, revealing a very low level of FT4 0.3 pmol/L (10-45 pmol/L), TT4 8.88 nmol/L (15-50 nmol/L), increased alkaline phosphatasis 208 U/L (10-101 U/L) and mild hypercholesterolemia of 269 mg/dl (116-254 mg/dl). The treatment for the pyodermatitis consisted of Marbofloxacin injectable 2 mg/kg sc/24 h, for five days, continued with oral administration for seven days, and for the otitis, topical solution 3 mg/ml, 10 drops, per ear, once daily. Also, daily baths with 3.5% clorhexidine gluconate shampoo or mousse were prescribed. Hypothyroidism therapeutic strategy consisted of levothyroxine 10µg/kg/12h. The dog was rescheduled for dermal revaluation and FT4 and TT4 monitoring within two weeks of starting treatment. The current paper presents a case of severe recurrent pyodermatitis, which failed to resolve on all previous correctly applied treatments, as it was secondary to an undiagnosed advanced state of hypothyroidism.*

Keywords: *bacterial otitis, canine hypothyroidism, staphylococcal pyodermatitis, Staphylococcus pseudintermedius.*

INTRODUCTION

Canine adult onset hypothyroidism is one of the most common endocrine diseases affecting middle aged and old dogs. The disease is defined as a decreased production of thyroxine (T4) and triiodthyronine (T3), as a result of any disruption in the hypothalamus-hypophysis-thyroid axis. The true prevalence of canine hypothyroidism worldwide is not yet known, as there are many controversies regarding the reliability of diagnosis techniques and hormonal reference range. Still, it is thought that 0.2 to 0.6% of the canine population might be affected by this endocrine disease. Hypothyroidism can affect both old dogs and young puppies, and can have a primary or central cause. Primary hypothyroidism in middle aged to old dogs is the most frequent form of hypothyroidism and appears secondary to lymphocytic thyroiditis or idiopathic thyroid atrophy. Other primary but rare causes, include neoplastic destruction of the thyroid lobes, antithyroid medication and congenital defects. Central hypothyroidism is rarely diagnosed in dogs and is caused by pituitary or hypothalamus

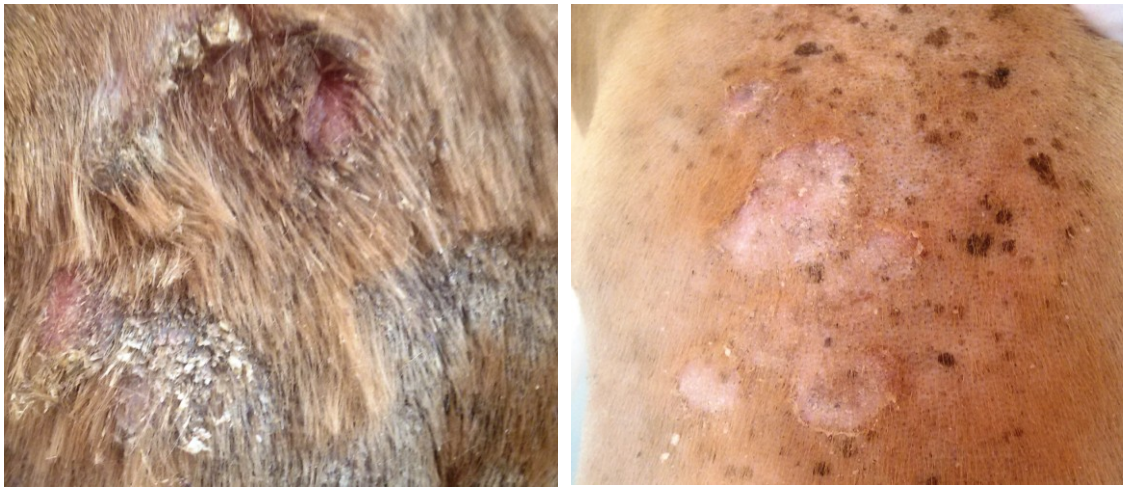
diseases. Alteration of thyroid hormones level affect almost all functions of the body, leading to a wide range of metabolic, dermatologic, neurologic, cardiovascular and reproductive sings. In up to 80% of cases, clinical sings include metabolic abnormalities and dermatological changes. Thyroid hormones are very important for the dermal health, thus observed features of hypothyroid dogs usually include dry, thin, brittle hair, scaling and scurfing, rat tail, skin hyperpigmentation in depilated areas, seborrhea sicca or oleosa, Malassezia folliculitis, myxoedema and tragic facial expression (Heripret, Guaguere et al. 2006, Mircean and Cozma 2014). Dermatological sings are progressing when thyroid specific treatment fails to be administered and the duration of the disease can be appreciated based on these manifestations. The cause of alopecia relies in the role of thyroid hormones for the initiation of the anagen phase of hair growth. A low level of thyroid hormones will lead to the persistence of telogen phase and cause brittle hair. Truncal alopecia (not necessarily symmetrical), rat tail, nasal alopecia and excessive wear of middle digits in advanced forms of the disease, where there is also lower motor neuron disease, can be observed. Usually all areas undergoing friction are affected, thus in dogs wearing collars and harnesses or after trimming, hair regrowth is slow or even absent. Dermatological signs are the most evident features of hypothyroid dogs and all other clinical manifestations can be discrete, with an insidious progression over time and can easily be overlooked.

Diagnosis relies on the observation of any of the specific clinical signs, followed by thyroid hormone level determination and thyroid imaging techniques (radiography, ultrasonography and scintigraphy) (Vulpe 2006). Unfortunately, none of the current available test is 100% specific and all can provide false positive or false negative results. More important, thyroid hormone levels are directly affected and decreased to the lower reference range by non-thyroidal illnesses and can have an influence in misleading and overdiagnosing the disease. As such, all dogs undergoing hypothyroidism suspicion need thorough evaluation before establishing a certain diagnosis and deciding for specific treatment (Falcă, Solcan et al. 2011, Mooney and Peterson 2012). The current paper presents a case of severe recurrent pyodermatitis, which failed to resolve on all previous correctly applied treatments, as it was secondary to an undiagnosed advanced state of hypothyroidism.

MATERIALS AND METHODS

Case report

A nine year old male entire Cocker Spaniel was presented at the Veterinary Teaching Hospital of Iasi, for microbiological investigations regarding severe recurrent pyodermatitis (Figure 1. a.) and bilateral external otitis. Purulent discharge was significantly richer in the right ear canal and palpation also indicated a higher level of pain, compared to the left ear. Dermal examination indicated dry and brittle hair, rat tail, hyperpigmentation and hyperkeratosis on axial and inguinal area, seborrhea oleosa (Figure 1 b), myxoedema and tragic facial expression. Clinical and physical examination also revealed a low heart rate of 70 bpm, lethargy, obesity (BSC: 7 of 9) and exercise and cold intolerance. Blood samples were collected for general biochemistry and hormonal parameters such as total thyroxine (TT4), free thyroxine (FT4) were performed in order to confirm hypothyroidism.



a. b.

Figure 1. a. Recurrent pyodermatitis on the left ear lobe b. Seborrhea oleosa, dry and brittle hair, hyperpigmentation and hyperkeratosis of depilated areas area.

Microbiological examination consisted in: sampling of purulent material from both external ear canals and sampling of seropurulent exudate from the dorsolumbar area, after scale removal. Special and selective culture media were used for collected purulent material and incubated for 24 hours at 37 °C, followed by antibiotic susceptibility tests. Also, dermal scrapings were performed for mycetes and parasitic mites such as Demodex.

RESULTS AND DISCUSSIONS

Laboratory findings

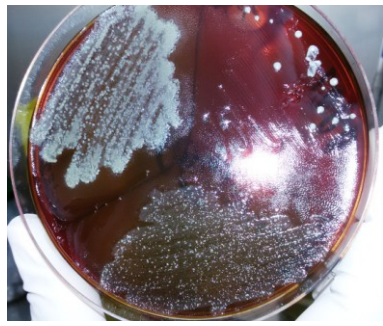
Serum biochemistry confirmed the suspicion of hypothyroidism, revealing a very low level of TT4 8.88 nmol/L (15-50 nmol/L) and FT4 0.3 pmol/L (10-45 pmol/L). Increased alkaline phosphatasis 208 U/L (10-101 U/L) and mild hypercholesterolemia of 269 mg/dl (116-254 mg/dl), also consistent with hypothyroidism, were observed.

Microbiologic diagnosis

From the dermal samples seeded on blood agar culture medium, *Staphylococcus pseudintermedius* was isolated and identified, and revealed accentuated hemolysis. Also, the isolate was confirmed to be coagulase-positive on the pathogenicity test (rabbit citrate plasma coagulation). Samples from the right external ear canal were seeded on usual and selective media (*Pseudomonas* agar Centrimide) and isolated *Proteus spp* (Figure 2.a.), *Pseudomonas spp* (Figure 2.b.) and reconfirmed *Staphylococcus pseudintermedius* (Figure 2.c.). Bacterial load from the left external ear canal was significantly lower than the one observed in the right ear, consistent with the clinical findings. As opposed to Gram-positive cocci, Gram-negative bacteria is not generally isolated from healthy ear canals and is frequently involved in the etiology of purulent external otitis. Moreover, it is known fact that the presence of *Proteus spp*. can emphasize the severity of otitis as it is a mobile bacterium, which can complicate the evolution towards middle or even internal otitis, leading to neurological deficits and permanent hearing loss (Rapuntean and Rapuntean 2005; Carp-Carare, Guguianu et al. 2015; Cole, Kwochka et al. 1998). For a clear microbiological diagnosis, two antibiotics susceptibility tests were performed for gram negative (Figure 3) and gram positive (Figure 4).

Common antibiotic susceptibility for both bacterial groups included Marbofloxacin and Lincomycin. Considering the signalments of the current case and that a series of previous treatment protocols have already included beta lactam antibiotic, Marbofloxacin was chosen as treatment agent. On the other hand, even if rarely, among the side effects of Lincomycin, cutaneous manifestations such as pruritus, redness, rash and Erythema Multiforme have been reported which could render confusion in pyodermatitis evolution along the treatment (Greene 2006). Dermal scrapings were negative for both mycetes and Demodex.

a. c.



b.

Figure 2. a. Stafilococus pseudintermedius; b. Pseudomonas spp.; c. Proteus spp. Swarming phenomenon.

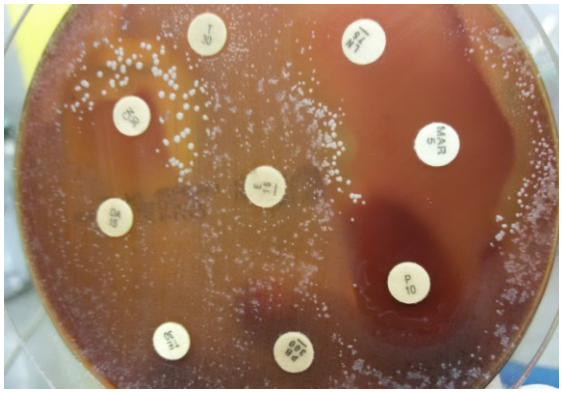


Figure 3. Gram negative antibiogram on blood agar photo at 48h;

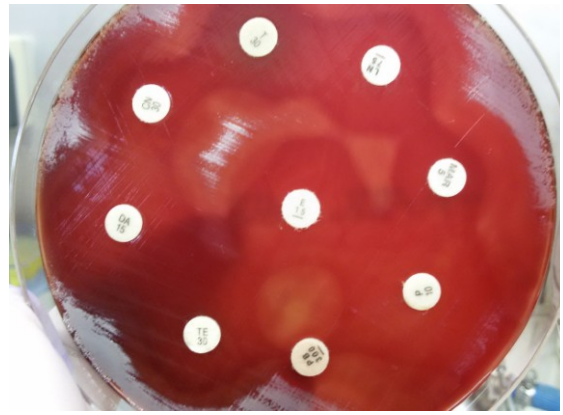


Figure 4. Gram negative antibiogram on blood agar photo at 24h.

Treatment

The treatment for the pyodermatitis consisted of Marbofloxacin injectable 2 mg/kg sc/24 h, for five days and continued with oral administration for seven days. For the otitis, topical solution 3 mg/ml, 10 drop per ear once daily was recommended. Also, daily baths with 3.5% clorhexidine gluconate shampoo or mousse were prescribed. Hypothyroidism therapeutic strategy consisted of levothyroxine 10 µg/kg/12 h. Within two weeks after the starting of treatment, the dog was rescheduled for clinical and FT4 and TT4 revaluation and readjusting the levothyroxine. Unfortunately, the dog was lost for monitoring for a period of eight weeks, after which he was presented back to the clinic with a considerable better

condition. Pyodermatitis and otitis were completely resolved, hair regeneration has been observed and axial and inguinal skin lesions and hyperkeratosis were well reduced (Figure 5).

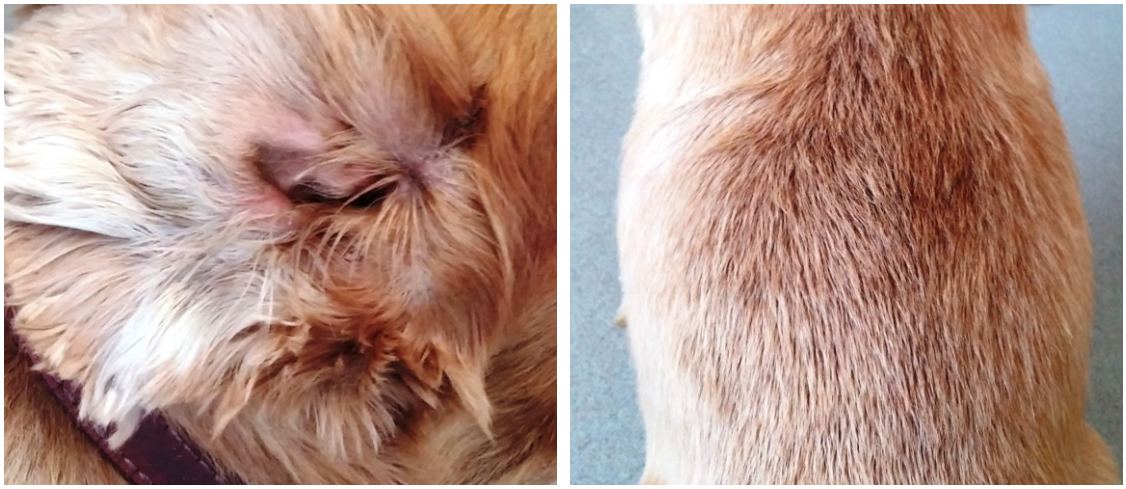


Figure 5. a. Pyodermatitis and otitis resolution; b. hair regeneration and skin lesions well reduced after eight weeks of treatment (Figure 5).

Hypothyroid dogs usually develop endocrine alopecia, which is usually non-pruritic symmetrical, bilateral, affecting the trunk and sparing the limbs. However, some dogs may be presented with asymmetrical patterns of alopecia and some with pruriginous lesions of the skin due to bacterial or Malassezia complications. A high proportion of dogs are presented for the initial consultation for the dermatological signs. In many cases, hypothyroidism is suspected and investigated as an underling cause of the dermal problems only after different prolonged and failing treatment protocols have been applied. Unfortunately, some clinicians rule out hypothyroidism based on the absence of the symmetrical pattern or presence of pruritus, misleading the correct diagnosis in a high number of cases and delaying specific treatment. After diagnosis establishment, besides thyroid hormone replacement, some dogs require the approach of other complications of the disease, such as otitis and pyoderma. In some of these cases, secondary bacterial infections are rendered resistant to usual antibiotics by repeated and intermittent antibiotic administration, making specific treatment even more difficult. The current paper described a case of long term evolving recurrent pyodermatitis, which resolved only after the diagnosis and specific therapeutic approach have been applied for an advanced state of hypothyroidism.

CONCLUSIONS

Many hypothyroid dogs are referred for the initial consultation for a series of dermatological manifestations. Unfortunately, in some cases dermal lesions are directly addressed, without considering an underling disease, leading to treatment failure, lesion progression and secondary resistance towards antibiotics after repeated administrations. Although currently there is a wide range of tests that can be performed in order to diagnose hypothyroidism, in most cases, specific clinical sings are often insidious and may take years until the disease is first suspected. As in the current case, not all hypothyroid dogs present the

entire panel of specific clinical manifestations, just as not all endocrine alopecia is non-pruriginous.

Hypothyroidism usually evolves with the decreasing of the immune system, thus, it is mandatory that all middle age to old dogs, presenting pyoderma suborned unilateral or bilateral bacterial otitis, to be tested for hypothyroidism.

REFERENCES

1. Carp-Carare, C., E. Guguianu and C. Rambu (2015). Bacteriologie speciala-Indrumator de lucrari practice, Ed. Ion Ionescu de la Brad.
2. Cole, L. K., K. W. Kwochka, J. J. Kowalski and A. Hillier (1998). "Microbial flora and antimicrobial susceptibility testing of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media." J Am Vet Med Assoc 212(4**)**: 534-538.
3. Falcă, C., G. Solcan, T. Moț, D. Morar, I. Papuc, V. Vulpe, C. Pop, C. Vlăgoiu, G. Giurgiu, M. Mircean and C. M. Braslașu (2011). Medicina internă a animalelor. Timișoara.
4. Greene, C. E. (2006). Infectious Diseases of the Dog and Cat, Elsevier.
5. Heripret, D., E. Guaguere and P. Prelaud (2006). Guide Practique de Dermatologie canine, Kalianxis.
6. Mircean, V. and V. Cozma (2014). Dermatologie Veterinara, AcademicPres.
7. Mooney, C. T. and M. E. Peterson (2012). BSAVA Manual of Canine and Feline Endocrinology, BSAVA.
8. Rapuntean, G. and S. Rapuntean (2005). Bacteriologie Veterinară Specială, AcademicPres, Cluj/Napoca.
9. Vulpe, V. (2006). Semiologie medicală veterinară - Semiologie speciala. Iași.

ULTRASONOGRAPHIC ASPECTS OF GASTROINTESTINAL DISORDERS IN DOGS

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Abstract:*Ultrasonography is a non-invasive imaging technique which provides useful real-time data regarding the functioning of the internal organs, especially of those from the abdominal cavity.*
Research has been conducted over one year period of time in Iasi, Faculty of Veterinary Medicine and Liverpool, Small Animal teaching Hospital, a total number of 47 dogs of different age and breed being investigated, all of them manifesting gastrointestinal specific symptoms. The study allowed to identify all major types of gastrointestinal disorders, inflammatory gastrointestinal disease being the most frequent identified disorder. All of these lesions had developed associated with or without other conditions.
Out of 47 subjects identified with gastrointestinal disorders, 16 had gastric specific symptoms (inapetence, vomiting), 13 manifested only intestinal symptoms (weight loss, diarrhea, constipation, melena) while 18 presented both gastric and intestinal lesions. All cases have been examined by ultrasonography in order to provide a secure diagnosis and to assess the existent lesions.

Keywords*: ultrasonography, gastritis, enteritis, dogs*

INTRODUCTION

Ultrasound is a noninvasive and painless imaging technique, which provides real-time data regarding the functioning of the internal organs, especially of those located in the abdominal cavity (4).

As a complementary diagnostic method, ultrasound has its suitability only if clinical examination cannot establish a definite diagnosis of the disease or condition, when aiming to detect the suspected lesions or whenever the owners want a thorough review on the health of their pets (1).

The importance of using ultrasonography to investigate the segments of the digestive tract is given by viewing parietal structures, shape and size, factors which can formulate a definite diagnosis. In order to interpret an ultrasound should be taken into account the topography of the main abdominal organs, the relations between them and the specificities of their echogenicity. Thus, after a detailed examination in order to establish the diagnosis of the condition, ultrasound technique can be used as a complementary diagnostic method (5).

MATERIAL AND METHOD

The research was undertaken over a period of one year, at the Medical Clinic of the Faculty of Veterinary Medicine Iasi and Small Animal Teaching Hospital Liverpool, investigating by ultrasonography a total number of 47 dogs of different breeds and ages, all showing symptoms of gastric or intestinal disorders, allowing the identification of main types of gastrointestinal lesions, most commonly diagnosed being the gastric and intestinal inflammatory processes, which have developed alone or as associated lesions (3).

The ultrasound examination was performed using Aquila Pro Vet with a convex probe of 5 and 7.5 MHz. The ultrasound machine is also equipped with ultrasound playback printer or memory card for later viewing of the stored images.

Ultrasound examination was also performed with Logiq 7, with a frequency between 5 to 14 MHz and 17-inch display, Doppler mode and 3D modeling which allows a tridimensional observation of the investigated organ.

The digestive tract contains a variable amount of gas or digestive juices and food particles. The presence of gas is an obstacle to the propagation of ultrasonic waves and thus artifacts are being created which prevents proper visualization and examination of the digestive segments.

Ultrasound evaluation of the digestive tract does not require special preparation. However, it is recommended a period of 12 hours of fastening before the examination in order to minimize the amount of air present in the digestive tract (1).

Both the stomach and the small or large intestine were systematically investigated in order to observe changes in thickness of the wall, assess the characteristic structure of the wall and observe the presence of regional lymphadenopathy. Of all imaging investigation techniques, the ultrasound is considered to be the most dependent on the examiner, the experience of using this technique being compared with the obtained results (2).

RESULTS AND DISCUSSIONS

Of the 47 subjects with gastrointestinal symptoms only 16 had gastric disorders (loss of appetite, vomiting), 13 cases showed symptoms of intestinal disorders (syndrome of weakness, diarrhea / constipation, blood in stool) and the remaining 18 dogs had disorders common to gastrointestinal diseases. The 47 cases were subjected to ultrasound examination to establish the diagnosis and assess the present lesions.

In Figure 1, a dog presented at the Medical Clinic with appetite loss and vomiting symptoms indicating a gastric disorder showed the chronic inflammation of the pyloric region which appears structurally altered without the normal stratification of the wall and with a hypoechoic ring around it. At 49 kg weight, the normal wall thickness at the pyloric should be around 5.5 mm. As can be observed, the wall thickness is 9.5 mm which attests a localized gastric inflammatory process.

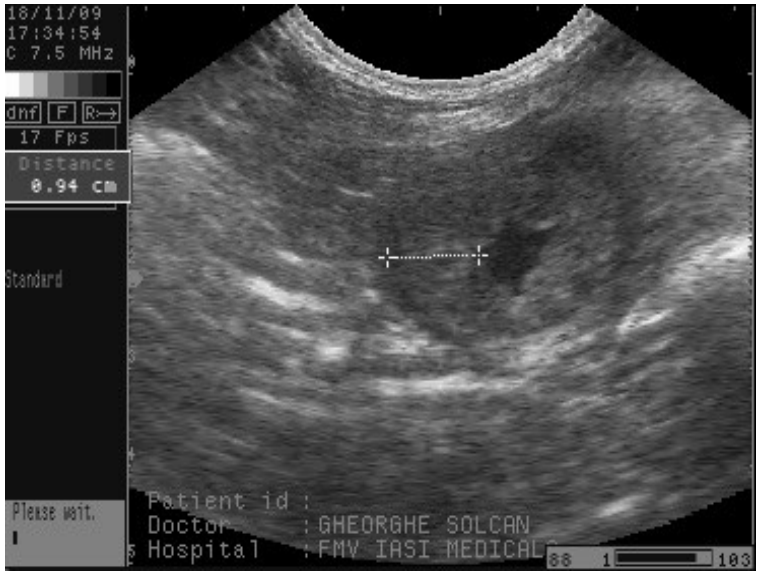

Fig. 1. Gastritis, Carpathian Shepard, male, 9 years old

The patient who was being carried out the ultrasound shown in Figure 2, a common breed dog aged 4 years old, was presented by the owner because the petmanifestedvomiting for the last week and an alarming drop in weight. Although there has not been recorded a change of the normal gastric wall dimension or diffuse or focal lesions of the gastric wall, loss of stratification in the stomach led to the diagnosis of gastritis. It can be seen that the stomach is deprived of food and has a small amount of gas. The diagnosis of gastritis can be suspected based on the loss of structural stratification, increase in the size of the wall or whenever focal or diffuse changes are seen at this level. All of these changes in ultrasound can be observed simultaneously or individually, leading to the assumption of a gastric inflammation. Of real importance is the assessment of echogenicity, aspect which can provide specific information about the existence of an inflammatory processes.

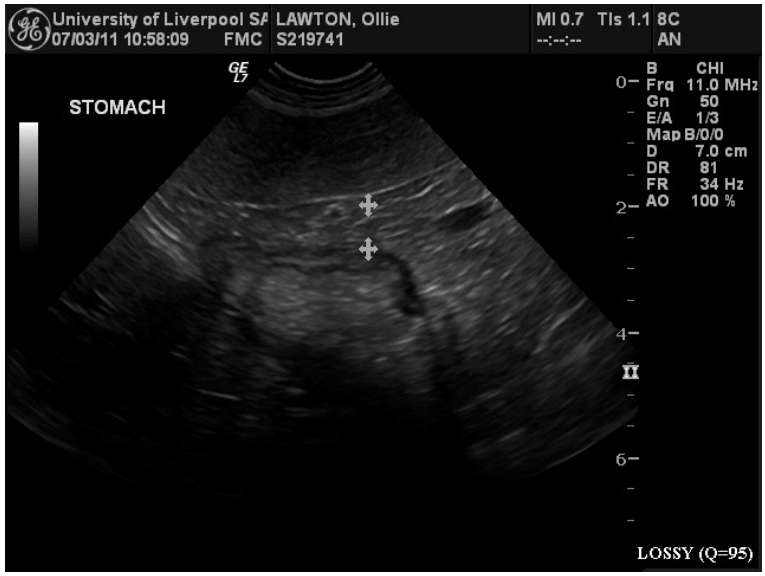

Fig. 2. Gastritis, crossbreed dog, male, 4 years old

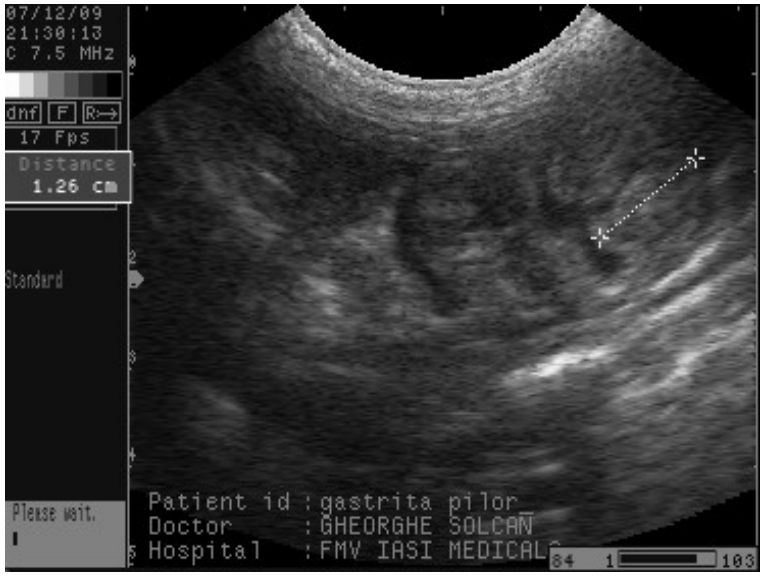

Fig. 3. Gastritis, German Brac, male, 14 months old

As can be observed in figure 3, the pylorus preserves its normal aspect, but the dimensions of the folds at this level is being increased.

Whenever interpreting the ultrasound images, should be taken into account the size of the patient and the echogenic appearance of neighboring structures but also the normal ultrasonographic appearance of the investigated segment. Thus, the above images have importance concerning the wall thickening of the pylorus, which shows the presence of irritation that have evolved over a period of time causing a corresponding increase in size of parietal layers or a neoplastic process that may lead to thickening of the section of the pylorus. Given that gastric wall layers retain individualization can lead to the conclusion that a chronic inflammatory process is present. Given the patient's age (14 months), the inflammatory process may be of parasitic or viral etiology, the latter manifested by subclinical infection, justified by the poor infection symptomsthat have been expressed: hyperthermia (39.3 ˚), light appetite loss for the last 10 days and the lack of vomiting or diarrhea.

In Figure 4 can be detected the characteristic appearance of gastritis with diffuse gastric wall thickening, accompanied by the presence of intraluminal content.

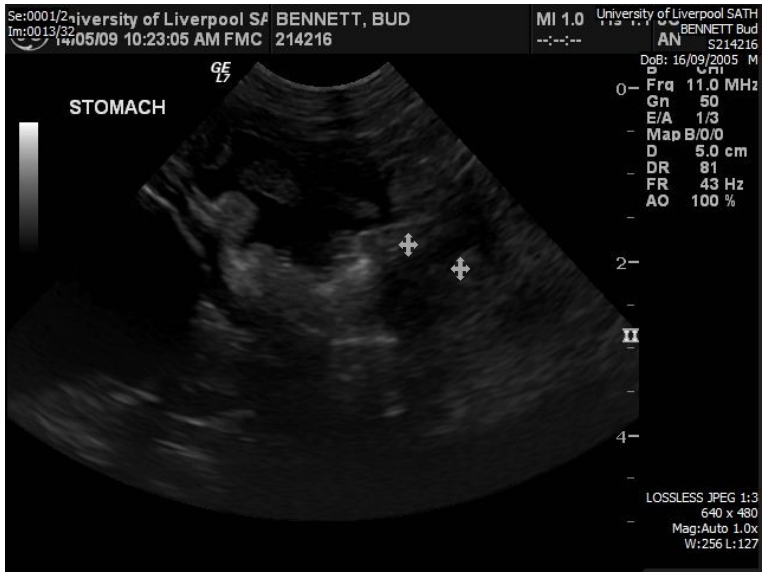

Fig. 4. Gastritis – Yorkshire Terrier

In the same figure can be seen the loss of specific stratification of the stomach wall, this change being reported throughout the section. In Figure 4 can be observed the presence of gastric folds and content in the gastric lumen.

In the same case can also be seen the inflammation of the proximal duodenum, immediately after the pyloric region. Intestinal wall loses its stratification and its size appears increased (Fig. 5.).

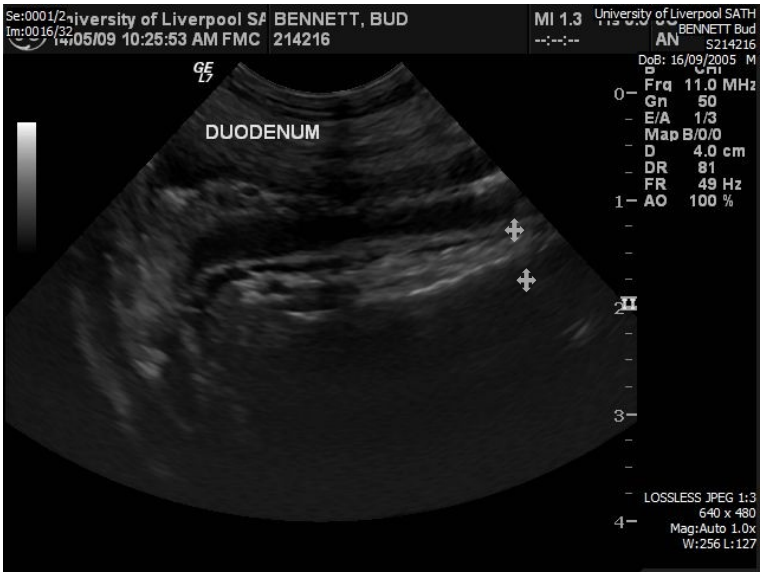

Fig. 5. Proximal duodenum inflammation

The presence of a gastric tumor can be detected in Figure 6, a heterogeneous formation with an anechoic edge and small sized hyperechoic center. The tumor, with a size of 36.65 x 30.64 mm, appears to be located in the lumen of the stomach, being surrounded by gastric contents in the lower part.

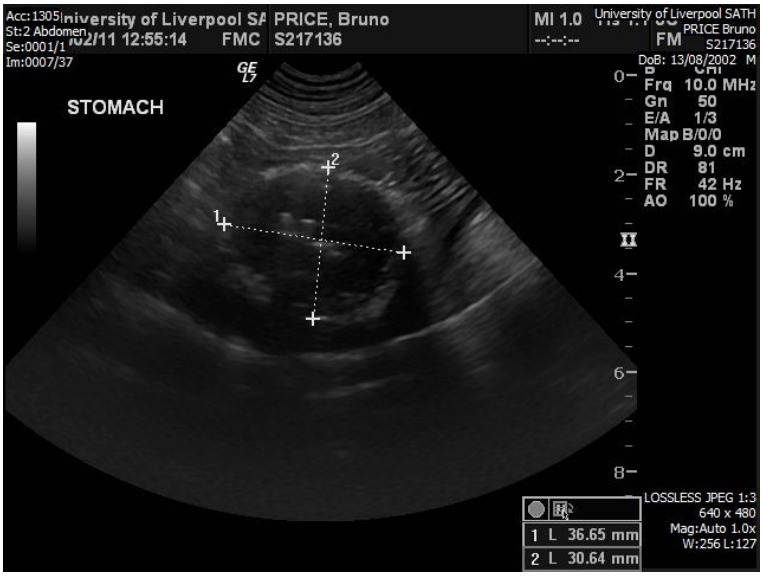

Fig. 6. Gastric neoplasia – Shetland Shepard, 9 years old

The gastric changes that are being most frequently identified by ultrasound technique were gastritis and disorders of the pyloric region of the stomach. Gastritis had uniform gastric wall thickening. Wall lesions were regular but the localization of the process that affected a particular layer of the wall was not detected by ultrasound in all the examined subjects. It was also discovered a gastric tumor which appeared as an asymmetrical, focal change of the stomach wall. In order to view it from different angles and to assess its origin, the peristaltic

movements were monitored by changing the position of the probe and patient to see if it was located in the wall (lesion remains fixed) or intraluminal.

Difficult to assess and locate were the gastric wall uneven thickenings (which normally has a thickness of 3-5 mm). Gastritis, both acute and chronic, can be recognized by focal or diffuse thickening of the gastric wall.

Chronic hypertrophic gastritis were consistent with the hypertrophy of the gastric mucosa (glandular hyperplasia), sometimes putting on an almost tumor like aspect.

Pyloric stenosis has been recognized based on thickening of the pylorus. The diagnosis of hypertrophic pyloric gastropathy was established based on the thickening of the pylorus (9-19 mm), in particular the thickening of the muscular layer (3 to 5.4 mm) accompanied by morphological changes in the stomach distension and strong peristaltic movements (4).

Intestinal disorders showed diffuse changes and were represented by enteritis, tumors or immune enteropathy. The main change in these conditions was represented by thickening of the intestinal wall. Some authors consider (as a rule) that the thickening of homogeneous large portions of the wall without losing its specific architecture is found in intestinal inflammatory processes and localized thickenings, asymmetric loss of stratification in the wall suggest a neoplastic process. Exceptions to this "rule" are the lymphomas (affecting a large intestinal area) and some localized enteritis (such as duodenitis associated to pancreatitis) (2).

Wall lesions were characterized by diffuse or localized thickening of the intestinal wall. It is estimated that ultrasound technique is superior to radiographic examination in the diagnosis of gastrointestinal tumors. Localized extramural lesions reduces the intestinal lumen by the compressions exercised by the adjacent formations (postoperative adhesions, regional lymphadenopathy, etc.) (1).

In figure 7can be observed the increase in thickness of the bowel wall withthe characteristic stratification and echogenicity preservation.

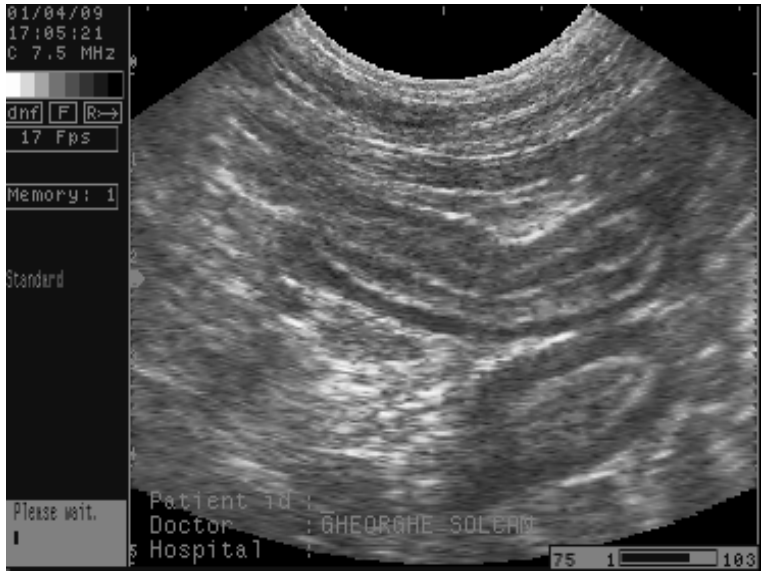

Fig. 7. Chronic enteritis, crossbreed dog, female, 6 years old

The patient, a 6 years old common breed dog, accused diarrheal episodes. The ultrasound examination made possible to highlight the enteritis lesions represented by the increased parietal thickness and changes in echogenicity.

As shown in Figure 7 and in figure 8 the intestinal loops are affected by lesions which appears diffuse. Very important to note is that the proportions are maintained between the specific layers of the intestinal wall, which reveals the existence of a chronic inflammation.

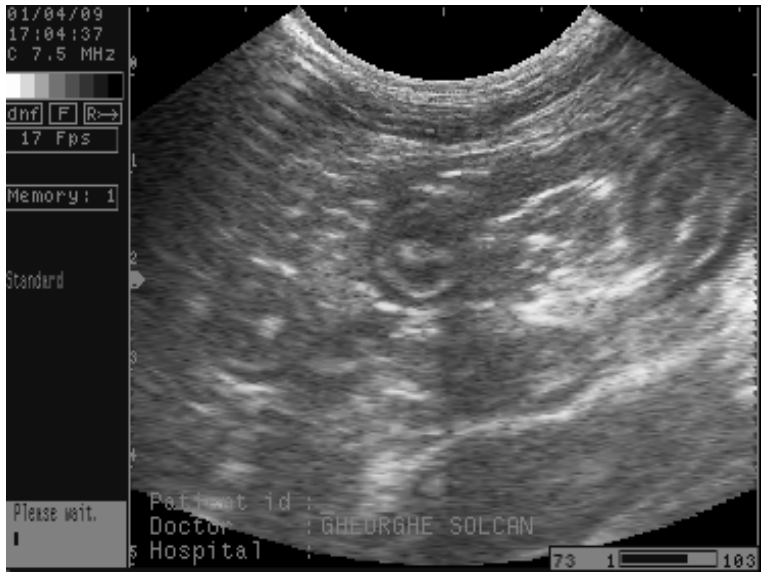

Fig. 8. Chronic enteritis, crossbreed dog, female, 6 years old

In figure 9 can be observed the specific aspect of colitis, with the presence of speckles within the large bowel wall.

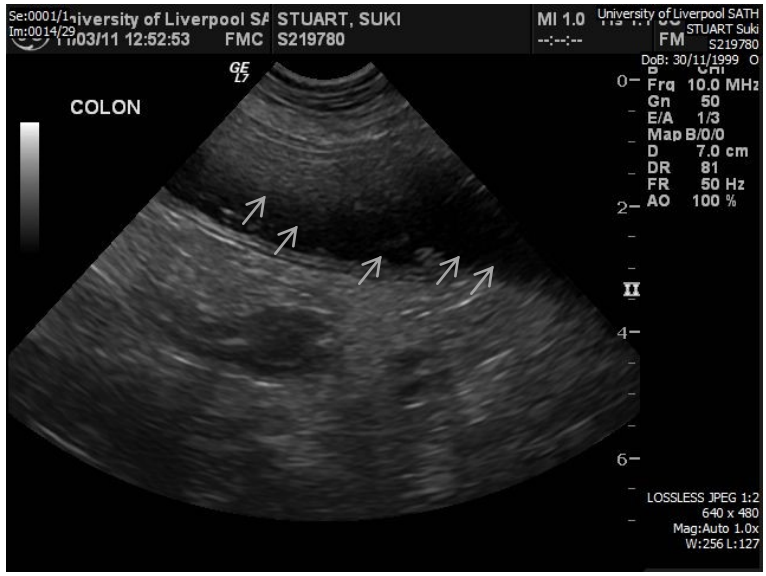

Fig. 9. Colitis with the presence of speckles

In the same ultrasound image can be revealed the presence of intestinal content, and two longitudinal sections of the small intestine.

In figures 10 is represented a particular case of intussusception in a German Shepherd dog aged 6 years old. Specific for the diagnosis of intussusception is the presence of the "ring sign" or "bull's eye", a term often used in foreign literature. Figure 10 clearly presents 2 intestinal loops without being able to detect the intestinal wall stratification. The invaginatedloop has a very small, hyperechoic lumen that is barely observable, surrounded by a predominantly hypoechoic structure given by the mucosal and muscular layers. The external loop is predominantly hyperechoic with a reduced in thickness, hypoechoic mucosal layer.

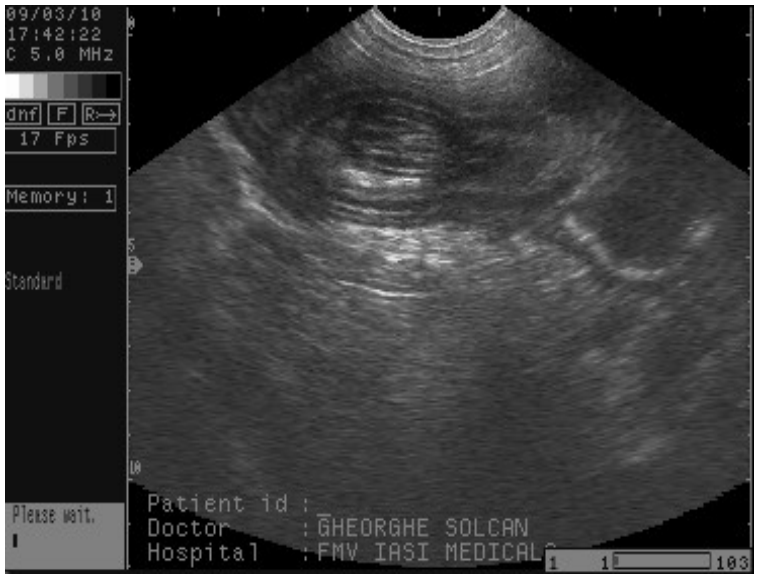

Fig. 10. Intestinal intussusception, GermanShephard, female, 6 years old

CONCLUSIONS

The ultrasound examination was performed in a number of 47 patients, the disorders that can be assessed by ultrasound technique including changes in the gastric and intestinal wall size, parietal stratification, the presence of foreign bodies or intraluminal masses, the presence of striations or speckles in the intestinal wall, changes in the echogenity of the investigated segments and the diffuse or localized appearance of the lesions;

Ultrasound technique has allowed to observe the peristaltic movements of the stomach. Its ultrasound examination can be performed both in the longitudinal and in the transverse plane, from left (cardia portion) to the right (pyloric portion);

The diagnosed disorders were represented by the gastritis, pyloric hypertrophy, gastric cancer, enteritis and intussusception;

Gastritis is characterized by diffuse increasing of the gastric wall dimensions with the preservation of normal stratification, in most cases;

Enteritis are described as entities characterized by increased thickness of the intestinal wall and altered echogenicity (hyperechoic structures);

In the study were identified by ultrasound two gastric tumors with focal increase in the size of the stomach wall, accompanied by loss of individualization of the component layers;

In the study was also identified a case of intussusception characterized by an increase in the number of parietal layers that can be ultrasonographically assessed.

REFERENCES

1. Codreanu M., 2000 - Diagnosticulecograficînbolile interne la animale, Ed. Coral Sanivet;
2. Haroutunian G., 1995 – Ecographie du chien et du chat, Edition Vigot, Paris;
3. Mălăncuș R.N., Gh. Solcan, Cristina Tofan (Malancus), 2010 - The use of ultrasonography in gastrointestinal disease in dogs, Lucr. Stiintifice USAMV Iasi, seriaMedicinaVeterinaravol 53, pag. 772-777;
4. Mălăncuș R.N., Gh. Solcan, V. Vulpe , 2009- The diagnosis of canine gastritis secondary to chronic nephritis, Lucr. Stiintifice USAMV Iasi, seriaMedicinaVeterinaravol 52, pag. 664-667;
5. Nyland T.G., Matoon J.S., 1995 – Veterinary Diagnostic Ultrasound, Saunders Company, U.S.A;

SOME ASPECTS REGARDING IN VITRO MATURATION OF BOVINE OOCYTES

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Abstract:*In vitro production of bovine embryos involves a lot of critical steps and in vitro maturation of oocyte is one of them. Harvesting and the process of cultivation in maturated media of immature oocyte leads to the completion of first meiotic division. For these reasons we intended to emphasize nuclear maturation of bovine oocyte through aceto - orcein staining and also to identify peculiar elements from the perspective of the maturated mediacomposition. The oocytes were classified based on morphological criteria in three groups resulting* ***cumulus cells–oocyte–complexes(COC)*** *of three qualities. These oocytes were the support for further maturation in TCM199 plus 10% ECS (Estrus Cows Serum) with or without FSH supplementation. The oocytes were subsequently denuded and stained with aceto–orcein. FSH supplementation influenced the rate of maturation as follows : 9.67 ± 3.18 vs 7.00 ± 0.58 for class C I, 9.00 ± 4.16 vs 5.67 ± 1.20 for class C II and 4.33 ± 1.33 vs. 2.33 ± 1.20 ($p \leq 0.05$) for class C III. Cultivation and maturation processes implies a perdition which is reflected in our case by the loss of 25.75% COC generated by the the steps of denudation, fixation and staining. FSH supplementation does not influence bovine COC maturation as it was proved only through evaluation of nuclear stages of female gamete. Due to its affinity to nucleic acids, the aceto–orcein staininginfluences further the quality and number of resulted COC as considered by the nuclear maturation and also making them useless for farther steps of in vitro fertilization. For a complete evaluation of oocyte maturation, besides assesment of nuclear maturation, cytoplasmic maturation also has to be considered.*

Key words*: in vitro maturation, oocytes, bovine*

In vitro production of embryos is an assisted reproduction technology (ART) used with good results in bovine species. *In vitro* maturation (IVM) is the first step in IVP technique and an important one because the success of *in vitro* embryos production depends on oocyte quality and on the composition of maturation media.

Serum (FCS, ECS) and hormons (FSH, LH, 17ß estradiol) are added in the maturation media in different concentrations in order to improve bovine fertilization and cleavage rate; for exemple 0.5µg/ml FSH [1], 1 µg/ml [2] or 20 µg/ml [3], although bovine pre-ovulatory surge FSH concentrations were reported in average 125 ng/ml; the same procedure worksfor LH, which is added 5µg/ml, although *in vivo* LH surge average is 200 ng/ml [4].

The aim of this paper it is to highlight the nuclear maturation of bovine oocytes using aceto-orcein staining, identifying the specific elements that evidences this process and to observe the influence of the composition of maturated media on oocyte maturation.

MATERIALS AND METHODS

Bovine ovaries (n=20) were collected from slaughterhouse and transported within two hours to the laboratory in containers consisting of 0.9% NaCl solution, at 35^0C. The handling medium for COC was Dulbecco-PBS (100 ml)[5] supplemented with 100 µl

Pen/Strep (17-602F, Lonza); 3.6 mg sodium piruvate, 30 mg BSA (A9647, Sigma-Aldrich), 100 mg glucose (G7021, Sigma-Aldrich). COC's were aspirated by puncturing thefollicles with 3-8 mm diameter with a 18G needle attached to a 5 ml syringe.

The classification of COC's based on morphological aspects was made with stereomicroscope[1] (Stemi 2000-C, ZEISS) with hot plate (33.4^0C) after the criteria of Hawk and Wall, 1994 [6] as follows : *class I* - CI (COCs with cumulus compact and unexpanded, with full or at least 5 layers of cumulus cells, cytoplasm clearly seen, dense and homogenous), *class II* – CII (COCs with cumulus compact, thick, 2-4 layers of cumulus cells, covering all zona pellucida, cytoplasm dense, with uniform granulation) and *class III* - CIII (oocytes partially denuded of cumulus cells, or with 1-2 complete layers of cumulus cells and/or with irregular shrunken cytoplasm).

The oocyte maturation media prepared in our laboratory consists in tissue culture medium 199 (TCM 199 HEPES modification medium, M2520, Sigma-Aldrich) with 10% ECS and with or without sheep FSH (F8174, Sigma-Aldrich)[7]. We have maturated 8-10 COC's tanks in 50µl TCM-199, with 0.5 µl FSH (0.88 µg/µl) or without FSH, in 35 mm Petri dishes (Greiner Bio-One, Germany) covered with mineral oil at 38.5^0C in 5% CO_2 humidified atmosphere air for 24h. After 24h since the cultivation, all oocytes were examined for maturation and signs like expansion and presence of mucus in cumulus cells were observed.

The oocytes were denuded using a fine capillary glass and examined for Ist polar bodies at stereomicroscope (5X). All oocytes denudated were stained with 1% aceto-orcein after the method of Khatun et al. (2011)[8] with minor modification (for fixing the cover slip, 18x18 mm, we made only two lines with Baysilone-Paste). The slides were examined for nuclear changes under microscope (Leica DM750) using 40X objective. Oocytes were considered nuclear maturated only if extrusion of the first polar body and the appearance of the metaphase plate (MII) were present.

RESULTS AND DISCUSSION

Out of a total of 20 cow ovaries were collected 171 COC, in average 8.55 COC/ovary, similar values with Mermillod [1]- 5-10 COC/ovary and with Zheng-GUANG WANG [9], who obtained 9.7 COC/ovary.

Based on morphological aspectss 101 COC were maturated in vitro during the experiment and were stained with orcein; from the total number, only 75 (74,25%) werecolored. The rest of 25,75% were lost during steps of denudation, fixing and staining. Similar results with PRENTICE-BIENSCH and co., 31% [10].

FSH supplementation influenced the rate of maturation as follows : 9.67 ± 3.18 vs 7.00 ± 0.58 for class C I, 9.00 ± 4.16 vs 5.67 ± 1.20 for class C II and 4.33 ± 1.33 vs. 2.33 ± 1.20 ($p \leq 0.05$) for class C III, without semnificative diferences (table 1) .

COC's quality had a positive effect on cumulus expansion rate based on morphological aspects observed after maturation. As it can be observed, in class C III there were significantly less COC maturated ($p<0,05$) (table 1) .

[1]The research was carried in the IVF (In Vitro Fertilization) laboratory from the Horia Cernescu Research Unit equiped through POSCCE 2669 program

Table 1. COC classification ($\overline{X} \pm$SX)before/after maturation in culture medium with/without FSH(where * represents $p \leq 0.05$)

	C1		C2		C3	
	Before maturation	**After maturation**	**Before maturation**	**After maturation**	**Before maturation**	**After maturation**
+FSH	11.33±4,06	9,67±3,18	12,00±3,61	9,00±4,16	10,00±1,15	4,33*±1,33
-FSH	8,33±0,88	7,00±0,58	8,33±1,86	5,67±1,20	7,00±1,73	2,33±1,20

We have noticed a low percentage of oocytes in stage MI and MII (figure 1), based on gamete maturation marker, with no difference between groups with/without FSH. From all oocytes stained, 7.14% and 9.52% were in MI, respectively MII in group with FSH and in the group without FSH 6.06% and 9.09% were in MI and MII, the rest of the oocytes were in germinal vesicle (GV) and germinal vesicle break down (GVBD) stages.

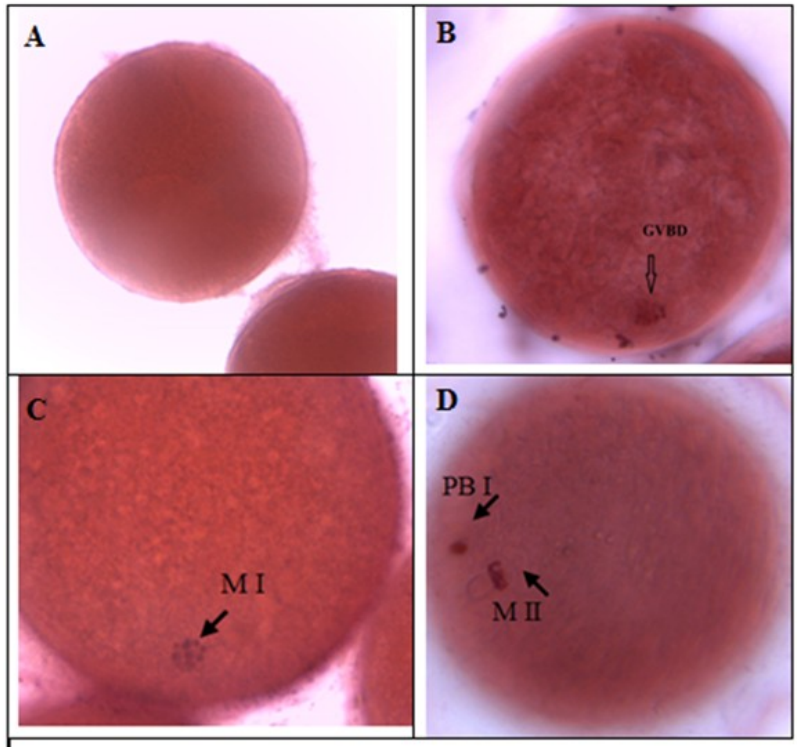

Figure 1. Oocyte nuclear stages: germinal vesicle (A), germinal vesicle break down (B), metaphase I (C) and metaphase II (D)(40X)

Thus 54,76%, respective 28,57% from oocytes maturated in medium with FSH were in GV stage, respective GVBD. Similar results were also present in the other group (without FSH) 54,54% remained in GV and 30,30% in GVBD (figure 2).

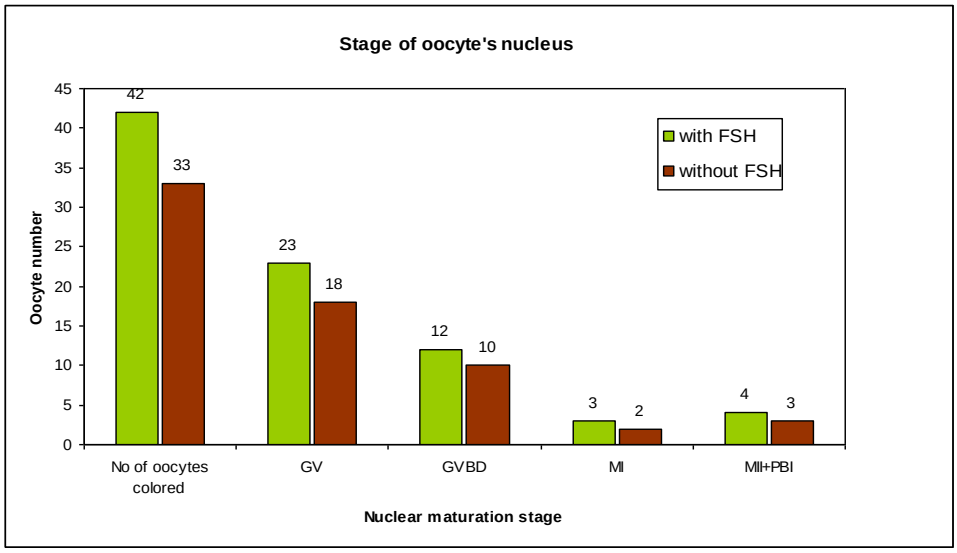

Figure 2. Stages of oocyte's nucleus

Kakkassery et al. (11), observed also a corelation between nuclear maturation percentage and oocyte class after aceto-orcein stain – 36%, 16%, 10 % from oocytes class I, II, respective III were in MII stage.

Fig. 1 Oocyte nuclear stages: germinal vesicle (A), germinal vesicle break down (B), metaphase I (C) and metaphase II (D)(40X)

CONCLUSIONS

- using FSH in the maturation medium did not influenced nuclear maturation of bovine COC's, but has to play a role in preparing the next stages of IVF by increasing the capacity of fertilization and development capacity of bovine oocytes.
- bovine oocytes stained with 1% aceto-orcein is an easy method which is used for assessing the nuclear maturation, but the fact that the stained oocytes could not be used further for IVF represents a disadvantage.
- to fully evaluate bovine oocyte maturation it has to be considered also cytoplasmic maturation

REFERENCES

1. P.Mermillod, A.Massip, F.Dessy, "In vitro production of cattle embryos: review and Belgian results", Int.J.Dev.Biol, vol.36, pp.185-195, 1992.
2. I.G. Smetanina, L.V. Tatarinova, A.S. Krivokharchenko, *"In vitro* fertilization of bovine oocystes in protein-free culture system", Russian Journal od Developmental Biology, vol. 37, 6, pp. 366-370, 2006.
3. M. Franco and P.J. Hansen, "Effects of hyaluronic acid in culture and cytochalasin B treatment before freezing on survival of cryopreserved bovine embryos produced in vitro", In Vitro Cell Dev Biol, vol.42, pp.40-44, 2006.
4. M.D. Calder, A.n. caveney, L.C. Smith, A.J.Watson. "Responsiveness of bovine cumulus-oocyte-complexes (COC) to porcine and recombinant human FSH, and the effect of COC quality on gonadotropin receptor and Cx43 marker gene mRNAs during maturation in vitro", Reproductive Biology and Endocrinology, I, 14, 2003.

5. I. Gordon, “Reproductive technologies in farm animals”, 2nd ed., in CABI Publishing, 2003, pp. 79-111
6. H.W. Hawk and R.J. Wall, “Improved yields of bovine blastocysts from in vitro-produced oocytes. I. Selection of oocytes and zygotes”, Theriogenology, vol 41, pp. 1571-1583, 1994.
7. J.J. Parrish, J.L. Susko-Parrish, M.L. Leibfried-Rutledge, E.S. Critser, W.H. Eyestone and N.L. First, ”Bovine in vitro fertilization with frozen-thawed semen”, Theriogenology, vol. 25, pp. 591–600, 1986.
8. M. Khatun, M.M. Udin Bhuiyan, J.U. Ahmed, A.Haque, M.B. Rahman, M. Shamsuddin, ”In vitro maturation and fertilization of prepubertal and pubertal black Bengal goat oocytes”, J.Vet.Sci, vol.12(1), pp.75-92, 2011.
9. ZHENG-GUANG, WANG, SONG-DONG, YU, ZI-RONG, XU (2007) – *Effects of collection methods and recovery efficiency, maturation rate and subsequent embryonic developmental competence of oocytes in Holstein cow*, Asian-Aust. J. Anim. Sci, 20, 4, 496-500.
10. PRENTICE-BIENSCH, J. R., SINGH, J., ALFOTEISY, B., ANZAR, M. (2012) - *A simple and high-throughput method to assess maturation status of bovine oocytes: Comparison of anti-lamin A/C-DAPI with an aceto-orcein staining technique,* Theriogenology, 78, 633–1638
11. KAKKASSERY, M. P., VIJAYAKUMARAM, V., SREEKUMARAN, T. (2010) - *Effect of cumulus oocyte complex morphology on in vitro maturation of bovine oocytes,* J.Vet.Anim.Sci., 41, 12-17.

DOGS AS POTENTIAL SENTINEL FOR LYME DISEASE RISK IN HUMAN PUPULATIONS

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Abstract:*Lyme borreliosis is one of the most threatening vector-borne diseases nowadays. Although it doesn't has long history, it did became shortly an emerging infection. The great ability of Borrelia burgdorferi sensu lato (s.l.) complex, the causative agent, and the tick vector (genus Ixodes) to adapt to different organisms, both vertebrate and nonvertebrate, it seems to be the key in the maintaining of infection in nature. In some of this hosts, Borrelia burgdorferi s.l. produces a multisystemic infection, such as in humans, dogs, horses and in lower percentage affects cats, goats, or cattle. Although, there are differences in the matter of clinical presentation in humans and animals, evaluating the seroprevalence of dogs or/and horses can help monitoring the infection risks for the human population. Dogs especially, can be used successfully as a sentinel because they mostly share the same environment as their owners and usually they have a better history of travels. Serologic evaluation based on antibodies determination by ELSISA or IFA are indicated methods to investigate the prevalence, while testing antibodies against protein ospC gives indication regarding the incidence. Ticks can also be investigated for their status of infectivity, so then predicts the risk of infection of both humans and animals population in a specific area, using molecular biology techniques. In this review we have integrated information from over twenty latest articles which reported results of testing over 3000 dog sera. Most of them were conducted on dogs without disease symptoms to establish the prevalence. Data were then compared with those reported in humans, where there were available, and emphasized their close connection. Therefore, it is concluded the utility of dogs as sentinel for early recognition, monitoring and prediction of the endemicity status of Lyme borreliosis in a predefined geographical area.*

Keywords: *Borrelia burgdorferi* sensu lato, dog, seroprevalence, Lyme disease risk

INTRODUCTION

Lyme disease is a tick-borne disease that affects human and domestic animals, particularly dogs and horses, caused by a group of spirochetes. This group comprise 19 *Borrelia* spp., known also as *Borrelia burgdorferi* sensu lato (s.l.) complex of which 12 *(Borrelia burgdorferi* sensu stricto (s.s.)*, Borrelia garinii, Borrelia afzelii, Borrelia japonica, Borrelia lusitaniae, Borrelia valaisiana, Borrelia tanukii, Borrelia tundi, Borrelia spielmanii, Borrelia carolinensia, Borrelia sinica and Borrelia americana)*- are proven to be implicated in producing Lyme boreliosis (Wang, 2015). It is a multi-systemic disease that in humans can start with skin lesions, fever, loss of appetite, influenza-like symptoms, in the acute stage and may advance to sever arthritis, glomerulonephritis, myocarditis and even neuroborreliosis. Some symptoms are associated with specific *Borrelia* species. For instance, skin lesions, such as *erythema migrans* (EM) and *acrodermatitis chronica atrophicans*, are mostly associated with *B. afzelii*, while arthritis with *B. burgdorferi* sensu stricto, and *B. garinii* is associated with neuroboreliosis (Hubálek, 2009). The major risk of the infection is the contact with infected ticks, the only vector for the causative agent. *Ixodes ricinus* is the most common tick (in Europe and North America) acting as vector for *Borrelia spp.*, followed by *Ixodes scapularis, Ixodes persulcatus, Ixodes trianguliceps*, or *Ixodes hexagonus* (Hubálek, 2009*).*

As the geographical distribution of ticks tend to increase due to multiple ecological changes, Lyme boreliosis (Lb) cases in human and dog populations do as well.

Nowadays, tick habitat covers a large part of Northern Hemisphere, subscribing between 35^0 N and 60^0 N, up to 1300 meters above the sea level and a temperate climate (Rizzoli et. al., 2011). Regarding tick distribution, spatial models should be developed to identify high-risk areas using environmental and climatic features (Estrada-Pena et. al., 2009). One such study showed increasing suitability for *I. ricinus* in some parts of Europe (Portugal, Spain, Italy, France, some areas of UK, Ireland), while other decreased suitability (Balkans, Southern Scandinavia and central part of Europe) (Estrada-Pena et. al., 2011). Geographic information systems (GIS) provide valuable information about the probability of encountering infected ticks based on environmental features, while serological survey detection of antibodies establish the prevalence of the disease in dog and human population.

Dogs are a good sentinel to asses the risks for Lyme disease in humans, as they are also susceptible for *B. burgdorferi* s.l. infection, they harbour more often ticks, are in closer contact with tick habitat, their residence is better define and usually their long distance travels are having a better history than their owners (Davoust, 1998; Doby, 1988; Bowman et al., 2009). These data are important when a geographical Lyme disease status is to be determined. Dog serum samples, as a surveillance can detect changes for the *B. burgdorferi* exposure with veterinary and public health significance (Goossens et al., 2001; Bowman et al., 2009).

The ecology of Lyme borreliosis

The maintenance of the infection in nature is given by a wide range of mammals and birds. Most of them are reservoir, as well. The bridge between the reservoir and the organisms where these spirochetes produce the disease is represented by hard-ticks, which through their blood meal acquire or infect the host. Larval stages of ticks attach to small or medium size vertebrate or non-vertebrate (rodents, lizards, birds etc.) for their first blood meal when they usually get infected. The nymphal stages look for medium or large vertebrate, as host. Companion animals and humans are at risk in equal measure, when infected nymphs chose them as a host. Establishing the rate of infection with *B. burgdorferi* s.l. in one of the hosts (ticks, companion animals e.g. dogs/ horses or humans) gives good indication about the risk of the infectivity for the other two.

Tick habitat is generally represented by deciduous, mixed forests, surrounding areas, clearings, marshes, forest plantations (40 nymps /m^2 in Swedeen) (Lindostrom and Jaenson, 2003). Forest ecosystems are acting as a "buffer", protecting ticks to extreme temperature exposure and keep a good humidity level (an annual average temperature of 8°C and UR of 95%, were correlated with a highest density of ticks (Montomoli, 2013). Positive correlations were also made between fertile soils and deer population, although the last has only role in maintaining tick populations without being a competent reservoir host (Vor et al., 2010). Land use and altitude are negatively correlated with LB incidence (Jouda et al., 2004). Although chances of tick bite are greater in this kind of areas, recent studies showed high density of ticks in urban and peri-urban areas, increasing the risk of tick-borne diseases for humans and companion animals (Rizzoli al., 2011). Urbanization has created good condition for rodents (dormice, mice, voles, squirrels, rats, shrews), rabbits, hedgehog, birds, lizards. Some of them are so well adapted and are found in higher density than in their natural

ecosystem (Pfaffle et al., 2013). They fulfil the role of tick-maintenance hosts and some of them are reservoirs for tick's pathogens as well (Mihalca et al., 2012).

Green areas, such as parks, neglected areas with vegetation, are an encounter place for humans, domestic animals and ticks. Different activities are associated with a potential threatening environment (Randolph, 2002; Smith et al., 1988). LB incidence is increasing in human and dog populations, particular if they spent time in tick's habitat, thus rangers, hunters, as hunting dogs, have a higher risk in contacting *Borrelia spp*. (Stefancikova et al., 1996).

Given that rural and urban areas are in a continue changing, especially in developing countries due to the economic or social factors (e.g. high density population), updated or even new information about potential risk for zoonotic diseases are even more valuable.

Lyme borreliosis in human and dog populations

In Europe, the mean of annual LB cases in human population is over 65,000 (Rizzoli et al., 2011). The incidence measured by country, ranges from less than one to about 350 per 100.000 inhabitants (Hubálek, 2009). Northern and central countries have higher prevalence then southern countries (Rizzoli et al., 2011). At local level, there are hotspots where yearly more than 100 new cases are reported (e. g. north-eastern Poland, some parts of Slovenia, Austria, Germany, southern Sweden, some Estonian and Finish islands) (Rizzoli et al., 2011).

Prevalence of LB in Europe varies also in dog populations: in Latvia has been reported to be 2.49 % (11/441- a healthy dogs group) (Berzina et al., 2013); similarly, the seroprevalence in dog population in Romania was reported as 6.72 % (Kiss et al., 2011), in Sweden 3,9%, in Czech Republic 6,5%, and France 1.09% (Pantchev et al., 2009).

Given that the rate of host's infection is strictly depending on tick habitat, which continues to enlarge, serologic surveillance screenings are needed to keep update data on the veterinary and public health risks for LB.

Dogs are a good sentinel to express the human Lyme disease risk as they largely share the same environment as their owners and they use the same outdoors (Goossens et al., 2001). IgG antibodies anti-*Borrelia* are lasting rather short time, about 1 year, maximum 2 years post infection (Guerra et al., 2001) in dogs, while in humans lasts for several years (Hovius et al., 1999). Thus, results from dog serologic surveillance studies are more likely to express new LB infections rather than prevalence.

Dog or human antibodies anti-*Borrelia* sera detection are both sensitive tools to express the risk for LB; however, in some regions, the use of dog as a sentinel is a more reliable method than keeping reports of LB incidence for human clinical cases (Goossens et al., 2001).

Establishing risk factors and certain correlations between them and incidence of the infection in both human and dog populations, were also goals for several studies. Information with local but also global relevance have been obtained. To identify antibodies anti-*Borrelia*, the enzyme linked immunoasorbent assay (ELISA) and IFA test are the most indicated methods for screening, although IFA is less sensitive (Voller et al., 1980).

Bowman et al. (2009) have reported the results of a national serologic survey study, in the USA, testing dogs for four vector-borne disease agents, including *B. burgdorferi*, using an ELISA-based method. The prevalence, varied between 4.0 % and 11.6% (Bowman et al., 2009). The results were then compared with those reported in human populations from the

areas, data provided by the Center for Disease Control and Prevention. Based on this analysis, it was concluded that in regions were the seroprevalence in dogs was lower, <1%, a correlation with an annual media of 0.2 at 100.000 inhabitants incidence was found; while, in areas where a seroprevalence in dogs was over 5%, it was correlated with an incidence of LB over 25.9 per 100.000 inhabitants (Bowman et al., 2009).

A similar study of Guerra et al. (2001), testing 1.077 dogs sera. From these, 234 were coming from asymptomatic dogs, and was obtain a 53.4% seropositive results in Southern Connecticut and 34.7% seropositive results in New Jersey. For the rest of the cases that presented indicating LB symptoms, the infection rate was higher: 76.2% in New York, and 66.5% in Connecticut. An overall prevalence of 52.2 % (562/1.077) tested positive for antibodies anti-*B. burgdorferi* s.l. Of these, 461 reacted to at least one of the immunoblot bands. Using a regression based method, it was established that a number of 105 dogs that were natural infected, 372 were classified as vaccinated and 16 were both (Guerra et al., 2001).

In the UK is considered that incidence of LB in humans have grown in the past 2 decades. If in the year 2000 it was considered to be 0.38 per 100.000, in 2009 the incidence was 1.79 per 100.000 inhabitants (https://www.gov.uk). It is believed that this rise is due to multiple factors: increasing of tick populations through their adaption to northern climate, new distribution of their hosts and reservoir in urban and peri-urban areas, the obligation of microbiology laboratories to report diagnosed LB cases, improvement of the diagnostic tools and also socio-economic factors (Schule-Spechtel et al., 2003; Skogman et al., 2008; O'Brien, 2009).

Studies on dog and tick populations were also performed. One study involved a total of 3534 dogs from which there were collected 810 ticks. 17 were PCR positive for *B. burgdoprferi* s.l. This gives an estimation of 0.5 of infected ticks per hundred thousand dogs (Smith et al., 2011).

In Scotland, prevalence of LB in human population varies between 1.25 and 16.5 per hundred thousand human populations (Joss et al., 2003).

In Romania Kiss et al. (2011) tested 276 dogs for anti-*Borrelia* antibodies and found an overall prevalence of 6.72 % (18/276); a higher prevalence (46.15%) was found in a midcountry region. They also tested 260 horse sera and obtained a prevalence of 11.92% (Kiss et al., 2011).

In a study conducted on 389 *I. ricinus* ticks by Briciu et al. (2014) it was reported a prevalence of *B. burgdorferi* s.l. infection of 11.1%. Rauter et al. (2005), following a meta-analysis of surveillance data, reported that in Europe, mean prevalence of *Borrelia* spp. infection in ticks was 13.7% (range: 0-49.1%) (Rauter et al., 2005).

Serologic surveys found no correlations between age, breed, gender, weight, vaccination or tick control products and the rate of *B. burgdorferi* s.l. infection in human or dogs populations (Guerra et al., 2001). Overall, serologic surveillance found positive correlations between LB cases in human or dog and tick populations (Guerra et al., 2001; Kitron and Kazmierczak, 1997). Higher incidences were found in human and dogs that spend longer periods outdoors (e.g. hunters, service dogs) (Vos et al., 1996; Kuiper et al., 1993; Kiss et al., 2011).

To conclude, the relevance of dogs as sentinel concerning risks for LB has been proven through many studies (Lindenmayer, 1991; Bowman et al., 2001; Olson, 2000; Mead

et al., 2011; Little et al., 2014); however, due to the continuous changing in the eco-epidemiology of the disease, serological survey are needed for early recognition, monitoring and prediction of LB endemicity status (Lastavica, 1989; Schulze, 1986; Goossens et al., 2001).

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REFERENCES

1. Balakrishnan N., Musulin, S., Varanat, M., Bradley, J.M., Breitschwerdt, E.B., 2014. Serological and molecular prevalence of selected canine vector borne pathogens in blood donor candidates, clinically healthy volunteers, and stray dogs in North Carolina. Parasites & Vectors, 7:116.
2. Berzina I., Capligina V., Bormane A., Pavulina A., Baumanis V., Granta R., Matise I, 2013. Assosiation between *Anaplasma phagocytophilum* seroprevalence in dogs and distribution of *Ixodides ricinus* and *Ixodes persulcatus* ticks in Latvia, TTBDis, 4: 83-88.
3. Bowman D., Little S.E., Loretzen L., Shields J., Sullivan M.P., Carlin E.P., 2009. Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic- based serologic survey. Veterinary Parasitology, 150(1-2): 138-148.
4. Briciu T. V., Meyer F., Sebah D., Tatulescu F. D., Coroiu G., Lupse M., Carstina D., Mihalca D. A., Hizo-Teufel C., Klier C., Hubrer I., Fingerle V., 2014. Real-time PCR-based identification of *Borrelia burgdorferi* sensu lato species in ticks collected from humans in Romania. Tick and Tick-borne Disease, 5 (5): 575–581.
5. Coipan E.C., Vladimirescu A.F., 2011. *Ixodes ricinus* ticks (Acari:Ixodidae): vectors for Lyme disease spirochetes in Romania. Experimental and Applied Acarology, 54 (3): 293-300.
6. Coipan E.C., Vladimirescu A.F., 2010. First report of Lyme disease spirochetes in ticks from Romania (Sibiu County). Experimental and Applied Acarology, 52(2): 193-197.
7. Durrani A. Z., Sagar M. G., Nadeem K., 2011. Retrospective Study on Seroprevalence of *Borrelia burgdorferi* Antibodies in Horses in Minnesota. Journal of Equine Veterinary Science, 31 (8): 427-429.
8. Davoust B., and Boni M., 1998, Lyme-disese in dogs- seroepidemiological survey in the south-east France, Medicine et Maladies Infectieuses, 28:408-409
9. Doby J., Chevrier J. S. Couatarmanach A., 1988. La spirochetose a tiques par *Borrelia burgdorferi* chez les chiens dans louest de la France, Rec. Med. Vet., 164: 367-374.
10. Hovius K.E., Rijpkema S.G., Westers P., van der Zeijst B.A.M., van Asten F.J.A., Houwers D.J., 1999. A serological study of cohorts of young dogs, naturally exposed to *Ixodes ricinus* ticks, indicates seasonal reinfection by *Borrelia burgdorferi* sensu lato. The Veterinary Quartely, 21 (1): 281-285.
11. Estrada-Pena A., Ortega C., Sanchez N, Desimone L, Sudre B, Suk J.E, Semenza J.C., 2011. Correlation of *Borrlia burgdorferi* Sensu Lato Prevalence in Questing *Ixodes ricinus* Tick with Specific Abiotic Traits in the Western Palearctic. Applied and Environmental Microbiology, 77 (11): 3838-3845.
12. Estrada-Pena A., 2009. Diluting the dilution effect: a spatial Lyme model provides evidence for the importance of habitat fragmentation with regard to the risk infection. Geospatial health, 3(2): 143-155.
13. Falco R.C., Smith H.A., Fish D., Mojica B.A., Bellinger M.A., Harris H.L., Hechemy K.E., 1993. The distribution of canine exposure to *Borrelia burgdorferi* in a Lyme-Disease endemic area. American Joumal of Public Health, 83 (9): 1305-1310.

14. Goosens H.A.T., van den Bogaard A.E., Nohlmans M.K.E., 2001. Dogs as sentinel for Lyme boreliosis in The Netherlands. Journal of clinical microbiology, 39 (3): 844-848.
15. Guerra M.A., Walker E.D., Kitron U., 2001. Canine surveillance system for Lyme borreliosis in Wisconsin and northern Illinois: geographic distribution and risk factor analysis. The American Journal of Tropical Medicine and Hygiene, 65 (5): 546-552.
16. Hubálek Z., 2009. Epidemiology of Lyme borreliosis. Current problems in dermatology, 37: 31-50.
17. Jouda F., Pettet J.L., Gern L., 2004. *Ixodes riscinus* density, and distribution and prevalence of *Borrelia burgdorferi* sensu lato infection along an altitudinal gradient, Journal of Medical Entomology, 41(2): 162-169.
18. Kiffner C., Zucchini W., Schomaker P. Vor T. Hagedorn P. Niedrid M., Ruhe F., 2010. Determination of tick-borne encephalitis in counties of southern Germany 2001-2008. International Journal of Health Geographics, 9: 42.
19. Kiss T., Cadar D., Krupaci A.F., Bordeanu A., Brudasca G.F., Mihalca A.D., Mircean V., Gliga L., Dumitrache M.O., Spinu M., 2011. Serological reactivity *to Borrelia burgdorferi* sensu lato in dogs and horses from distinct areas in Romania. Vector Borne Zoonotic Disease, 11(9): 1259-1262.
20. Kitron U., Kazmierczak J.J., 1997. Spatial analysis of the distribution of Lyme disease in Wisconsin. American Journal of Epidemiology, 145 (6): 558-566.
21. Kuiper H., van Dam A.P, Moll van Charante A.W., Nauta N.P., Dankert J., 1993. One year follow-up study to assess the prevalence and incidence of Lyme borreliosis among Duch forestry workers. European Journal of Clinical Mocobiology & infectios diseases, 12 (6): 413-418.
22. Lambin E.F., Vanwambeke T.A., Linard S.O., Soti V., 2010. Pathogenic landscapes: interaction between land, people, desease vectors, and their animal host, International Journal of Health Georgraphics, 9: 54.
23. Lastavica C.C., Wilson M.L., Berardi V.P., Spelman A., Deblinger R.D., 1989. Rapid emergence of a focal epidemic of Lyme disease in coastal Massachusetts. The New Englang Journal of Medicine, 320: 133-137.
24. Lindenmayer J.M., Marshall D., Onderdonk A.B., 1991. Dogs as sentinels for Lyme Disease in Massachusetts. American Joumal of Public Health, 81 (11): 1448-1455.
25. Lindstrom A., Jaeson T.G., 2003. Distribution of the common tick*, Ixodes ricinus* (Acari*: Ixodidae*), in different vegetation type in southern Sweden. Journal of Medical Entomology, 40 (4): 375-358.
26. Little S., Beall M.J., Bowman D.D, Chandrashekar R., Stamaris J., 2014. Canine infection with *Dirofilaria immitis, Borrelia burgdorferi, Anaplasma* spp., and *Ehrichia* spp. in the United States, 2010-2012. Parasites & Vectors, 7: 257.
27. Mead P., Goel R., Kugeler K., 2011. Canine serology as adjunct to human Lyme disease surveillance. Emerging Infectious Diseases, 17 (9): 1710-1712.
28. Mihalca A.D., Dumitrache M.O., Sándor A.D., Magdaş C., Oltean M., Györke A., Matei I.A., Ionică A., D'Amico G., Cozma V., Gherman C.M., 2012. Tick parasites of rodents in Romania: host preferences, community structure and geographical distribution. Parasites & Vectors, 5: 266.
29. Montomoli E., 2009. A new seroepidemiological survey in Italy. Lecture, ISW Vienna, htpp://www.isw-tbe.info.
30. O' Brien E.A., Morris J., 2009. Journal Active England: the woodland project. Edinburgh, UK: Forestry Commission.
31. Olson P.E., Kallen A.J., Bjorneby J.M., Creek J.G., 2000. Canines as sentinels for Lyme disease in San Diego County, California. Journal of Veterinary Diagnostic Investigation, 12 (2): 126–129.
32. Pantchev N., Schaper R., Limousin S., Norden N., Weise M., Lorentzen L., 2009. Occurrence of *Dirofilaria immitis* and tick-borne infections caused by *Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato and *Ehrichia canis* in domestic dogs in France: results of a countrywide serologic survey. Parasitology Research, 105 (1): S101-114.
33. Pfaffle M., Littwin N., Muders V.S., Petney N.T., 2013. The ecology of tick-borne diseases. International Journal of Parasitology, 43: 1059-1077.
34. Randolph S., 2002. Predicting the risck of tick-borne disease. The international journal of microbiology, 291 (33): 6-10.
35. Rauter C., Hartung T., 2005. Prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes riscinus* in Europse: a metaanalysis. Applied and environmental microbiology, 11: 7203-16.

36. Rizzoli A., Haufe H.C., Carpi G., Vorch G., Neteller M., Rosa R., 2011. Lyme borreliosis in Europe, Eurosurveillance, Volume 16, issue 27
37. Rizzoli A., Silaghi C., Obiegala A., Rudolf I., Hubálek Z., Földvári G., Plantard O., Vayssier Taussat M., Bonnet S., Spitalská E., Kazimirová M., 2014. *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Frontiers in Public Health, 2: 251.
38. Schulte-Spechtel U., Lehnert G., Liegl G., Fingerle V., Heimerl C., Hohnson F., 2003. Significant improvement of the recombination *Borrelia*-specific immunoglobin G immunoblot test by addition of VlsE and DbpA homologue derived from *Borrelia garinii* for diagnostic of early neuroborreliosis. Journal of Clinical Microbiology 27: 1299-3013.
39. Schulze T.L., Shisler J.K., Bosler E.M., Lakat M.F., Parkin W.E., 1986. Evolution of a focus of Lyme disease, Zentralblatt Bakteriol. Microbiol. Hyg. A., 263 (1-2): 65-71.
40. Schurer J.M., Ndao M., Queswezance H., Elmore S. E., Jenkins E.J., 2014. People, pets and parasites: one health surveillance in Southestern Saskatchewan. The American Journal of Tropical Medicine and Hygiene, 90(6): 1184-1190.
41. Skogman B.H., Croner S., Forsberg P., Ernerudh J., Lahdenne P., Sillanpaa H., et al., 2008. Improved laboratory diagnostics of Lyme neuroborreliosis in children by detection of antibodies to new antigens in cerebrospinal fluid. Pediatric Infection Disease Journal, 27:605-12.
42. Skotarczak B, 2002. Canine borreliosis - epidemiology and diagnostics. Annals of Agricultural and Environmental Medicine, 9 (2): 137–140.
43. Smith F.D., Ballantyne R., Morgan E.R., Wall R., 2011. Prevalence, distribution and risk associated with tick infestation of dogs in Great Britain. Medical and Veterinary Entomology, 25(4): 377-384.
44. Sperling J.L.H., Middelveen M.J., Klein D., Sperling F.A.H., 2012. Evolving perspectives on Lyme borreliosis in Canada. The Open Neurology Journal, 6: 94-103.
45. Strle F., Ruzic-Sabljic E., Cimperman J., Lotric-Furlan S., 2006. Comparison of Findings for Patients with *Borrelia garinii* and *Borrelia afzelii* isolated from Cerebrospinal Fluid, Clinical Infectious Diseases, 43 (6): 704-710.
46. Stefanickova A., Skardova I., Petko B., Janovska D., Cyprichova V., 1996. IgG antibodies to Borrelia in dogs in the area of Kosice. Veterinary Medicine (Praha), 41 (3): 83-86.
47. Stone E.G., Lacombe E.H., Rand P.W., 2005. Antibody testing and Lyme disease risk. Emerging Infectious Diseases, 11 (5): 722-724.
48. Stricker R.B., Johnson L., 2011. Lyme disease: the next decade. Infection and Drug Resistence, 4: 1-9.
49. Voller A., Bidwell D., Bartlett A., 1980. Enzyme-liked immunoasorbent assay, Rose NR, Friedman H., EDS. Manual of Clinical Immunology. Second edition, Washington, DC: American Society for Microbiology, 359-371.
50. Vor T., Hagedorn P., Niedring M., Ruhe F., 2010. Tick burden on European roe deer (*Capreolus capreolus*). Experminetal and Applied Acarology, 51 (4): 405-417.
51. Vos K., van Dam A.P., Kuiper H., Spanjaard L., Dankert J., 1994. Seroconvertion of Lyme borreliosis among Duch military. Scandinavian Journal of Infection Disease, 26:427-434.
52. Wagner B., Freer H., Rollins A., Garcia-Tapia D., Erb N. H., Earnhart C., Marconi R., Meeusc P.,2012. Antibodies to *Borrelia burgdorferi* OspA, OspC, OspF, and C6 antigens as markers for early and late infection in dogs. Clinical and Vaccine Immunology: CVI, 19 (4): 527-535.
53. Wagner B., Erb H.N., 2012. Dogs and horses with antibodies to outer-surface protein C as on-time sentinels for ticks infected with *Borrelia burgdorferi* in New York State in 2011. Preventive Veterinary Medicine, 107(3-4): 275-279.
54. Wang G., 2015. *Borrelia burgdorferi* and Other *Borrelia* Species. Molecular Medical Microbiology, 3:1867-1909.

HORMONAL EFFECT OF THE VARIOUS TREATMENTS APPLIED DURING THE MATURATION AND DEVELOPMENT OF BOVINE OOCYTES IN VITRO

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Abstract:*The aim of this research was to evaluate the effects of different hormonal treatments on bovine oocytes in the maturation, fecundation and, development stages. For this experiment we used three groups of bovine oocytes: two experimental groups with different hormonal treatment and control. For all working groups we used Fetal Bovine Serum (FBS). Also, for hormonal treatment we used Follicle-Stimulating Hormone (FSH) for the first experimental group and Human Chorionic Gonadotropin (hCG) for the second one. The rate of maturated oocytes in the Metaphase II varied from 73.3% for control to 85.7% for second group (with hCG), with significant differences (P<0.01) between the hormonal treatment groups and the control group. The maturated oocytes in Tissue Culture Medium (TCM) supplemented with hCG (second experimental group) displayed a better cumulus cell expansion compared to maturated oocytes in TCM supplemented with FSH (first experimental group). Fertilization rate was significant affected (P<0.01) after the treatment with FSH (81.25%) and after treatment with hCG (93.33%) compared to the control (85.7%). The blastocyst rate obtained after 168 hours post-insemination was significant higher (P<0.01) for oocytes maturated with gonadotropin (second experimental group – with hCG). The blastocyst rate after 7 days of culture in B2-Menezo medium and hormonal treatment varied between 41.66% and 44.11% compared to the control (37.5%). Our results emphasized that bovine oocytes maturated in vitro presents a high maturation and fertilization rate, both in the presence or absence of gonadotropin. Also, the treatment with hCG it seems to be the most beneficial for the cytoplasm and nuclear maturation, leading to a high rate of obtained blastocyst in vitro.*

Key words: *oocytes maturation, development, FSH, hCG*

INTRODUCTION

Under physiological conditions cow ovulated one oocyte in each cycle and therefore can only produce a calf each year. When trying to obtain eggs for in vitro fertilization and embryo production must get mature oocytes in vitro. The maturation of oocytes can be defined as the set of changes that must undergo these cells before they can respond to fertilization and support the early stages of embryonic development. This process involves at least tree events: nuclear maturation, cumulus expansion and cytoplasmic maturation (Kakkassery et al., 2010).

Supplementation of the culture medium with gonadotropins and steroids has shown a favorable effect on bovine oocyte maturation (First and Parrish, 1987; Sirard et al., 1988).

In recent years we have greatly improved methods of culture of embryos (Milovanov and Herradon, 1996). They can develop some genetic engineering techniques and micromanipulation of embryones. The culture media used contain all the vitamins,

essential amino acids and other products necessary for cell growth, ensuring the development of high percentage of embryos to the 8-cell stage.

However these means do not allow embryos exceed the critical phase of its evolutionary process of 8-16 cells (Sirard et al., 1991) and at this stage is a change in the control of cell functions. Until now such control has been exercised by the RNA inherited from the mother, present in the egg at the time of fertilization and already from this stage, activation of the embryo genome occurs, so that RNA becomes embryonic origin (Eyestone and First, 1989; Camous et al., 1984).

MATERIAL AND METHODS

Maturation in vitro (IVM)

Oocytes were selected and transferred to micro drops of maturation medium. Maturation medium consist of: culture medium TCM-199, 10% FBS, 0.2mM pyruvate and 50 µg/ml gentamycin sulfates. This medium was supplemented with the following hormones: ovine FSH (0.5µg/ml; NIADDK), hCG (10µg/ml; NIADDK). For this experiment we used three groups of bovine oocytes: two experimental groups with different hormonal treatment and control. For all working groups we used Fetal Bovine Serum (FBS). Also, for hormonal treatment we used Follicle-Stimulating Hormone (FSH) for the first experimental group and Human Chorionic Gonadotropin (hCG) for the second one.

The duration of the incubation period was 24 hours and was held at a temperature of 38.5^0C, 5% CO_2 and maximum relative humidity. Maturation of oocytes was evaluated by observing the expansion of the cumulus cells and the expulsion of the first polar body.

Fertilization in vitro (IVF)

IVF was performed according to the procedure of Parrish et al. 1986, and 1988. After 18 hours of incubation, we proceeded to a new examination of oocytes, considering that the fertilization was successful in all those which contained two pronuclei.

Development in vitro

Newly obtained zygotes were washed and then the young embryos were placed in B2 medium supplemented with 10% cow serum in heat and oviductal epithelial cells. At 48 hours of culture was verified the rate of segmentation and the development of blastocyst was completed after 7 days.

RESULTS

The proportion of oocytes that matured to Metapfase II in the 3 experiment used varied from 73.3% to 85.7% (table 1) with statistically significant differences ($P<0.01$) between the media containing hormones and those that do not contained oocytes matured in TCM-199 supplemented with hCG and FBS showed good expansion of the cluster (cumulus) compared to the other groups.

When the oocytes were examined after 18 hours of co-culture with sperm, we found that the percentages of penetrated and polyspermic oocytes showed statistically significant differences ($P<0.01$) as a function of their maturation had occurred in the presence or absence of gonadotropine (table 1).

Fertilization rate was significant affected (P<0.01) after the treatment with FSH (81.25%) and after treatment with hCG (93.33%) compared to the control (85.7%). The blastocyst rate obtained after 168 hours post-insemination was significant higher (P<0.01) for oocytes maturated with gonadotropine (second experimental group – with hCG). The blastocyst rate after 7 days of culture in B2-Menezo medium and hormonal treatment varied between 41.66% and 44.11% compared to the control (37.5%). Our results emphasized that bovine oocytes maturated *in vitro* presents a high maturation and fertilization rate, both in the presence or absence of gonadotropine. Also, the treatment with hCG it seems to be the most beneficial for the cytoplasm and nuclear maturation, leading to a high rate of obtained blastocyst *in vitro*.

Table 1. The effects of different hormonal treatment applied to bovine oocytes in maturation, fertilization, and development *in vitro* [%]

Treatment*	**IVM****	**Fertilization**				**Development**	
	Nr of maturated oocytes from total	**Nr of penetrated from total**	**Nr with 2 pronuclei / penetrated**	**Nr of polyspermic from total**	**Nr of normal fertilized from total**	**Nr with 2-4 cell from total**	**Nr of blastocyst from total divided**
1	73.3	87.5	85.7	12.5	75	66.6	37.5
2	84.6	100	81.25	18.75	81.25	70.58	41.66
3	85.7	93.75	93.33	6.25	87.50	82.92	44.11

* All treatments included 3 replications; ** IVM – *in vitro* maturation; (P<0.01)

1 – TCM-199 with FBS, without hormones; 2 – TCM-199 with FBS with FSH (0.5μg/ml); 3 – TCM-199 with FBS with hCG (10μg/ml)

DISCUSSIONS

Some researches demonstrate that bovine oocytes are capable to arrive, *in vitro*, at a well maturation stage – when this process takes place in the absence of gonatropine (Goto et al., 1988). However, in other studies it was showed that addition of gonadotropine has beneficial effect *in vitro* oocytes maturation.

Our results indicate that the bovine oocytes matured *in vitro* have a good rate of fertilization and maturation, both in the presence and absence of gonadotropins. However, the modifications that occur during maturation influence evidently the ability to sustain the embryo development. Thus, the oocytes cultured in the presence of gonadotropine showed a greater ability to induce formation of male pronucleos and a higher rate of development to 2-4 cell stage and blastocyst.

The addition of gonadotropine to the culture medium accelerates the resumption from the Meiosis of bovine oocytes, similarly with that observed in other species (Johnston et al., 1989; Moor et al., 1981; Wolfenson et al., 2004). As well, the gonadotropine influences favorable the oocytes rate that arrive to Metaphase II. However, the mechanism of positive influence of gonadotropine of oocytes maturation it is not well known. Based on our results, we can confirm that hCG seems to be the most beneficial for nuclear and cytoplasm maturation – which is related with the rate of obtained blastocyst. This data match the observations made by Brackett et al. (1989) that states that hCG acts selectively improving the quality of oocytes maturated *in vitro*. The same

author demonstrates later that the effects of LH and hCG of *in vitro* maturation of bovine oocytes are comparable (Brackett et al., 1994).

Thus, our results indicate that the oocytes cultivated *in vitro* tend to reassume to the Meiosis spontaneously, while the cytoplasm maturation take place. Also, the addition of gonadotropine to the culture medium accelerates the meiotic progression and improves the quality of oocytes maturated *in vitro*.

Data presented above indicate the need to further investigate the hormonal mechanisms involved in the process of maturation of the ovocidos, having the ultimate goal the obtaining of new procedures for maturation and, thus, to improve the performance of the technical of bovine embryos obtaining *in vitro*.

The zygotes presumptive obtain *in vitro* fecundation were cultivated with different successful rate, both with a great variety of cellular types (granulosa cells, oviduct epithelial cell, trophoblast and fibroblast, Buffalo rat liver cells – BRL).

However, the best results were obtained using the oviduct epithelial cells and a culture medium supplemented with serum and gonadotropine. Based on these data it was suggested the possibility of using the estrus cow serum because of its high content of gonatropine ascertain its capacity of assure the favorable environment for embryo development. Thus, the results demonstrate that the simultaneously addition of serum and somatic cells increase significantly the potential of development of zygotes, leading us to the opinion that one or more benefic factors are not specific. Moreover, it is also not known whether the beneficial effect of serum and somatic cells is a result of contribution to the culture medium of components specific required for development or of the elimination of inhibitory substances of the components from the culture medium (Bavister et al., 1992).

More actual were made some research on the elimination of somatic cells from culture medium and these were supplemented with essential and non-essential amino acids with satisfactory results (Trounson et al., 1994; Milovanov and Herradon, 1996).

The TCM-199 was successful used in cultivation of bovine and sheep embryos (Gandolfi and Moor, 1987; Eyeston and First, 1989). Meanwhile, the B2 medium started to be used in the *in vitro* fecundation, and later for the cultivation of human embryos (Menezo et al., 1984), and then for cultivation of bovine embryos (Marquant-Le Guienne et al., 1989).

Improving the culture medium allows to obtain transferable embryos with normal characteristics, ensuring they are free of contact to specific pathogens for the bovine species. All these findings provide a great hope for the rapid development of scientific knowledge and will allow in the future, the application of the *in vitro* fecundation to advance animal reproduction and genetic improvement.

REFERENCES

1. Bavister, B.D., Rose-Hellekant, A., Pinyopummintr, T.(1992). Development of in vitro matured in vitro fertilized bovine embryos into morula and blastocysts in defined culture media. Theriogenology 37: 127-146.
2. Brackett, B.G., Younis, A.I., et Fayer-Hosken, R.A.. (1989). Enhanced viability after in vitro fertilization of bovine oocytes matured in vitro with high concentrations of luteinizing hormone.Fertil.Steril.52: 319-324.

3. Brackett, B. et KESKINTEPE, l. (1994). Aspects of bovine oocyte maturation and embryo production in vitro.7th International Meeting on Animal Reproduction Murcia.Spain.27-3
4. Camous, S., Heyman, Y., Meziou, W. et Menezo, Y. (1984). Cleavage beyond the block stage and survival after transfer or early bovine embryos cultured with trophoblastic vesicles. J. Reprod. Fertil.72:479-485
5. Eyestone, W.H. et First, N.L. (1989). Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or in conditioned medium. J. Reprod. Fertil. 85:715-720.
6. First, N.L. et Parrish, J.J.(1987). In vitro fertilization in ruminants. J. Reprod. Fertil. 34 (Suppl):151-165.
7. Gandolfi F. et Moor, R.M. (1987). Stimulation of early embryonic development in the sheep by coculture with oviduct epithelial cells. J. Reprod. Fertil. 81:23-28.
8. Goto, K., Kajihara, Y., Kosaka, S., KOBA, M., Nakanishi, Y. et OGAWA, K.(1988). Pregnancies after co-culture of cumulus cells with bovine embryos derived from in–vitro fertilization of in-vitro matured follicular oocytes. J. Reprod. Fertil. 83:753-758.
9. Johnston, L.A., O'brien, S.J. et Wildt, D.E. (1989). In vitro maturation and fertilization of domestic cat follicular oocytes. Gamete Res. 24:343-356
10. Kakkassery, M.P., Vijiyakumaran, V., Sreekumaran, T. (2010), Effect of cumulus oocyte complex morphology on in vitro maturation of bovine oocytes, J. Vet. Anim. Sci., 41:12-17.
11. Marquant-Le Guienne, B., Gerard, M., Solari, A., et Thibault, C.(1989). In vitro culture of bovine egg fertilized either in vivo or in vitro. Reprod. Nutr. Dev. 29:559-568.
12. Menezo, Y., Testart, J et Perrone, D (1984). Serum is not necessary in human in vitro-fertilization, early embryo culture, and transfer .Fert. Steril. 42:750-755
13. Moor, R.M., Osborn, J.C., Cran, D.C. et Walters, D.E.(1981). Selective effect of gonadotrophins on cell coupling, nuclear maturation and protein synthesis in mammalian oocytes. J. Embryol. Exp. Morph.61:347-365
14. Milovanov, C et Herradon, P.G. (1996). In vitro development of IVM/IVF bovine embryos in B2 medium supplemented with albumin (BSA) or polyvinyl alcohol (PVA). Theriogenology 45:215.
15. Parrish, J.J., Susko-Parrish, J.L., Leibfried-Rutledge, M.L., Critser, E.S., Eyestone, W.H., et First,N.L. (1986). Bovine in vitro fertilization with frozen-thawed semen. Theriogenology 25:591-600
16. Parrish, J.J., Susko-Parrish,J.L., Winer, M.A., et First, N.L.(1988). Capacitation of bovine sperm by heparin. Biol. Reprod. 38:1171-1180.
17. Sirard, M.A., Parrish,J.J., Ware, C.B., Leibfried-Rutledge ,M.L., et First,N.L.(1988). The culture of bovine oocytes to obtain developmental competent embryos. Biol. Reprod.39:546-552
18. Sirard, M.A., Gagne, M., Belanger, C., Leclerc, P., Milovanov, C et Coenen, K.(1991). Le development des embryons bovins en culture in vitro. Med. Vet. Quebec 22:7-11
19. Trounson, A., Pushett, D., Maclellan, L.J., Lewis, I., et Gardner, D.K. (1994). Current status of IVM/IVF and embryo culture in humans and farm animal. Theriogenology 41:57-66.
20. Wolfenson, D., Inbar,G., Roth, Y., Kaim, M., Bloch, A.,Braw-Tal, R., (2004). Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nuliparous heifers. Theriogenology 62: 1042-1055

EFFECT OF CYSTEINE SUPPLEMENTATION ON SOW CUMULUS CELLS AND ON BCL2 GENE EXPRESSION DURING IN VITRO MATURATION

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Abstract:*In vitro maturation (IVM) is the first step for IVP technique and the most important because the success of in vitro embryos production depends on oocyte quality. In order to protect the cell from oxidative damages and to improve fertilization rate and embryo developement, precursors of GSH such as cysteine, cysteamine, 2-mercaptoethanol are added to maturation medium. Another important role in oocyte development and maturation via intercellular communications are the cumulus cells, having an important role during maturation, ovulation, fertilization and embryonic development. Previous cumulus cells studies suggested that an increased expression of BCL2 gene family in cumulus cells succeed in being fertilized and developed into many cell embryos. The aim of our study was to observe the effect of cysteine supplement during in vitro maturation of sow COCs on morphological aspects of COCs after maturation and also its effect on cumulus cells BCL2 gene expression. Another purpose of the research was to compare expression of this gene in cumulus cells before and after maturation. The Bcl2 gene expression was evaluated by Quantitative Revers-Transcription PCR, having as target the total ARN isolated from cumulus cells maturated in media with or without Cysteine. The obtained data was interpreted by 2 ΔΔ C(T) method. Our results suggest that Bcl2 gene expression varies in different evaluative stages of oocyte but also depending on culture media composition. There is an increase of Bcl2 gene expression in the case of mature oocyte cumulus cells compared with immature oocyte cumulus cells and also, in the case of mature oocyte cumulus cells obtained from COC complexes cultivated in culture media supplemented with cysteine when compared to cumulus cell cultivated in culture media with no cystein addition although, at the morpho-structural level no differences were recorded between studied cumulus cells.*

Key words*: cumulus cells, in vitro maturation, gene expression*

In vitro maturation (IVM) represents a key step for in vitro fecundation (IVF), thus the environment in which the process of IVM will develop it will directly influence the IVF outcome. Removing the cumulus cells before IVM has negative effects on swine oocyte maturation (Chian and Niwa, 1994). This is why cumulus cells are considered very important for oocyte maturation, ensuring meiotic arrest, triggering meiosis resumption and supporting citoplasmic maturation. All these cumulus cells key functions during oocyte maturation relies on gap junctions network.

Oocyte-cumulus cells complex (COC) has a dynamic evolution during oocyte maturation. The loss of gap junctions from cumulus cells is related to meiosis resumption in preovulatory follicle. This process is happening perhaps due to the block of meiosis inhibition signals from external cumulus cells to oocyte (Isobe et al, 1998). In the second part of maturation, the cooperation between cumulus cells and oocyte is limited to corona radiata, while external cumulus cells are disconnected (Mattioli and Barboni, 2000). The gap junction downregulation is a prerequisite for cumulus cells expansion (Sutovsky et al., 1995).

Cumulus cells play an esential role in cumulus cells metabolism during cytoplasmic maturation of oocyte. Cumulus cells reduces cystine to cysteine, promoting also cysteine absorbtion to oocyte during IVM (Takahashi et al., 1993). As a result, oocytes surrounded by cumulus cells do contain more intracellular glutathione (GSH) than the denuded one (Geshi et al., 2000). Stable levels of GSH in IVM oocytes ensure a larger number of oocyte to be fertilized and reaching the blastocist stage. Glutathione is crucial for maintaining the redox state of cells and also protecting them against oxydative process (Lim et al., 1996). In swine, cumulus cells defend the oocyte againts oxidative stress by rising the oocyte GSH content (Tatemoto et al., 2000). Moreover, adding GSH precursors such as cysteamine or cystein to the culture medium can improve the IVM outcome in denuded oocyte, mainly by increasing the oocyte GSH content (De Matos et al., 1997).

In order to estimate the viability of pig oocytes after cysteine supplementation we have evaluated the morphology after IVM and also assayed the BCL2 gene expression before and after IVM.

BCL2 gene (cell B lymphom 2) is a member of Bcl-2 regulating proteins and its role is to regulate the cell death (apoptosis) byinducing or inhibit it. BCL-2 is considered a specific anti-apoptotic protein beeing classified as an oncogene. The apoptosis of granulosa cells process is implicated in ovarian folliculogenesis. In the follicles, cumulus cells are intimate connected to the oocyte creating the cumulus-oocyte complex (COC) and these cells are crucial for oocyte maturation and fertilization through bidirectional signals (Senbon et al., 2003). The studies regarding cumulus cells genes expressions (McKenzie et al., 2004) and cumulus cells apoptosis (Corn et al., 2005) emphasized the fact that cumulus cells could reflect the developmental potential of human embryo during IVF. The rise of number of apoptotic cells was related to a decrease of mature oocytes and also to a reduced fertilization rate during IVF (Host et al., 2000). The oocyte BCL-2 content reverberates the oocyte competence (Filali et al., 2009).

Swine oocytes were clasified using the criteria proposed by Antosik et al (2009), based on cytoplasm aspect and also on features of cumulus oophorus cell layers. Class I oocytes displayed a homogenous cytoplasm and also the cumulus cell layers was intact and compact, with more than 5 cell layers. These oocytes are accepted as suitable and do posses a high developmental and IVF potential. Commonly only class I oocytes are used for in vitro techniques, considering the fact that morphological features of COC is convenient for selecting the competent oocytes for IVF (Alvarez et al, 2009).

MATERIAL AND METHODS

The ovaries were obtained from slaughterhouse, from where were transported within one hour in a saline solution supplemented with antibiotics (Penstrep, 1ml/0.5 l) at 35-37º C. Oocytes were harvested by puncturing the ovaries with 18G needles, the follicular liquid beeing aspired into a 5 ml syringe and then introduced in sterile 50 ml tubes containing PBS. The sediment was removed using sterile pipetes and transferred in Petri dishes containg PBS for the first washing. Using the stereomicroscope, the intact oocytes surrounded by cumulus cells were removed and transferred in another Petri dish for the second wash. For the third wash the oocytes were transferred in TCM solution and finally, in droplets of TCM medium, supplemented (50%) or not (50%) with cysteine. They were incubated for 44 hours in a CO_2

5% medium, at 38.5º C. The TCM maturation medium consisted of 10 ml TCM199 with Earle`s salts ; 10 mg/ml BSA ; 0.1 mg/ml cysteine and 0.5 µg/ml FSH. On a 30 mm Petri dish were placed 4 droplets of 80 µl (3.6 µl FSH and 76.4 µl maturation media), covered with mineral oil. It remained in the incubator for 4 hours, at 38.5º C and CO_2 5%, for equilibration. For further use, unmatured, matured with or without cysteine, oocytes were in equally proportions denudated. The denuding process consisted in introducing the oocytes in Ependorf tubes containing PBS, and then the oocytes were aspirated and repressed consecutively. The denuded oocytes were removed and the liquid with cumulus cells was transferred in another Ependorf tube and was centrifuged for 5 minutes at 200G (1060 rpm). The sediment was recovered, suspended in PBS and again centrifuged using the same parameters. The supernantant was removed and the samples were dipped in liquid nitrogen for 1 minute and after were stored at -80º C until total RNA isolation.

Biological samples that were selected to undergo processing in this study are two of each: I - unmature oocytes; II - mature oocytes cultivated in cystein containing culture media; III – mature oocytes cultivated in cystein containing media.Prior to proceeding with RNA isolation and purification the cell samples were carefully washed with PBS buffer and centrifuged at 3000 x g for 5 minutes obtaining a sediment. Total ARN was isolated and purified from the sedimented cells using *SV Total RNA Isolation System* (Promega, US) commercial kit following the producers indications.

Quantity and quality of extracted RNA was assesed by measurements with *NanoDrop 8000* spectrophotometer (Thermo Scientific). From isolated RNA, the cDNA was synthesized using *First Strand cDNA Synthesis Kit* (Fermentas), following the producer indications, and oligo dT(8) primer, also provided with the kit. The cDNA obtained was used as template in qPCR reactions using Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific), according to provided protocol with a *Stratagene Mx3000P* (Agilent) real time PCR equipment. The primers sequences (table 1) used in this study were obtained from the reference literature (Jeon et al, 2012) and were synthesized by *Eurogentec* (Belgium).

Table 1.Sequences of primers used in this study

Expression marker	**Primer sense 5'-3'**	**Primer antisense 3'-5'**
BCL2	GAAACCCCTAGTGCCATCAA	GGGACGTCAGGTCACTGAAT
Reference gene (β-Actin)	CTCGATATGAAGTGCGACG	GTGATCTCCTTCTGCATCCTGTC

Each sample was analyzed in duplicate. As a negative control for each primer a sample, a template without DNA was tested. For the relative quantification the Δ (ΔCt) method was used (Livak and Schmittgen, 2001).For all of the samples the number of cycles(Ct) were determined. For relative quantification was used the Δ (ΔCt) method. According to this method the R (the relative ratio between the control and stressed variant) is calculated with the following formula: $R = 2^{-\Delta\Delta Ct}$

RESULTS AND DISCUSSIONS

It has been proven that many factors enhancedoocytes fertility and development when cultivated in vitro. Cysteine, as a precursor of the antioxidant glutation, is wide known to be

beneficial in oocyte maturation and male pro-nucleus formation. Also, glutation helps preventing the alteration of sperm cells membrane in many species. A study carried in 2001(Jeong and Yang) had shown thatthe presence of cystein in the culture media had significantly improved the blastocists development. Total RNA was successfully isolated from all biological samples and the quantity and quality were considered optimum to undergo qPCR experiments. The ratio of mRNA was normalized with β – Actin house keeping gene expression. The obtained data was interpreted with ANOVA software (table 2).

Table 2.Bcl 2 gene expression I - unmature oocytes; II - mature oocytes cultivated in cystein containing culture media; III – mature oocytes cultivated in cystein containing media.

Sample	Expression ratio	Average
Ia	1	1
Ib	1	
Ic	1	
Id	1	
IIa	3,68	3,765
IIb	4,5	
IIc	3,61	
IId	3,27	
IIIa	5,06	6,3375
IIIb	7,73	
IIIc	6,06	
IIId	6,5	

From our obtained data, resulted that Bcl2 gene expression varies in different stages of oocyte evolution, but also is positively influenced by culture media composition. Paradis et al. (2010) reported that bovine oocyte prevent apoptosis by modulation of BAX and BCL2 expression in the cumulus cells.

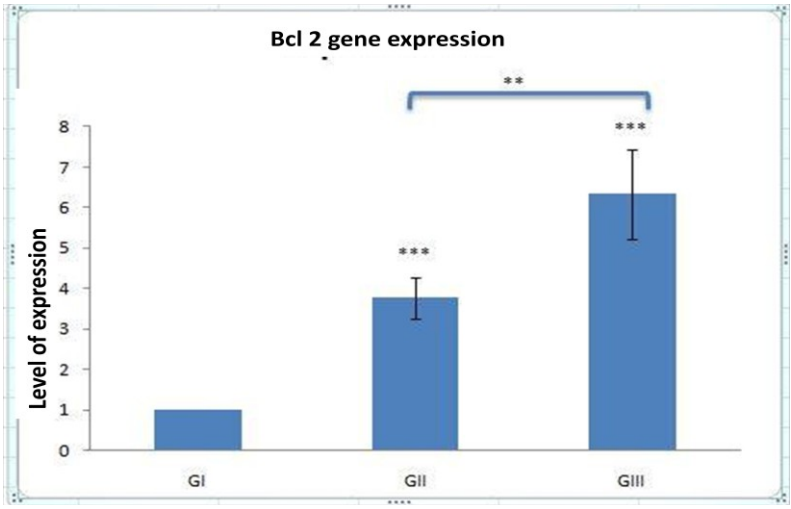

Figure. 1: Bcl-2 gene expression in cumulus cells becoming from COC I: before maturation, II: after maturation without Cysteine addition, III: after maturation with Cysteine addition.

Also, in the case of cumulus cells obtained from COC complexes cultivated in culture media with Cysteine addition, we have emphasized that the expression of Bcl2 is almost double compared with cumulus cells cultivated in culture media without Cysteine addition despite the fact that there were no differences at morpho-structural level (figure 1).

There is a increase in Bcl 2 gene expression in case of cumulus cells of mature oocyte compared with cumulus cells of immature oocyte. In a study from 2011, Feugang et al. reported that anti-apoptotic genes (Bcl2-like1) are critical for the survival of embryos and also that Bcl2-like1/Bax ratios were always in the direction of Bcl2-like1 transcripts which is favorable to embryo survival.

Based on *p* intervals (table 3) it is noticed that the obtained results are statistically significant.

Table 3. Statistic significance of obtained data

Ratio	*p* value	Signifiance
p I/II	0,0017	Significantly higher
p I/III	0,0023	Significantly higher
p II/III	0,0118	Significant

These results are in accordance with data published in 2014. Studying the effect of hormonal estrus induction on maternal effect and apoptosis-related genes expression in porcine cumulus-oocyte complexes, Bogacki et al. (2014) reported that the expression of apoptosis-related gene BCL-2 was significantly higher ($p \leq 0.05$) in COCs derived from gilts treated with PMSG/hCG group compared to PMSG/hCG + PGF2α treated gilts or control.

CONCLUSIONS

Cysteine supplementation in the maturation media does not increase the number of maturated oocytes and also no structural morphological changes were emphasized during maturation process.

Bcl2 gene expression registered a significant increase in COC cultivated in cysteine supplemented medium.

Bcl2 gene expression could be used as a molecular marker to assess viability and hence the rate of fertilization of the oocytes.

REFERENCES

1. ALVAREZ,G.M., DALVIT, G.C., ACHI, MARIA VERONICA, MIGUEZ M.S., CETICA P.D. (2009)-Immature oocyte qualityand maturational competence of porcine cumulus-oocyte complexes subpopulations, BIOCELL 33: 167-177
2. ANTOSIK P., KEMPISTY B., JACKOWSKA M., PIOTROWSKA H., BUKOWSKA D., LIANERI M., BRUSSOW K.P., WOZNA P., JASKOWSKI J.M. (2010)Assessment of transcript and protein levels contributing to cell cycle control and gap junction connections in morphologically variable groups of porcine cumulus-oocyte complexes. Veterinarni Medicina , 55(10):154-162
3. BOGACKI M, MARTA WASIELAK, ANNA KITEWSKA, IWONA BOGACKA, BM JALALI. (2014). The effect of hormonal estrus induction on maternal effect and apoptosis-related genes expression in porcine cumulus-oocyte complexes. Reproductive Biology and Endocrinology 12:32
4. CHIAN RC, NIWA K. (1994) Effect of cumulus cells present during different periods of culture on maturation in vitro of bovine oocytes. Theriogenology 41: 176.

5. CORN CM, HAUSER-KRONBERGER C, MOSER M ETAL. (2005) Predictive value of cumulus cell apoptosis with regard to blastocyst development of corresponding gametes.Fertility and Sterility84, 627–633.
6. DE MATOS DG, FURNUS CC, MOSES DF. (1997) Glutathione synthesis during IVM of bovine oocytes: role of cumulus cells. Biol Reprod 57: 1420-1425.
7. FEUGANG JM, JM GREENE, ST WILLARD, PL RYAN. (2011). In vitro effects of relaxin on gene expression in porcine cumulus-oocyte complexes and developing embryos. Reproductive Biology and Endocrinology 9:15
8. FILALI M, N FRYDMAN, MP BELOT, L HESTERS, F GAUDIN, G. TACHDJIAN, D EMILIE, R FRYDMAN, V MACHELON. (2009)Oocyte in-vitro maturation: BCL2 mRNAcontent in cumulus cells reflects oocyte competency. Vol. 19 Suppl. 4 Reproductive BioMedicine Online; www.rbmonline.com/Article/4309
9. GESHI M, TAKENOUCHI N, YAMAUCHI N, NAGAI T. (2000) Effects of sodium pyruvate in nonserum maturation medium on maturation, fertilization and subsequent development of bovine oocytes with or without cumulus cells. Biol Reprod 63:1130-1734.
10. HOST E, MIKKELSEN AL, LINDENBERG S, SMIDT-JENSEN S (2000) Apoptosis in human cumulus cells in relation to maturation stage and cleavage of the corresponding oocyte.Acta Obstetricia et Gynecologica Scandinavica79, 936–940.
11. ISOBE N, MAEDA T, TERADA T. (1998) Involvement of meiotic resumption in the disruption of gap junctions between cumulus cells attached to pig oocytes. J Reprod Fertil 113: 167-172.
12. JEON RYOUNG-HOON, GEUN-HO MAENG, WON-JAE LEE, TAE-HO KIM1, YEON-MI LEE, JEONG-HYEON LEE, BASAVARAJAPPA-MOHANA KUMAR, SUNG-LIM LEE AND GYU-JIN RHO, (2012), Removal of cumulus cells before oocyte nuclear maturation enhances enucleation rates without affecting the developmental competence of porcine cloned embryos, Japanese Journal of Veterinary Research, 60(4): 191-203.
13. JEONG BS , YANG X. (2001)Cysteine, glutathione, and Percoll treatments improve sow oocyte maturation and fertilization in vitro. Mol Reprod Dev. 59 (3) : 330-335
14. LIM JM, LIOU SS, HANSEL W. (1996) Intracytoplasmatic glutathione concentration and the role of β-mercaptoethanol in preimplantation development of bovine embryos. Theriogenology 46: 429-439.
15. LIVAK KJ, SCHMITTGEN TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 ΔΔ C(T) Method. Methods 25 (4): 402–408
16. MATTIOLI M, BARBONI B.(2000)Signal transduction mechanism for LH in the cumulus oocyte complex. Mol Cell Endocrinology 161: 19-23.
17. MCKENZIE LJ, PANGAS SA, CARSON SA ET AL.(2004) Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Human Reproduction19, 2869–2874.
18. PARADIS F, HS MOORE, A PASTERNAK, SUSAN NOVAK, MK DYCK, WT DIXON, GR FOXCROFT. (2010). Pig preovulatory oocytes modulate cumulus cell protein and gene expression in vitro. Molecular and Cellular Endocrinology 320:87-96
19. SENBON S, HIRAO Y, MIYANO T (2003) Interactions between the oocyte and surrounding somatic cells in follicular development: lessons from in-vitro culture. Journal ofReproduction and Development49, 259–269.
20. SUTOVSKY P, FLECHON JE, PAVLOK A. (1995) F-actin is involved in control of bovine cumulus expansion. Mol Reprod Dev 41: 5621-5629.
21. TAKAHASHI M, NAGAI T, HAMANO S, KUWAYAMA M, OKAMURA N, OKANO A. (1993) Effect of thiol compounds on in vitro development and intracellular glutathione content of bovine embryos. Biol Reprod 49: 228-232.
22. TATEMOTO H, SAKURAI N, MUTO N. (2000) Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during in vitro maturation: role of cumulus cells. Biol Reprod 63: 805-810.

USING PERCOLL AND SWIM-UP METHODS FOR BOVINE IN VITRO FERTILIZATION

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Abstract:*The succes rate of in vitro fertilization depends on oocyte and semen quality, on properties of culture media and also on the perfect coordination of working steps. The properties of frozen semen varies depending on many factors and its assessment after thawing in order to select the living spermatozoa is crucial. We have routinely used the Swim-up method in our laboratory but the inconstant results oriented us to Percoll method which is commonly used in human assisted reproduction. We tried to adapt the Percoll method for selecting and testing bovine sperm viablity, comparing the obtained results with those generated through Swim-up method. Sperm separation using Percoll and Swim-up techniques decreased the number of immature and primary abnormality spermatozoa but increased the proportion of secondary abnormality sperm. Percoll method generated 40-52 % living spermatozoa while Swim-up method, 72-74 %. Thawed semen displayed a 40 % mobility. Percoll method generated 80 % mobile spermatozoa and Swim-up method only 70 %. The concentration of thawed semen decreased from 100 milion/ml to 30-60 % after using Percoll method and dropped to 10-20 milion/ml when Swim-up method was used. We concluded that Percoll method generates better spermatozoa concentration and mobility compared to Swim-up method. The use of Percoll method is more suitable for obtaining more spermatozoa with a better motility.*

Key words*: Percoll method, Swim-up method, thawed semen, bovine in vitro fertilization*

In vitro fertilization (IVF) although representes a well defined assisted reproduction technique, already used for decades worldwide both in human and animals, sometimes generates insufficient results especially in domestic mammals (Tanase and Nacu, 2011). The IVF outcome depends mainly on gametes quality, culture media composition and respecting the steps of the procedure. As long as all laboratory techniques tend to mimic as much as possible the physiological events it is crucial to ensure as much as possible elements resembling the natural frame of each phenomenom. Before entering the IVF protocols, the sperm has to be separated from the seminal plasma (Jaquet, 2003) or diluants and also is essential to obtain only the living and motile spermatozoa. All the techniques have to ensure the optimal medium in order to allow the sperm to go through capacitation and hyperactivation processes. Capacitation consists in all the physiological, chemical and biochemical changes covered by spermatozoa in the female genital tract in order to achieve fertilization capacity (Beaulieu, 2006). Besides motility and hyperactivation, the sperm needs also specific proteins (such as Spam-1 , Fraser -2013) to penetrate the cumuls cell layers. All these reasons involve the propper laboratory methods to ensure the optimal spermatozoa selection from thawed semen. Considering the fact that the Swim-up method has generated variable results in our IVF attempts we have decided to compare this wide-used technique with the results generated by Percoll separation tehnique which is usually used in human assisted reproductive approaches.

MATERIALS AND METHODS

We used various solutions, either purchased or prepared in our laboratory. As a base for cell culture we have chosen Earl`s solution (1943) prepared in our laboratory following the original receipe (for 100 ml solution we used Sigma products: 0.0265 g $CaCl_2x2H_2O$, 0.02 g $MgSO_4x7H_2O$, 0.04 g KCl, 0.68 g NaCl, 0.01586 $NaH_2PO_4x2H_2O$, 0.1 g $C_6H_{12}O_6$, 0.0011 Phenol Red and 0.22 g $NaHCO_3$). All ingredients were solubilized in ultrapure water (Millipore) and subsequently sterilized by filtering with 22 µm filters (Millex GS Filter Unit) and kept at 4ºC until using. Initially a Earl 10X solution was prepared and for obtaining Earl 1X, 10 ml of Earl 10X were mixed with 90 ml ultrapure water and 0.22 g of NaHCO3, generating a change in the initial colour, from yellow to magenta. *Spermac* staining was used for sperm morphology and structural abnormalities assessment.

Percoll solution is widely used for cell, cell organelles or viruses isolation through centrifugation based on concentration gradient. Percoll consists in coloidal silicium particles covered with polivynilpirolydone (PVP) and is suitable due to its low viscosity, osmolarity and toxicity compared to other solutions. The Percoll stock solution was obtained by mixing 9 ml of Percoll with 1 ml Earl 10X. In order to avoid precipitation, the solutions were mixed through a continous and very gentle stirring. There were necessary multiple attempts in order to obtain a perfect cristal clear solution.For gradient concentration centrifugation, were also prepared Percoll 90% and 45% by dilution with Earl 1X. SPERM-TALP solution is used for sperm preparation by swim-up method. Sperm-Talp solution was prepared in our laboratory by mixing 125 ml of bidistilated water, 725 mg NaCl, 28.75 mg KCl, 261.25 mg $NaHCO_3$, 5.07 mg $NaH_2PO_4xH_20$, 295.5 mg TCM-199 HEPES, 460 µl of sodium lactate syrup 60%, 38.75 mg $MgCl_2x6H_2O$, 1.25 mg Phenol Red and 36 mg of $CaCl_2x2H_20$.

Semen sample consisted in straws from two bulls : Blazer 831559 and Bonaqua 52584 (BVN Neustadt a.d. Eisch, Germany). The straws were thawed at 37ºC for one minute and their content was subsequently analyzed.Morphological features of the semen were estimated subsequently to Spermac staining, using a 40X objective (Leica M3350). The semen concentration was assessed by hemocytometric method using a Marienfeld counting chamber and the mobility of spermatozoa was examinated with a decimal system (Bara, 2012). Its viability was estimated following the eosine-negrosine (VitalScreen) staining.

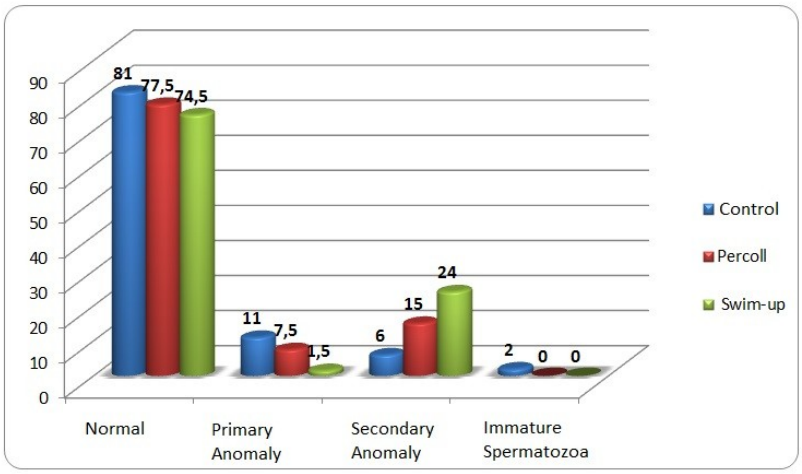
There is no unanimously accepted Percoll protocol for use in veterinary assisted reproduction. The method principle consists of semen centrifugation in a concentration gradient followed by precipitate sampling and semen concentration, morphology, viability and motility assesment. 200 µl of semen were slowly introduced in the centrifuge tube already containing Percoll 90% - 45% previously warmed at 37ºC for four hours. The mixture was centrifugated (Hettich 350R) at 600g (1950 RPM) for 20 minutes.The supernatant was removed and 2 ml of EarlX1 solution was added, than the mixture was centrifuged another 20 minutes at 200g (1060 RPM). Again,the supernatant was removed and 700 µl of Earl 1X were added, followed by semen assesment. The Swim-up method is usually used to separate sperm from the bovine diluted semen in order to practice the IVF. This method is based on the movement of viable spermatozoa to the surface of the Sperm-Talp solution, supported by sperm motility and their feature of getting free from seminal plasma. 1 ml of Sperm-Talp solution was poured in four centrifuge tubes and the semen was placed at bottom using insuline syringes. The tubes were maintained oblique at 45º (for enlarging the surface) for

one hour in an controlled atmosphere incubator (Panasonic inCu Safe), at 37ºC with 5% CO_2 and 100% humidity. After one hour, the liquid from surface was aspired with a micropipette and mixed with 1 ml Sperm-Talp solution in a 15 ml centrifuge tube. The mixture was centrifugated (Hettich 350R) for 10 minutes at 350g (1000 RPM). The precipitate was rinsed twice and centrifuged in the same conditions. After the last rinse, over the sediment was added 60 µl of Sperm-Talp and the mixture was used for semen assesment.

RESULTS AND DISCUSSIONS

The results of the morphological exam for Bonaqua bull are presented in graph 1.

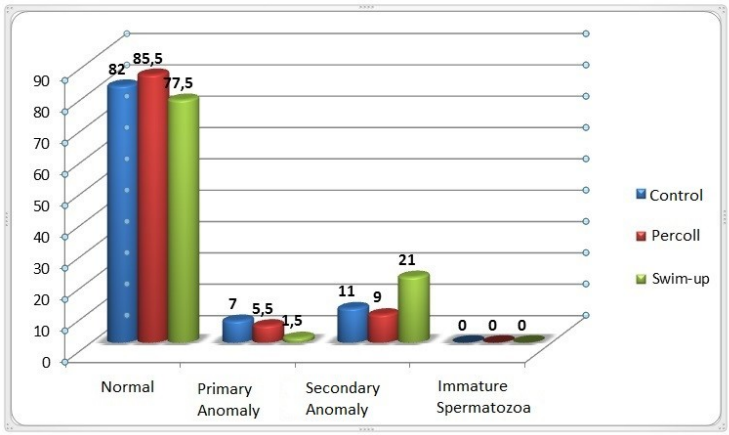
Graph 1. Morphological exam of sperm from Bonaqua bull



The initial exam revealed 82% normal sperm, 7% of primary and 11% secondary abnormalities. Subsequently to Percoll separation resulted 85.5% of normal spermatozoa, 5.5% with primary and 9% with secondary abnormalities. For comparison, Swim-up method revealed 77.5% normal spermatozoa, 1.5% with primary and 21% with secondary abnormalities.

Graph 2 includes morphological features of Blazer bull semen, comprising 82% normal spermatozoa, 7% with primary and 11% with secondary abnormalities. Percoll separation revealed 85.5%, 5.5% and 9% while Swim-up displayed 77.5%, 1.5% and 21% for normal, primary respectively secondary abnormalities.

Graph 2. Morphological sperm exam for Blazer bull



In order to characterize sperm mobility were considered spermatozoa with forward movements. For both bulls, 40% of the sperm showed forward movements before separation while Percoll emphasized 80% and Swim-up a light decreased percentage -70. These results are proving the efficiency of the methods for viable sperm selection, with better results for Percoll, as Getz (1999) and also Ding et al. (2000) reported. A better motility increases the sperm ability to penetrate zona pellucida (Suarez and Ho, 2003) and this is why motility is an important criteria for comparing the two separation methods.

Sperm viability was 40% for Bonaqua bull before separation. Percoll rised the percentage of viable sperm to 56%, while Swim-up climbed to 72%. A similar growth was emphasized in Blazer bull. From the originally 38%, viability increased to 40% after Percoll and to 74% following Swim-up. Somfai et al. (2002) reproted a large number of viable spermatozoa with intact acrosome subsequent to Percoll use, compared to Swim-up method in the case of using frozen-thawed semen.The difference of percentage for viable spermatozoa could be explained through centrifuge force action which can affect motility and sperm membrane integrity (Verberckmoes et al., 2000) and also by Swim-up method principle which relies on spermatozoa movements.

Semen concentration after thawing was the same (100 millions/ml) for both bulls. After Percoll use, spermatozoa concentration was 30 millions/ml for Bonaqua and 60 millions/ml for Blazer. Consequential using Swim-up method, concentration decreases at 10 millions/ml for Bonaqua and to 20 millions/ml. These findings are similar with those reported by Englert et al. (1992) who obtained fewer spermatozoa in human by using Swim-up compared to Percoll method. Parrish et al (1995) obtained more bovine spermatozoa using Percoll method but with a lower capacity of zona penetration.

The values of semen parameters of Blazer and Bonaqua bulls are presented in table 1. Regarding morphology, the normal spermatozoa ranged between 81-82%, while primary anomalies varies between 7-11%, the secondary anomalies between 6-11% with a 0-2% percentage of immature spermatozoa.

Table 1. Semen parameters for Blazer and Bonaqua bulls

	Morphology				Viability (%)	Motility	Concentration (milions/ml)
	Normal (%)	Primary anomalies (%)	Secondary anomalies (%)	Immature (%)		Forward movement	
Control	81-82	7-11	6-11	0-2	38-40	40%	100
Percoll	77,5-85,5	5,5-7,5	9-15	0	40-52	80%	30-60
Swim-up	74,5-77,5	1,5	21-24	0	72-74	70%	10-20

Using both Percoll and Swim-up methods for separation lowered the percentage of immature and primary anomalies spermatozoa meanwhile increasing the proportion of spermatozoa displaying secondary anomalies. The proportion of living spermatozoa was ranged between 38-40% in the thawed semen. Subsequent to Percoll method resulted 40-52% living spermatozoa, the highest values being generated by Swim-up method : 72-74%. In the frozen-thawed semen motility was 40%. Using Percoll method resulted 80% while consecutive to Swim-up method only 70% of spermatozoa displayed that feature. The

concentration decreases from 100% in thawed semen to 30-60 milions/ml after Percoll and to 10-20 milions/ml after Swim-up method.

CONCLUSIONS

Percoll method generates better results regarding spermatozoa concentration and motility.Both methods improved viability but Swim-up was superior to Percoll.

Either Percoll or Swim-up decreased the number of spermatozoa with primary anomalies, but in the same time can induce spermatozoa tails alterations and sequential secondary anomalies.Percoll method is useful for obtaining a larger number of high motility spermatozoa.

REFERENCES

1. BARA SILVIU-SABIN M. (2012) - *Performanțele productive și reproductive ale raselor de tauri pentru carne importate în România*, Teza de doctorat, Universitatea De Ştiinţe Agricole și Medicină Veterinară Cluj-Napoca, Şcoala Doctorală, Facultatea De Medicină Veterinară.
2. BEAULIEU M. (2006) - *Implication des extracellular signal-regulated kinases dans la capacitation des spermatozoïdes porcins,* teză de doctorat, Ecole vétérinaire d'Alfort.
3. DING DC., LIOU SM., HUANG LY., LIU JY., WU GJ. (2000) - *Effects of four methods of sperm preparation on motion characteristics and nitric oxide concentration in laboratory-prepared oligospermia,* Zhonghua Yi Xue Za Zhi, 63, 822–7, Taipei.
4. EARLE W. (1943) - *Production of Malignancy In Vitro. IV. The Mouse Fibroblast Cultures and Changes Seen in the Living Cells*, J.N.C.I. 4, 165-169.
5. ENGLERT Y., VAN DEN BERGH, M., RODESCH, C., BERTRAND E., BIRAMANE J., LEGREVE A. (1992) - *Comparative auto-controlled study between swim-up and Percoll preparation of fresh semen samples for in vitro fertilization*, Hum. Reprod, 7, 399–402.
6. FRASER B. (2013) - *Liaison de la proteine spermatique bovin Spam-1 à la zone pellucide de l'ovule,* disertatie, Universitate Laval.
7. GETZ I. (1999) - *In vitro maturation, fertilization and culture of bovine oocytes*, Master Thesis, Zagreb.
8. JACQUET M. (2003) - *Contribution à l'étude des protocoles de capacitation in vitro et de congélation des spermatozoïdes chez le chien,* teză de doctorat, Ecole vétérinaire de Lyon.
9. SOMFAI T., BODO S., NAGY S., PAPP AB., IVANCSICS J., BARANY B. (2002) - *Effect of Swim up and Percoll treatment on viability and acrosome integrity of frozen–thawed bull spermatozoa,* Reprod. Domest. Anim., 37, 285–290.
10. SUAREZ S., HO H. (2003) - *Hyperactivated motility in sperm*, Reprod. Domest. Anim., 38, 119–124.
11. TĂNASE D., NACU G. (2011) - *Biotehnologii De Reproducţie*, Universitatea de Ştiinţe Agricole Şi Medicină Veterinară "Ion Ionescu De La Brad", Material De Studiu I.D Iaşi Facultatea de Zootehnie Iaşi.
12. VERBERCKMOES S., DE PAUW I., VANROOSE G., LAEVENS H., VAN SOOM A., DE KRUIF A. (2000*) - Influence of ultracentrifugation on motility and membrane integrity of fresh bull sperm*, Theriogenology 53, 490.

INFLUENCE DEGREE OF A SMALL HYDROENERGETIC RESERVOIR ON A LARGE DOWNSTREAM RESERVOIR REGARDING THE PHYTOPLANKTON COMPOSITION

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Abstract:*The study is aimed to see if the phytoplankton strong development in a upper reservoir during the summer is having any impact on the composition of the phytoplankton from another downstream reservoir. The study showed that there is small similarity between the two reservoirs and the species developed in the enclosure of the small upstream reservoir are simply using the available ecological niches created by the dam while the water that passes through the turbines and it is evacuated closer to the downstream reservoir does not bring much live algal material into the river before its entrance in the downstream reservoir.*

Keywords: *phyto-plankton, diatoms, algae, reservoir, hydropower plant*

INTRODUCTION

The subject of the present paper regards the level of influence of an upstream small hydropower reservoir on a downstream large hydropower reservoir from the algal flora composition point of view. Both reservoirs were built in mountainous region on the course of a river, large enough to support a series of hydropower plants. The problem that we raised was the possibility that the small reservoir may be a source of populating of the downstream reservoir with certain algal species as in general, the downstream reservoir has a good water quality but its trophic state suddenly turned from oligo-mesotrophic in the last years to eutrophic.

We hypothesized that the reduced depth of the accumulation, the extreme environmental conditions in the summer as high light intensity and temperature, and the degree of water stagnation in this conditions, may lead to the development of certain algal species with high potential of invasion and bloom. Connected to the late increase in trophic level of the downstream reservoir, we wanted if there are any species that may reach the reservoir and develop, becoming viable, in the newly trophic conditions.

MATERIALS AND METHOD

Study area. Both studied reservoirs were built on the Bistriţa River in the Oriental Carpathians (eastern of Romania) as water supply for hydro-electrical power plants. The downstream reservoir, is Izvoru Muntelui-Bicaz, [length-33.3 km; maximum depth: 92 m volume 1200 x 10^6 m^3; surface of 31.25 $km^{2)}$; catchment surface: 4025 km^2 (Miron et al., 2010)]. The upper reservoir, Poiana Teiului, is located in upstream from Bicaz Reservoir being at the altitude of 527.9 m. Its

afferent power plant, Topoliceni, is located at 538.6 m alt., and the water released from the turbines is eliminated in a channel that meet the Bistriţa River at 506.6 m alt. The reservoir has a surface of 21 ha, a volume of 0.7 x 10^6 m^3 and a length of 2 km. The maximum depth is 7 m.

Material and Method. Sampling was carried out during the summer of 2013, in a period of time when the algal components has fully develop under the influence of the environmental factors. The weather average was of 29ºC), and the days before the sampling sessions had temperatures between 30 and 32ºC. The samples were collected from the surface and 5 m depth (the maximum depth we were permitted before reaching the benthos) in key locations. The first sampling station (1- in the paper) is located at the point where Bistriţa River enters the upper reservoir ; the second sampling station (2) is located next to the dam before the absorption tunnel opening; the third sampling location (3) was where a part of the water is released as the main course of Bistriţa river. The fourth sampling station (4) is located at the upper point of the Izvoru Muntelui-Bicaz reservoir. Before this point, the river receives the water eliminated by the hydropower plant, after passing the turbines.

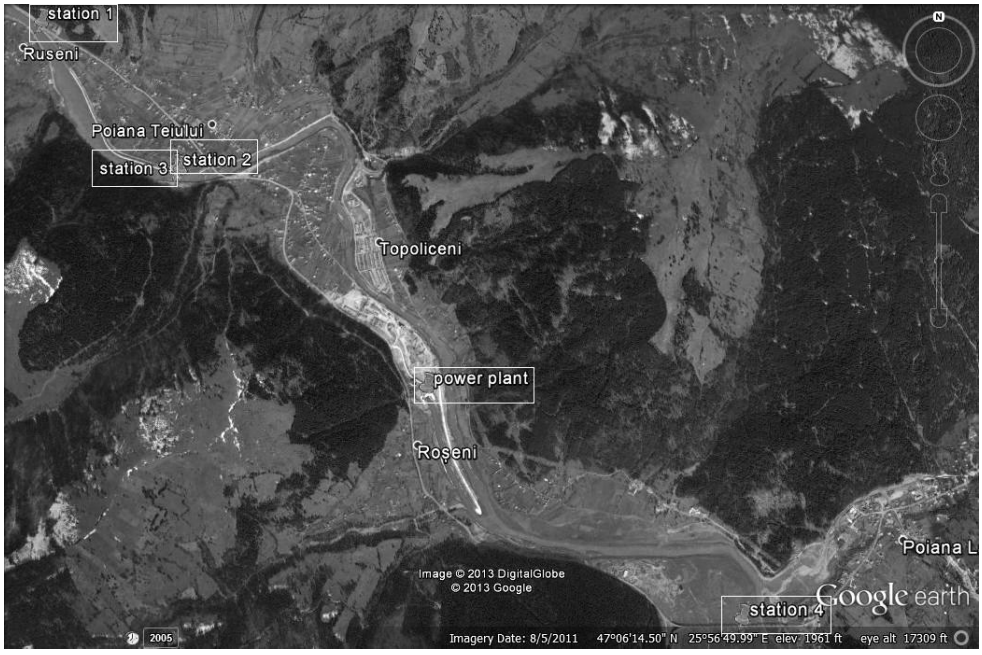

Figure 1. Sampling stations on the Topoliceni Reservoir (1, 2); Bistriţa River (3) and upper region of Izvoru Muntelui – Bicaz Reservoir (4).

Sampling was made with the help of a Wildco Van – Dorn 2.2 l horizontal water sampler. The transparency was identified using the Secchi disk. The phytoplankton samples were preserved at site with 5 ml of 37% formaldehyde and Lugol solution. The water samples for physical-chemical analysis were preserved

through special methods and stored in a frigorific box until reaching the laboratory. The temperature, dissolved oxygen (D.O.) and ammonia concentration were analyzed and registered at site later being compared to the back-up laboratory analysis. For trophy level assessment we used chlorophyll-*A* concentration (μg L^{-1}/mg m^{-3}). The extraction was performed using a vacuum pump on a 300 ml water volume and the filter with the concentrated algae was placed in 50 ml of 90% ethylic alcohol. The samples were heated in a water bath for 5 minutes at 75ºC for the extraction of the chlorophyll. The samples were than filtered and additional 90% ethylic alcohol was added until the sample-to-be-read had a 50 ml volume. The chlorophyll-*A* concentration readings were made using a Shimadzu UV-Vis spectrophotometer at 665 nm and 750 nm in comparison with a control sample containing the solvent (90% ethylic alcohol). Mineralization of the samples was made with 0.01 ml hydrochloric acid 3 mol/l for 10 ml of extract, agitated and let to rest for 5 up to 30 minutes then measured again at 665 nm and 750 nm. The values, read with the spectrophotometer were included in the formula:

$$Chl.\ a\ (\mu gL^{-1}) = A_w - A_z / 82 \times 2{,}42 \times X \times 10^3 / Y \times Z$$

Where: A_w = the reading before HCl mineralization = A1(650 nm) - A2 (750 nm); A_z = the reading after HCl mineralization = A1 (650 nm) - A2 (750 nm); X = ethylic alcohol quantity (ml); Y = filtered water (liters); Z = dimension of vat (cm).

The trophic state assessment was made using Ryding and Rast (1994) chlorophyll-*A* concentration as follows: ultra-oligotrophic: 0-1 μgL^{-1}; oligotrophic: 1-2.5 μgL^{-1}; meso-trophic: 2.5-8 μgL^{-1}; eutrophic: 8-25 μgL^{-1}; hyper-eutrophic: 25-75 μgL^{-1}. The water analysis identified the following physic-chemical characteristics: pH, nitrate, nitrite, phosphorous, total phosphorous (TP), ammonia, carbonate hardness, free carbonic acid, total hardness, residual hardness (Table 1). Based on the analysis the water was characterized (Table 1) by the means of trophy level (meso-trophic to eu-trophic) and WQI (Water Quality Index) and diagnosed as good (WQ diagnosis).

Regarding the algal flora qualitative and quantitative analysis, we used, for diatom identification the mineralization of samples and mounting in a high refractive index medium (Karthick et al., 2010) while for the quantitative analysis we used the Utermöhl method (Lars and Elbrächter in Karlson et al., 2010, Chapter 2: 13-20). The identification guides used for determinations are represented by Hindàk et al. (1975, 1978), Prescott (1951), Nagy-Tóth and Barna (1998) and Prygiel and Coste (2000).

RESULTS AND DISCUSSION

The reservoir is thermally stratified, dimictic (Miron et al., 1983) and, nowadays is included in the meso-eutrophic class of trophy, according to the chlorophyll-*A* concentration readings (Aoncioaie et al., 2013). One characteristic

feature of the reservoir that derives from its use is the continuous fluctuation of water level, which makes impossible the development of a macrophytes belt. The physico-chemical analyses are presented in table 1 next to trophic levels and water quality diagnosis.

Table 1. Physico-chemical analyses of the water samples, trophy levels and water quality diagnosis

Analysis \ Station		1	2	3	4
Chlorophyll-*A* (µg L^{-1}/mg m^{-3})		5.41	10.33	9.84	9.34
Trophy diagnosis		Mesotrophic	Eutrophic	Eutrophic	Eutrophic
Biomass (mg m^{-3})		362.5	692.1	659.3	625.8
Secchi disk depth (m)		1.25	1.20	1.20	2.40
Photic area depth (m)		3.125	3	3	6
Temperature	**0m**	18.5	20.2	20.0	22.0
(°C)	**5 m**	18.0	20.0	19.8	21.0
Dissolved	**0m**	7.6	7.3	7.3	7.0
oxygen (mg L^{-1})	**5 m**	7.4	7.0	7.2	6.8
CBO_5 mg L^{-1}		0.50	0.65	0.60	0.60
NO^-_3 (mg L^{-1})		18	20	20	20
NO_3-N (mg L^{-1})		4.08	4.54	4.54	4.54
NO_2^- (mg L^{-1})		0.010	0.010	0.010	0.03
NO_2-N (mg L^{-1})		0.003	0.003	0.003	0.009
NH^+_4 (mg L^{-1})		0.03	0.05	0.05	0.05
NH_4^+-N (mg L^{-1})		0.024	0.04	0.04	0.04
PO^{3-}_4 (mg L^{-1})		0.03	0.15	0.15	0.15
TP (mg L^{-1})		0.0098	0.049	0.049	0.049
pH		7.9	7.9	7.8	7.9
Carbonate	**°d**	5.6	5.7	5.7	6
Hardness	**ABC mmol L^{-1}**	2.05	2.1	2.1	2.2
Free carbonic acid (CO_2, mg L^{-1})		3.075	3.15	3.99	3.3
Total	**°d**	7.4	7.5	7.1	7.8
Hardness	**mg $CaCO_3$ L^{-1}**	133	134	130	140
Residual Hardness (°d)		0.5	0.5	0.5	0.5
WQI (Water Quality Index)		76	74	75	74
WQ diagnosis		Good			

Phytoplankton

The qualitative composition of the phytoplankton is represented by 9 phylums (Bacillariophyta, Cyanophyta, Chlorophyta, Euglenophyta, Dinophyta, Chrysophyta, Cryptophyta, Choanozoa and Heterokontophyta) with 90 genera that comprise 172 taxa plus the group of autotrophic picoplankton. The qualitative analysis shows 90 taxa identified in sampling station 1; 92 taxa in sampling station 2; 102 taxa in sampling station 3 and 111 taxa in location 4. To these numbers we add the autotrophic picoplankton colonies that may or may not be composed of several or just one taxa that we could not identify.

The resumed quantitative data is presented in table 2 and gives a total of 260.4 x 10^5 cells L^{-1} for sampling station 1; 1003 x 10^5 cells L^{-1} for sampling station 2; 630.4 x 10^5 cells L^{-1} for location 3 and 557.5 x 10^5 cells L^{-1} for the fourth sampling location, next to information on taxa numbers and density (cells L^{-1}) for each phylum and sampling station. The Shannon diversity index (Tab. 3) shows that the highest diversity and entropy was at sampling station 4 (3.055; 4.407) and the lowest at sampling station 2 (1.492; 2.152). The Sorensen similarity index (Tab. 3) calculated for total phytoplankton shows the highest similarity between 1 and 3 (0.722) and the lowest between 1 and 4 (0.591).

Table 4. Phytoplankton quantitative analysis:

Phylum	**1**		**2**		**3**		**4**	
	Taxa	**Cells x 10^5 L^{-1}**	***Taxa***	**Cells x 10^5 L^{-1}**	***Taxa***	**Cells x 10^5 L^{-1}**	***Taxa***	**Cells x 10^5 L^{-1}**
Bacillariophyta	*52*	158.9	*52*	239	*63*	204.7	*50*	390.6
Cyanophyta	*6*	2.75	*9*	7.25	*11*	27.9	*5*	3.86
Chlorophyta	*23*	11.4	*24*	14.97	*25*	14.24	*30*	37.7
Euglenophyta	*5*	1.7	*4*	2.77	*2*	0.7	*9*	11.3
Dinophyta	*3*	0.64	*1*	0.3	*1*	0.29	*6*	7.6
Cryptophyta	*0*	0	*0*	0	*0*	0	*3*	2.3
Chrysophyta	*1*	0.21	*2*	0.615	*0*	0	*5*	10.6
Choanozoa	*0*	0	*0*	0	*0*	0	*2*	6.27
Heterokontophyta	*0*	0	*0*	0	*0*	0	*1*	0.57
Autotrophic picoplankton	*colonies*	84.8	*colonies*	738.1	*colonies*	382.6	*colonies*	86.7
Total	*90+pico*	260.4	*92+pico*	1003	*102+pico*	630.4	*111+pico*	557.5

Table 3. Diversity Indexes and Sorensen Similarity Index for the sampling stations

	1	**2**	**3**	**4**
Shannon-Wiener Diversity Index	2.817	1.492	2.072	3.055
Species Richness (S)	91.0	93.0	103.0	112.0
Total Abundance (x $10^5 L^{-1}$)	266.08	1003.04	630.36	557.45
Simpson Diversity Index				
D:	0.143	0.545	0.377	0.131
1-D:	0.857	0.454	0.623	0.868
1/D:	7.002	1.833	2.653	7.619
Evenness	0.624	0.329	0.447	0.647
Shannon Entropy	4.064	2.152	2.989	4.407

Shannon-Wiener Diversity Index/Entropy: higher the value – higher the diversity
Evenness: 0 to 1 – less variation in communities between the species, the higher the Evenness
Simpson's Index (D) 0 – infinite diversity; 1 – zero diversity: D high – low diversity
Simpson's Index of Diversity (1-D) – values 0 to 1: 1-D high – high diversity
Simpson's Reciprocal Index (1/D): 1 – minimum: 1/D high – high diversity

Sorensen similarity index	2	3	4
1	0.674	*0.722*	*0.591*
2		0.663	0.634
3			0.595

In all four sampling stations the dominance in the total phytoplankton quantitative composition was divided between phylum *Bacillariophyta* and the autotrophic picoplankton. In sampling stations 2 and 3, the locations with the highest degree of standing water duration, the autotrophic picoplankton topped the diatoms (up to three times in sampling station 3). In sampling station 1 and 4, where the ecosystem is in transition from lotic to lentic but the water is still visibly flowing, the diatoms were about 2 up to 4.5 times more numerous that the picoplankton. The picoplankton densities were similar in these two locations, being of 84.8 (1) and 86.7 x 10^5 cells L^{-1} (2).

Phylum *Bacillariophyta* was quantitatively represented by similar groups of species in all four sampling stations in various densities. We considered dominant all taxa with a density of minimum 10 x 10^5 cells L^{-1} (Tab. 6). In sampling station 1, *Achnanthes affinis*, *Cymbella ventricosa* and *Diatoma hyemale* represent the main constituent of the phytoplankton. As visible, these are species not primarily planktonic but benthic species pulled from the support and able to live as plankton (Cărăuş, 1979). In sampling station 2, these three diatoms are present but with smaller densities, except *D. hyemale* that slightly exceeds the density from 1 (Tab. 4). Next to these three diatoms, also occurred in high densities *Achnanthes minutissima*, *Diatoma vulgaris*, *Fragilaria capucina* and *Synedra vaucheriae* (Tab. 6), all highly colonial organisms, many observed in the undisturbed colonial form, that sustaining the calm and slow water regime. In sampling station 3, the location where water is released from the dam as a continuity of the Bistriţa River, the dominant diatoms are the same with the exception of some reduce in *D. vulgaris* numbers (<10 x 10^5 cells L^{-1}) and an increase of *Gomphonema olivaceum* (Tab. 4). In sampling station 4, one kilometer down from where Bistriţa River enters the upper region of Izvoru Muntelui – Bicaz Reservoir, and the mainstream where the waters from sampling station 3 are directly dumped after passing through the hydropower plant turbines, the dominant diatom composition maintains similar, *D. vulgaris* increases slightly in numbers while the rest regress (Tab. 4) and to the dominant taxa list, 3 more species with high densities are added (*Asterionella formosa* – 14.13 x 10^5 cells L^{-1}; *Cyclotella comta* – 44.25 x 10^5 cells L^{-1}; *C. distinguenda* var. *unipunctata* – 117.21 x 10^5 cells L^{-1}). As a specification, the number of diatom taxa reduces from sampling point 1, 2 and 3 to 4 (Tab. 4) where their densities increase. Regarding phylum *Bacillariophyta*, the highest Shannon diversity and entropy was at sampling station 3 (4.515; 3.129) and

the lowest at sampling station 4 (3.529; 2.446). The Sorensen similarity index, calculated particularly for *Bacillariophyta*, is above 0.740, and shows the best similarity between 1 and 3 (0.817) and the lowest between 1 and 4 (0.745).

Table 4. Dominant phytoplanktonic elements in the study period:

Cells L^{-1} x 10^5	**1**	**2**	**3**	**4**
Autotrophic picoplankton	84.4	738.1	382.6	86.7
Achnanthes affinis	23.1	17.16	23.32	+
Achnanthes minutissima	+	26.4	19.47	11.96
Asterionella formosa	+	+	+	14.13
Cycloytella comta	+	+	+	44.25
Cyclotella distinguenda var. *unipunctata*	+	+	+	172.21
Cymbella ventricosa	16.9	12.57	19.35	12.47
Diatoma hyemale	40.7	42.3	35.72	+
Diatoma vulgaris	+	10.1	+	11.05
Fragilaria capucina	+	15	16.85	+
Gomphonema olivaceum	+	+	10.7	+
Synedra vaucheriae	+	21.45	+	+

Phylum *Cyanophyta* is represented by a total of 14 taxa (Tab. 3), with variation depending on location (Tab. 4). The highest Cyanophyta densities were identified in sampling station 3 where the composition was mostly represented by large, dense colonies of four species: *Chroococcus limneticus* – 5.7 x 10^5 cells L^{-1}, *Gloeocapsa sanguinea* – 7.8 x 10^5 cells L^{-1}, *Gloeocapsopsis magma* – 5.3 x 10^5 cells $L^{-1}$and *Gloeothece rupestris* – 4.4 x 10^5 cells L^{-1}; while in sampling station 1 the dominant cyanophytic algae was *Oscillatoria tenuis* (0.84 x 10^5 cells L^{-1}), in sampling station 2 was *Synechococcus elongatus* (1.8 x 10^5 cells L^{-1}) and in sampling station 4 was *Oscillatoria formosa* (0.75 x 10^5 cells L^{-1}). Regarding phylum Cyanophyta, the highest Shannon diversity and entropy was at sampling station 2 (2.033; 2.933) and the lowest at sampling station 4 (1.510; 2.178). The Sorensen similarity index, calculated particularly for Cyanophyta, is above 0.540, and shows the best similarity between 2 and 3 (0.700) and the lowest between 1 and 4 (0.545).

Phylum *Chlorophyta* has a total of 54 taxa with variation depending also on location. The highest Chlorophyta densities were identified in sampling station 4 where the base line was mostly given by *Eudorina elegans* (10.06 x 10^5 colonies L^{-1}) and *Botriococcus braunii* (6.79 x 10^5 colonies L^{-1}) out of a total of 37.7 x 10^5 chlorophyte cells/colonies L^{-1}. In sampling station 1 and 2 the dominant chlorophyte is *Cosmarium obtusatum* (1.49 and 1.47 x 10^5 cells L^{-1}) and in sampling station 3 the dominant was *Cosmarium undulatum* (1.42 x 10^5 cells L^{-1}). Shannon diversity index for phylum Chlorophyta showed that the highest diversity and entropy was at sampling station 3 (3.062; 4.418) and the lowest at sampling station 4 (2.708; 3.907). The Sorensen similarity index, calculated for Chlorophyta is very low, in the range of 0.188 (between 1 and 4) to 0.271 (between 1 and 4).

The euglenophytes are mainly represented by *Euglena gracilis* (**1**: 0.64 x 10^5 cells L^{-1}; **2**: 0.96 x 10^5 cells L^{-1}; **3**: 0.34 x 10^5 cells L^{-1}) and *Lepocinclis ovum* (**2**: 0.95 x 10^5 cells L^{-1}; **3**: 0.34 x 10^5 cells L^{-1}) while *Trachelomonas planktonica* is representative for sampling station 4 (3.2 x 10^5 cells L-^{1}). The Sorensen similarity index is highest between 2 and 3 (0.800) and lowest for 3 and 4 (0.364).

The dinophytes are represented by six species in sampling station 4, while less species are identified in the upper three locations. In sampling station 1 only *Ceratium hirundinella*, *Peridinium cinctum* and *Sphaerodinium cinctum* were present (0.22 x 10^5 cells L^{-1} each), in sampling station 2 only *Glenodinium oculatum* was identified (0.32 x 10^5 cells L^{-1}) and in location 3 only *C. hirundinella* (0.28 x 10^5 cells L^{-1}).

Phylum *Chrysophyta* is represented by 5 species in sampling station 4 while the upper three are little represented by *Mallomonas acaroides* (**1**: 0.21 x 10^5 cells L^{-1}, **2**: 0.31 x 10^5 cells L^{-1}) and *Dinobryon divergens* (**2**: 0.31 x 10^5 cells L^{-1}) while no chrysophyte was identified in sampling station 3.

Phylum *Cryptophyta*, *Choanozoa* and *Heterokontophyta* were represented by a reduced number of species only in sampling station 4 (Tab.3; 4). The identified cryptophytes are *Cryptomonas marsonii*, *C. ovata* and *Rhodomonas lacustris*. *Desmarella moniliformis* and *Salpingoeca amphoridium* from *Choanozoa* group are epiphytic species primarily found on larger algal cells, in the present situation on *Actinochoris sphaerica* (*D. moniliformis* was identified next to few cells of the euglenophyte *Colacium sideropus*) and on colonial *Asterionella formosa* (*S. amphoridium*). The heterokontophytic colonies of *Synura ulvella* are planktonic and were identified in all the samples collected in location 4.

Regarding the topics we raised at the beginning of this study it looks like there is none or little influence coming from the upper reservoir. The similarity between station 4 and the upper three is small and is based on the baseline of algae that are commonly present in the river and that are characteristic to the waters in the region. A higher similarity occurs between stations 4 and 2 due to the fact that water is extracted directly from station 2 and after passing through the turbines it is evacuated on the river course closer to station 4 than station 3 is. Although, the life span of algae is cut shot by the passing through the turbine process, so little viable content is present in the water reaching the evacuation channel as shown in previous studies made at Stejaru power plant (Cărăuş, 1964). *Nitzschia sigma*, *Surirella linearis* var. *constricta*, *Spirulina nordstedtii*, *Trachelomonas oblonga*, *Koliella longispina* and *Glenodinium oculatum* are some of the species common only to station 2 and 4 while species like *Closterium littorale*, *Gloeocapsa sanguinea*, *Gloeocapsopsis magma* from station 3 were never identified in Izvoru Muntelui-Bicaz Reservoir. The same thing can be said about *S. nordstedtii* (station 2) that was identified for the first time in Izvoru Muntelui-Bicaz Reservoir. Species like *Batrachospermum moniliforme* (station 2), *Gloeocapsa sanguinea*, *Gloeocapsopsis magma*, *Protoderma viride*

(station 3), *Draparnaldia glomerata* (station 1), *Gloeothece rupestris*, *Uronema confervicolum* (stations 2, 3) are fixed on substrate and thus, even if pulled and transported to Bicaz Reservoir, due to its depth would not be able to survive. *Coleochaete irregularis* was identified indeed in station 4 (first time ever) but we consider that it may have been brought from the surrounding aquatic ecosystems. The main result of our work is that there is no important effect of the upper reservoir onto the lower reservoir. The identified species from the second and third stations are present in the ecological niches the Topoliceni reservoir creates, like the stagnant water before the suction tunnel or immediately after the dam. The species that manage to exit alive after passing through the power plant turbines are apparently in small numbers and most of them are the species common in the region. The smallest similarity was found between station 1 and 4 as the river is populated mostly with benthic species and even if the diatoms are the dominant category in both locations, in station 4 their number almost doubles and is represented by planktonic species next to the benthic species that are also adapted to planktonic life.

REFERENCES

1. Aoncioaie, C., Erhan, M.-G., Miron, M., 2013. New observations on the biological features of Izvoru Muntelui – Bicaz Reservoir. An. St. Univ. Al. I. Cuza, Iaşi, s. I Biol. Anim. LVIII. Supplement. 55-67;
2. Cărăuş, I., 1979. Investigations on the phytoplankton of Vidra dam reservoir (Southern Carpathians, Romania). Trav. Stn. Stejarul, Limnol. 7. 111 – 124.
3. Cărăuş, I., 1964. Influenţa trecerii apei prin turbinele hidrocentralei "V.I.Lenin" de la Stejarul asupra planctonului conţinut. / The influence of water passing through the turbines of „V.I.Lenin" hydropower plant from Stejaru on the planktonic content. Bul. Inst. Cercet. Pr. Piscic., XXIII, 4. 64-66
4. Hindàk, F., Cyrus, Z., Marvan, P., Javornický, P., Komárek, J., Ette, H., Rosa, K., Sladečková, A., Popovský, J., Puncochárová, M., Lhotský, O., 1978. *Sladkovodne Riasy (Freshwater Algae)*, Vol. I, II. Slovenske Pedag. Necl. Bratislava. 724 p.
5. Hindàk, F., Komárek, J., Marvan, P., Růžička, J., 1975. *Kľúč Na Určovanie Výtrusných Rastlín.* Vol. I, II. *Riasy*. Slovenske Pedag. Necl. Bratislava. 396 p.
6. Karlson, B., Cusak, C., Bresnan, E., 2010. Microscopic and molecular methods for quantitative phytoplankton analysis. Intergovernmental Oceanographic Commission. Manuals and Guides. UNESCO. Available from
7. Karthick, B., Taylor, J.C., Mahesh, M.K., Ramachandra, I.V., 2010. Protocols for collection, preservation and enumeration of Diatoms from aquatic habitats for water quality monitoring in India. Icfai Univ. J. Soil and Water Sci., **III**, *1*: 25-60.
8. Miron, I., Cărăuş, I., Măzăreanu, C., Apopei, V., Grasu, C., Ichim, I., Mihăilescu, F., Rujinschi, R.-I., Rujinschi, C., Simalcsik, F., Misăilă, C., Ţăruş, T., Apetroaiei, N., 1983. *Lacul de acumulare Izvoru-Muntelui Bicaz. Monografie Limnologică.* / *Izvoru-Muntelui Bicaz Reservoir. Limnological monograph.* Ed. Academiei R.P.R. Bucureşti.
9. Nagy-Tóth, F., Barna, A., 1998. *Alge unicelulare (Chlorococcales). Determinator. / Unicellular algae (Chlorococcales). Identification guide*. Presa Universitară Clujeană. 200 p.
10. Prescott, G.W., 1951. *Algae of the Western Great Lakes area*. W.M.C. Brown Company Publishers, Dubuque, Iowa, U.S.A. 977 p.

11. Prygiel, I., Coste, M., 2000. *Guide Methodologique pour la mise en œuvre de l'Indice Biologique Diatomées. I. Les Diatomées: Présentation générale*. 340 p. (134 p. + plates).
12. Ryding, S.-O., Rast, W., 1994. *Le contrôle de l'eutrophisation des lacs et des réservoirs*. Masson Ed., Paris: p. 294.
13. Surugiu, V., 2008. *Limnobiologie şi saprobiologie. Compendiu de lucrări practice*. / *Limnobiology and saprobiology. Compendium tutorial*. Ed. Tehnopress, Iaşi: p. 331.
14. Zamfirescu, S.R., Zamfirescu, O., 2008. *Elemente de statistică aplicate în ecologie*./ *Statistical elements used in ecology*.; "Alexandru Ioan Cuza" University Ed., Iaşi: p. 218.
15. http://www.changbioscience.com/genetics/shannon.html - site for calculation of Shannon-Wiener Diversity Index; Simpson Diversity Index; Evenness; Shannon entropy; October 30, 2013.
16. http://www.water-research.net/watrqualindex/index.htm - site for calculation/explanation of Water Quality Index (WQI); October 30, 2013.
17. http://www.le.ac.uk/bl/gat/virtualfc/Stats/soeren.htm - site for calculation/explanation of Sorensen Similarity Index; October 30, 2013.
18. http://www.countrysideinfo.co.uk/simpsons.htm - site for calculation/explanation of Simpson Diversity Index; October 30, 2013.

INFLUENCE OF TWO THERMAL STRATIFICATION PHASES ON THE PHYTOPLANKTON AND PLANKTONIC CRUSTACEANS IN A DEEP DIMICTIC MOUNTAIN RESERVOIR

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Abstract*. The paper focuses on the direct and inverse thermal stratification phases and the changes induced by these on the phytoplankton and planktonic crustaceans from the Izvoru Muntelui – Bicaz reservoir-Romania. These thermal stratification phases were recorded during four months in winter and spring (December 2011, January, March and May 2012) and quantitative and qualitative changes upon the studied biological features were recorded.*

Keywords*: direct thermal stratification, inverse thermal stratification, phytoplankton, diatoms, planktonic crustaceans, reservoir.*

INTRODUCTION

The present study analyze the modifications that occur in the qualitative and quantitative composition of the phytoplankton and planktonic crustaceans during the thermal stratification phases. These modifications along the water column lead to a continuous change in the environment where the organisms need to survive.

The aquatic ecosystem is represented by Izvoru Muntelui – Bicaz Reservoir which is thermally stratified, dimictic, has at maximum volume a depth of 90 m deep (Miron et al., 1983) and was presented as oligo-mesotrophic during the last years (Aoncioaie and Erhan, 2010; Darabă, 2008) while the current study identified the water as mesotrophic (when the reservoir was covered with ice) and eutrophic during the other three periods of time.

Thermal stratification is a natural phenomenon that occur in dimictic reservoirs from temperate regions (Wetzel, 2001) and are consecutive during a year; most of the time being represented by the direct thermal stratification that implies that the temperature decreases along the water column from the surface to the bottom where the temperature is around 4ºC and the thermocline is clearly visible. The inverse thermal stratification phase takes place in the coldest period of winter while the surface layer is frozen or near to the freezing temperature. The main characteristic of this phase is the increase of the water temperature from the surface (≈ 0 – 1ºC) to the bottom of the reservoir (4ºC). In this moment there is almost no mixing in the water column due to the higher density of the warmer water at the bottom (≈ 4ºC) as the literature specifies that in the upper layers beneath the ice, a certain amount of mixing may occur (Fietz et al., 2005).

MATERIAL AND METHODS

Study area

Izvoru-Muntelui Bicaz Reservoir is located in the Eastern Romania, in the Oriental Carpathians on the Bistriţa River. Its main purpose is as water supply for a hydro-electrical power plant. At full volume the maximum depth is reached at the dam -90 m and the reservoir contains a quantity of approximate 1.23 x 10^9 m^{-3} of water (Miron et al., 1983).

Methods

Sampling was made in different years in order to have a more detailed picture regarding the occurrence of these thermal stratification phases (December 20, 2011; January 31, March 8 and May 5, 2012) in multiple points of the reservoir. The physical and chemical analyses were made using the Multiparameter Water Quality Sonde Type 6600 V2-2. The recorded parameters were depth (m), temperature (ºC), dissolved oxygen (D.O., mg L^{-1}), pH, oxidation-reduction potential (O.R.P., mV) and conductivity (mS cm^{-1}). The photic area depth was determined by multiplying the Secchi depth with the factor *2.5* (Surugiu, 2008).

For phytoplankton analysis, water samples were collected from 3 depths (0, 5, 10 m) as the photic area is rarely deeper than 10 meters. From each depth was collected 1 liter of water with a Wildco Van – Dorn horizontal water sampler (volume: 2.2 liters). The collected samples were stored in clean plastic recipients and preserved at site with 5 ml of 37% formaldehyde and Lugol solution. The samples were let to sediment for two weeks and the sediment was stored in special recipients. For diatom identification the samples were mineralized and mounted in a high refractive index medium (Karthick et al., 2010). The biomass was calculated based on the biovolume (μm^3 L^{-1}) of the algal cells (Hillebrand et al., 1999; Hutorowicz, 2005; Comptage-PHYTO-v7-4 IS soft). Chlorophyll-*A* analysis was made following the spectrophotometer method using 90% alcohol for extraction. Planktonic crustaceans' samples were collected by dividing the water column in 5 m layers up to 40 m depth using a Birge closing net (mesh size 80 μm) for the quantitative analysis and simple plankton net of 100 μm mesh size for qualitative analysis. Samples were preserved at site with 96% ethanol. Concentration of sample, identification and counting represent the laboratory stage and involved specific procedures like dissection, binocular and microscope analysis.

For the graphical representations, the scientific names of the phytoplankton species were abbreviated as following: *Asterionella formosa*: *A. f.* and *Cyclotella distinguenda* var. *unipunctata*: *C. d. u.*

RESULTS AND DISCUSSION

In December 20, 2011 the water column was in the direct thermal stratification state (Fig. 2 A: D1), very close to becoming a homothermy phase. The depth reached at the Dam of the reservoir was around 60 m. The Secchi disk depth average on the reservoir was of 3.16 m and the photic area reached a maximum of 10.8 m (7.9 m average). The temperature at the surface was of 6.54ºC (6.34ºC average) and slowly decreased until the bottom where it had a

value of 4.5ºC (4.9ºC average). The D.O. concentration varied from a maximum of 11.38 mg L^{-1} to a minimum of 6.37 mg L^{-1} (bottom). The pH fluctuated from 7.79 at surface to 7.22 at the bottom. The O.R.P. varied largely depending on location from 144 to 47 mV (surface) to 140 to 67 mV (bottom). Conductivity varied in the water column between 0.25/0.249 mS cm^{-1} (surface) and 0.255/0.243 mS cm^{-1} (bottom). The trophic state was evaluated by chlorophyll-*A* concentration which reached an average of 16.56 mg m^{-3} and according to Ryding and Rast (1994) the water was eutrophic.

In January 31, 2012, the temperatures along the water column begin to rise from the surface to the bottom of the reservoir (minimum: 1.75 ºC to a maximum of 3.9ºC), thus entering in the inverse thermal stratification phase (Fig. 2 A: I1). The highest depth among the sampling points was reached again at the Dam of the reservoir (65 m). The Secchi disk depth average on the reservoir was of 3.9 m and the photic area reached an average of 9.75 m. The D.O. concentration varied from 13.58 mg L^{-1} (surface, maximum registered) to a minimum of 11.15 mg L^{-1} (bottom). The pH fluctuated from 7.77 at surface to 7.61 at the bottom. The O.R.P. varied largely depending on location from 48 to 85 mV (surface) to 71 to 83 mV (bottom). Conductivity varied in the water column between 0.255 mS cm^{-1} (surface) and 0.253 mS cm^{-1} (bottom). The trophic state was evaluated by chlorophyll-*A* concentration which reached an average of 9.5 mg m^{-3} and according to Ryding and Rast (1994) the water was also eutrophic.

In March 8, 2012 analyses were made in one single sampling point where the ice cover was thick enough (25 cm), the other three sampling points not being reached due to its instability. The temperatures along the water column begin to rise from the surface (0.9ºC) to the bottom of the reservoir (3.56ºC). The minimum temperature registered this month was of 0.75ºC at 0.152 m and in the first meter the temperature begun to rise until 1.21 ºC then from 10 m depth the temperature maintained constant until bottom (Fig. 2 A: I2). The maximum depth was of 42 m. The Secchi disk depth was of 2.9 m and the photic area reached 7.25 m. The D.O. concentration varied from 13.92 mg L^{-1} (surface, under ice) to a minimum of 9.71 mg L^{-1} (bottom). The pH fluctuated from 7.73 at surface to 7.51 at the bottom. The O.R.P. varied from 53 mV (surface) to 70 mV (bottom). Conductivity varied in the water column between 0.281 mS cm^{-1} (surface) and 0.26 mS cm^{-1} (bottom). The trophic state was evaluated by chlorophyll-*A* concentration which reached an average of 6.39 mg m^{-3} and according to Ryding and Rast (1994) the water was mesotrophic.

In May 5, 2012, the temperatures along the water column begin to decrease from the surface to the bottom (Fig. 1 A: D2) of the reservoir thus entering again in the direct thermal stratification phase (15.29ºC at surface to 4.04ºC at the bottom). The maximum depth among the sampling points was reached again at the Dam of the reservoir (67.61 m). The Secchi disk depth average on the reservoir was of 0.78 m and the photic area reached a maximum of 3.25 m at the Dam area and a minimum of 0.25 m at the end of the reservoir (1.95 m average). The D.O. concentration varied from a maximum of 10.23 mg L^{-1} (Dam, surface) to 2.77 mg L^{-1} (Dam, bottom) while the averages for the reservoir are between 9.96 mg L^{-1} (surface) and 5.3 mg L^{-1} (bottom). The pH fluctuated from 8.01 (maximum registered) at surface to 7.14 at the bottom (minimum registered). The O.R.P. varied largely depending on location from 52 to 36 mV (surface) to 19 to 76 mV (bottom). Conductivity varied in the water column, depending on location, from 0.16 mS cm^{-1} to 0.251 mS cm^{-1} (surface) and 0.155 mS cm^{-1} to 0.325 mS

cm^{-1} (bottom). The trophic state was evaluated by chlorophyll-*A* concentration which reached an average of 10.8 mg m^{-3} and according to Ryding and Rast (1994) the water was again eutrophic.

Table 1 presents a series of analyses made in order to present the picture of the physical, chemical and biological features of the water column in the studied thermal phases: phosphate and total phosphorous (T.P.), water hardness, biomass, chlorophyll-*A*, transparency (Secchi disk depth) and photic area. Figure 2 contains the graphic representation of chlorophyll-*A*, biomass, transparency and photic area.

Table 1. Bicaz reservoir features during the study period:

Registered features		**D1 XII 20, 2011**	**I1 I 31, 2012**	**I2 III 8, 2012**	**D2 V 5, 2012**
Chlorophyll – *A* (mg m^{-3})		16.56	9.5	6.39	10.8
Biomass (mg m^{-3})		1109.5	636.5	426.12	723.6
Secchi disk depth (transparency, m)		3.16	3.9	2.9	0.78
Photic area (m)		7.9	9.75	7.25	1.95
PO_4^{3-} (mg L^{-1}) / TP (mg L^{-1})		0.15/0.049	0.15/0.049	0.15/0.049	0.15/0.049
Carbonate hardness	ºd	7.08	6.65	7	5.23
	ABC (mmol L^{-1})	2.6	2.9	2.6	1.9
Total hardness	ºd	8.53	8.3	8.5	7.08
	$CaCO_3$ (mg L^{-1})	158.3	160	160	128.75
Water assessment		Medium hard			
Residual hardness (ºd)		0.5	0.5	0.5	0.5

ABC – acid binding capacity; ºd – German degree.

The figure below (figure 1 A-F) presents the physical and chemical parameters for the two thermal phases. The Pearson correlation index showed that the dissolved oxygen had a very strong negative correlation with the temperature (r=-0.971), pH had a very strong positive correlation with the dissolved oxygen (r=0.947); the oxidation–reduction potential had a weak negative correlation with the pH and moderate positive with the dissolved oxygen (r_{pH}=-0.233; $r_{D.O.}$=-0.529); and conductivity was moderately positive correlated with the turbidity (r=0.659) as the increase of suspended material in water leads to higher conductivity.

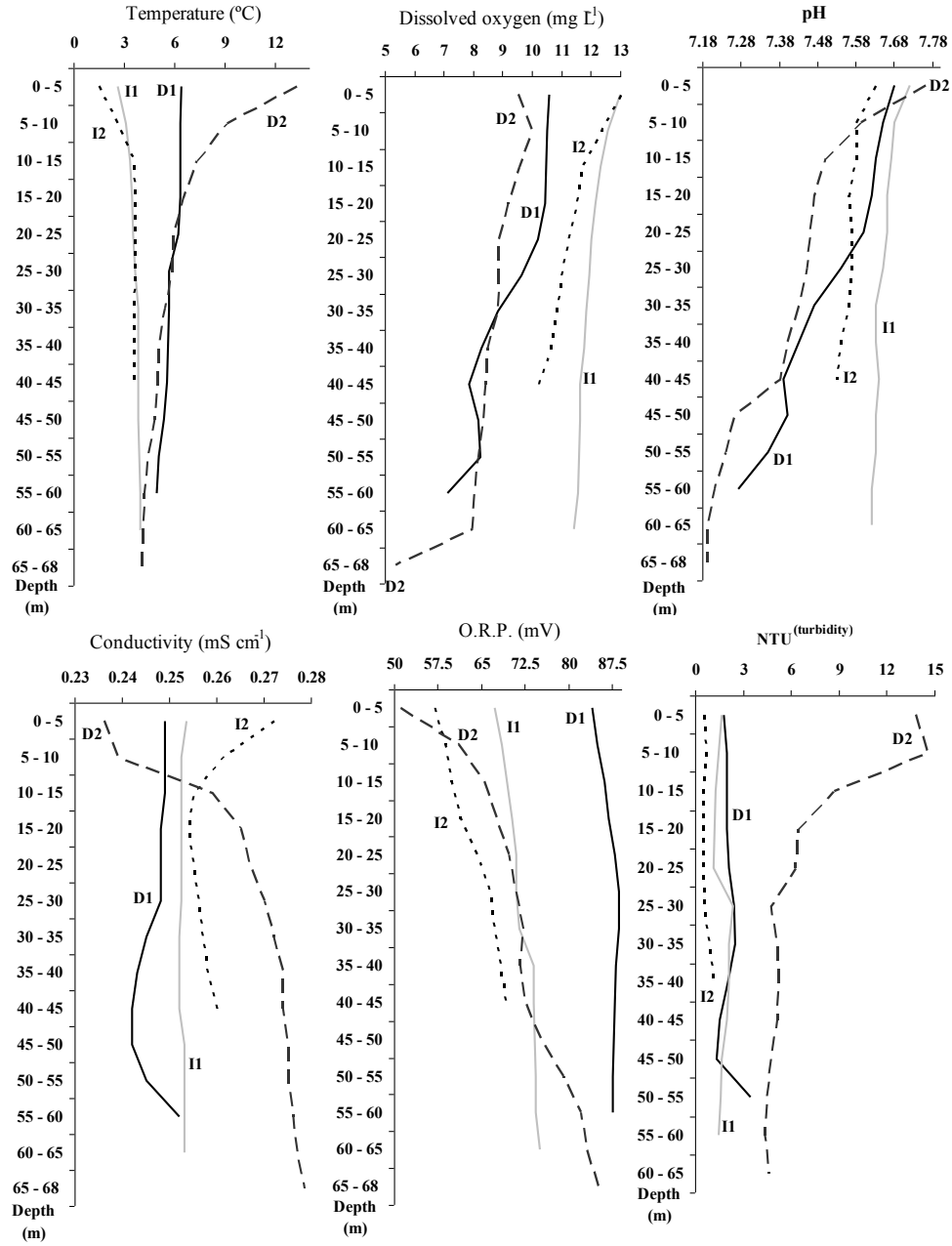

Figure 1. **A**: Temperature (ºC); **B**: Dissolved oxygen (D.O., mg L^{-1}); **C**: pH; **D**: Conductivity (mS cm^{-1}); **E**: Oxidation reduction potential (O.R.P., mV); **F**: Turbidity (NTU)

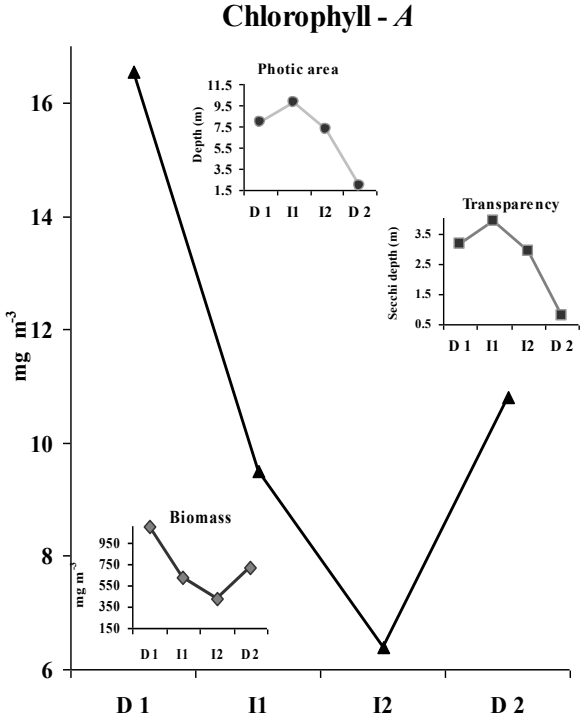

Figure 2. **A.** Chlorophyll-*A*; **B.** Transparency; **C.** Photic area; **D.** Biomass

Phytoplankton

Dominant in the phytoplankton was the group of autotrophic picoplankton that had a high density of colonies which represented about 24.26 up to 69.99% of the total phytoplanktonic density. Phylum *Bacillariophyta* was the second in dominance with 12 up to 49.9% of the total density. The diatoms are represented, in all the studied periods, by two species: *Asterionella formosa* Hassal and *Cyclotella distinguenda* var. *unipunctata* (Huestedt) Hakansson & J.R.Carter (Table 4) while the remaining diatom taxa cover about 18.6 – 32.6% of the total diatom density. In March and May, 2012 phylum Chlorophyta becomes an important part of the phytoplankton with densities that covered 11.4 and 23.45% of the total; in May, 2012 being close to the diatom density. Also in May, 2012 phylums like *Cryptophyta* and *Chrysophyta* had strong developments, mainly due to higher temperatures, from 0 to 1.41% (*Chrysophyta*) and from 0.2 to 4.1% (*Cryptophyta*). The detailed phytoplankton analysis (phylums, taxa and density – cells L^{-1} x 10^5) is presented in Table 2. Table 3 presents the genera identified for each algal phylum.

Table 2. Phytoplankton analysis:

Phylum	D1: XII 20, 2011		I1: I 31, 2012		I2: III 8, 2012		D2: V 5, 2012	
	Taxa	Cells L^{-1} x 10^5	Taxa	Cells L^{-1} x 10^5	Taxa	Cells L^{-1} x 10^5	Taxa	Cells L^{-1} x 10^5
Bacillariophyta	58	11.6	30	18.85	19	23.4	47	34.4
Cyanophyta	2	0.6	2	0.11	1	0.12	3	0.32
Chlorophyta	7	0.36	4	0.56	3	11	10	31.54

Euglenophyta	3	0.11	1	0.15	1	0.05	3	0.23
Dinophyta	2	0.23	1	0.23	2	0.76	3	1
Cryptophyta	0	0	1	0.15	1	0.16	3	11.4
Chrysophyta	0	0	0	0	0	0	3	3.92
Choanozoa	2	0.68	2	0.61	1	0.03	0	0
Bygira	0	0	0	0	0	0	1	0.21
Autotrophic picoplankton	colonies	31	colonies	53.3	colonies	11.38	colonies	193.6
Total	74+pico	44.48	41+pico	73.98	28+pico	46.9	73+pico	276.58

Table 3. Phytoplanktonic genera in each phylum

Phylum	Genera
Bacillariophyta	Achnanthes, Amphipleura, Asterionella, Caloneis, Ceratoneis, Cocconeis, Cyclotella, Cymbella, Denticula, Diatoma, Diploneis, Fragilaria, Gomphonema, Gyrosigma, Meridion, Navicula, Nitzschia, Surirella, Synedra;
Cyanophyta	Oscillatoria, Rhabdoderma;
Chlorophyta	Ankistrodesmus, Botriococcus, Carteria, Chlorella, Elakatothrix, Kirchneriella, Micractinium, Monoraphidium, Mougeotia, Pandorina, Scenedesmus, Siderocystopsis;
Euglenophyta	Trachelomonas;
Dinophyta	Glenodinium, Gymnodinium, Peridinium;
Cryptophyta	Cryptomonas;
Chrysophyta	Dinobryon, Mallomonas, Stelexomonas;
Choanozoa	Salpingoeca;
Bygira	Bicoeca.

Table 4. Dominant phytoplanktonic elements in the study period:

Cells L^{-1} x 10^5	**D1: XII 20, 2011**	**I1: I 31, 2012**	**I2: III 8, 2012**	**D2: V 5, 2012**
Asterionella formosa	3.66	5.69	12.57	3.64
Cyclotella distinguenda var. *unipunctata*	4.16	7.46	3.46	24.35
Autotrophic picoplankton	31	53.3	11.38	193.6

Planktonic crustaceans

Regarding the planktonic crustacean populations a number of seven species were identified and three categories of young forms (Tab. 5). The dominant organisms at all times are the young forms of *Copepoda*, especially the nauplii that cover the spectrum from 16.47% up to 50.76% in May 2012. The adult taxa that are constantly present and dominate the spectrum in the thermal phases are *Daphnia galeata* (Sars.) and *D. cucullata* (Sars.) from *Cladocera* and *Eudiaptomus gracilis* (Sars.) and *Cyclops vicinus* (Uljanin) from *Copepoda*. All these four species occur at all times in the thermal phases. *C. vicinus* (Uljanin) reached in May, 2012 (D2) the highest densities while in the first three thermal phases had very small number of individuals/m^{-3}. *D. cucullata* (Sars.) strongly reduced its densities in the I2 and D2 phases and also did *D. galeata* and *E. gracilis* even if the densities did not reduce extremely.

Table 5. Analysis of the planktonic crustacean populations:

Taxa	D1: XII 20, 2011 Ind. m^{-3}	I1: I 31, 2012 Ind. m^{-3}	I2: III 8, 2012 Ind. m^{-3}	D2: V 5, 2012 Ind. m^{-3}
Diaphanosoma orghidani (Negrea)	17.2	0	0	0
Daphnia galeata (Sars)	477.65	1420.7	214.2	348.15
Daphnia cucullata (Sars)	314.95	147.2	20	22.1
Bosmina longirostris (O. F. Müller)	38.65	1.25	0	23.3
Chydorus sphaericus (Mueller)	0	0	0	0.47
Cladocera (juveniles)	31.15	0	0	389.35
Eudiaptomus gracilis (Sars)	279.5	363.15	145.8	124.6
Cyclops vicinus (Uljanin)	66.05	11.5	30.8	699.13
Cyclops sp. (juvenile)	405.7	124.05	47.5	1320.6
Nauplii	450.65	407.85	149.2	3018.76
Total	2081.5	2475.7	607.5	5946.46

Discussions

The analysis shows that the majority of the planktonic crustaceans are not developing well under ice at the minimum of temperature (I2), and only *E. gracilis* had medium densities under the ice layer (0-5 m: 665 ind. m^{-3}; 5-10 m: 160 ind. m^{-3}; 10-30 m: average 12.5 ind. m^{-3}) where the phytoplankton had the highest densities (0-5 m: average of $50x10^5$ cells L^{-1}; 5-10 m: average of $40x10^5$ cells L^{-1}). In the cold period without ice (I1) *D. galeata* and *E. gracilis* had the maximum densities. In D1 (cold temperatures, not extreme 6-5ºC, no ice), *D. cucullata*, *Diaphanosoma orghidani* and *Bosmina longirostris* had the maximum development. In D2, when the temperatures grew (13.22 – 4.04ºC) species like *C. vicinus*, the cladocerans juvenile, *Cyclops* juvenile and nauplii (all young forms present in the water) developed well. The Pearson correlation index showed a strong positive correlation of the planktonic crustaceans with the increasing temperature (r=0.712)

An important find is that none of the above planktonic crustaceans reached high densities in I2 when *Asterionella formosa* had its highest development. Higher densities of crustaceans were noticed when *A. formosa* was not quantitatively important in the phytoplankton (D1, I1) and after it begun to decline due to the rise in temperatures (D2). This diatom is hard to consume as the bone-like shape of each cell, and the size and form of the stellate colonies, correlated with the high content of silica that pennate diatoms have, does not create a good food stock for zooplankton. The Pearson correlation index showed a moderate to strong negative correlation between the planktonic crustaceans and *A. formosa* (r=-0.697). Also, *A. formosa* is a species characteristic to very cold water (Pearson correlation index of the *A. formosa* densities and the water temperature showed a strong negative correlation of this algae with the increasing temperature; r=-0.802) and low intensity of light (Goldman et al., 1963). As specified by Salmaso (1996), *A. formosa* belongs to the winter group of algae with high developments in December and February (Flint, 1949), being a species favored by low temperatures (Løvestad, 1983) and daily sunshine under 4 hours (Flint, 1949). There are cases which present *A. formosa* as reaching high densities in March (Pasztaleniec & Lenard, 2008), as it is in the present situation, and from December to March, *A. formosa* is gradually increasing.

The other dominant algal components of the reservoir showed a moderate positive correlation with the increasing temperature: $r_{C.d.u}$=0.594; $r_{a.p.}$=0.636; r_{Phyto}=0.590.

The medium values that the planktonic crustacean *E. gracilis* had in I2 may be due to the highest densities of *Chlorophyta* ($10.9x10^5$ cells L^{-1}) and autotrophic picoplankton ($11.4x10^5$ cells L^{-1}) in the first 10 m of the water column. The strong development in D2 of the planktonic crustaceans (especially the numerous young forms) relate to a strong growth of small size phytoplankton including autotrophic picoplankton and *Cyclotella distinguenda* var. *unipunctata*, very small centric diatom, easy to consume. Pearson correlation index showed that the planktonic crustaceans have a very strong positive correlation with the development of *C. distinguenda* var. *unipunctata*, autotrophic picoplankton and the increasing quantity of total phytoplankton ($r_{C.d.u}$=0.969; $r_{a.p.}$=0.983; r_{Phyto}=0.954)

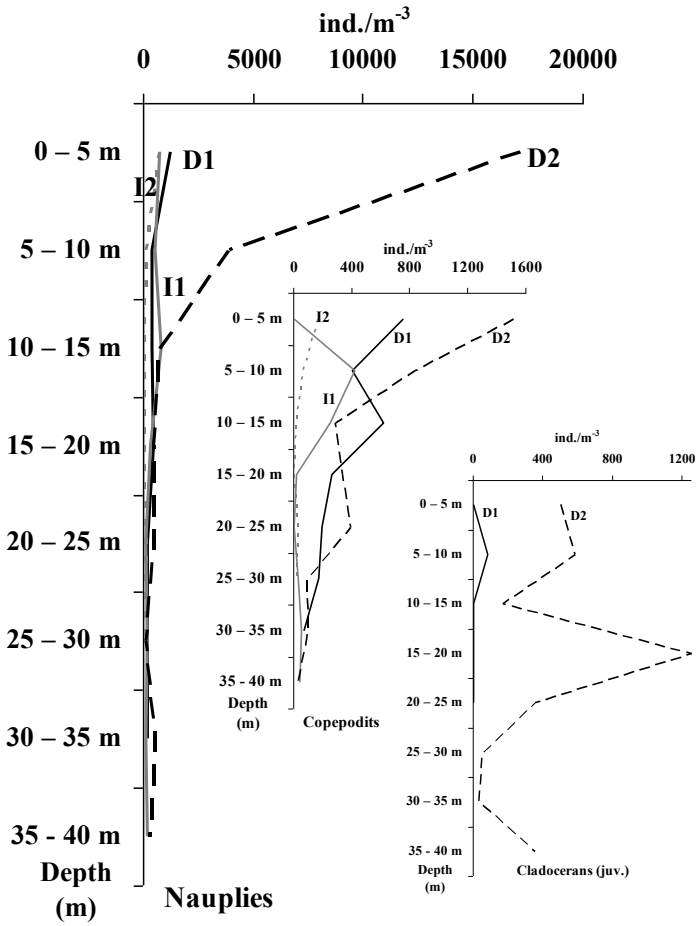

Figure 3

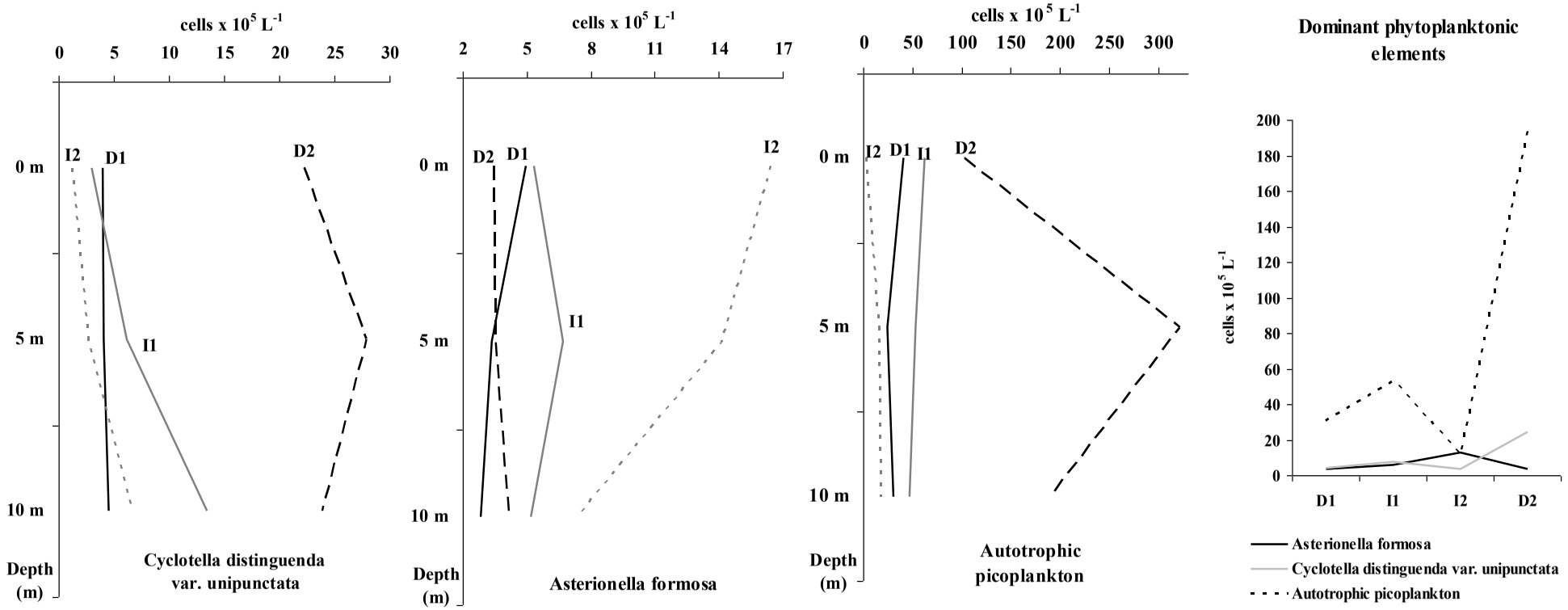

Figure 3. Detailed development of the dominant elements of the phytoplankton in the first 10 m of the water column; dominant phytoplanktonic elements in the study period

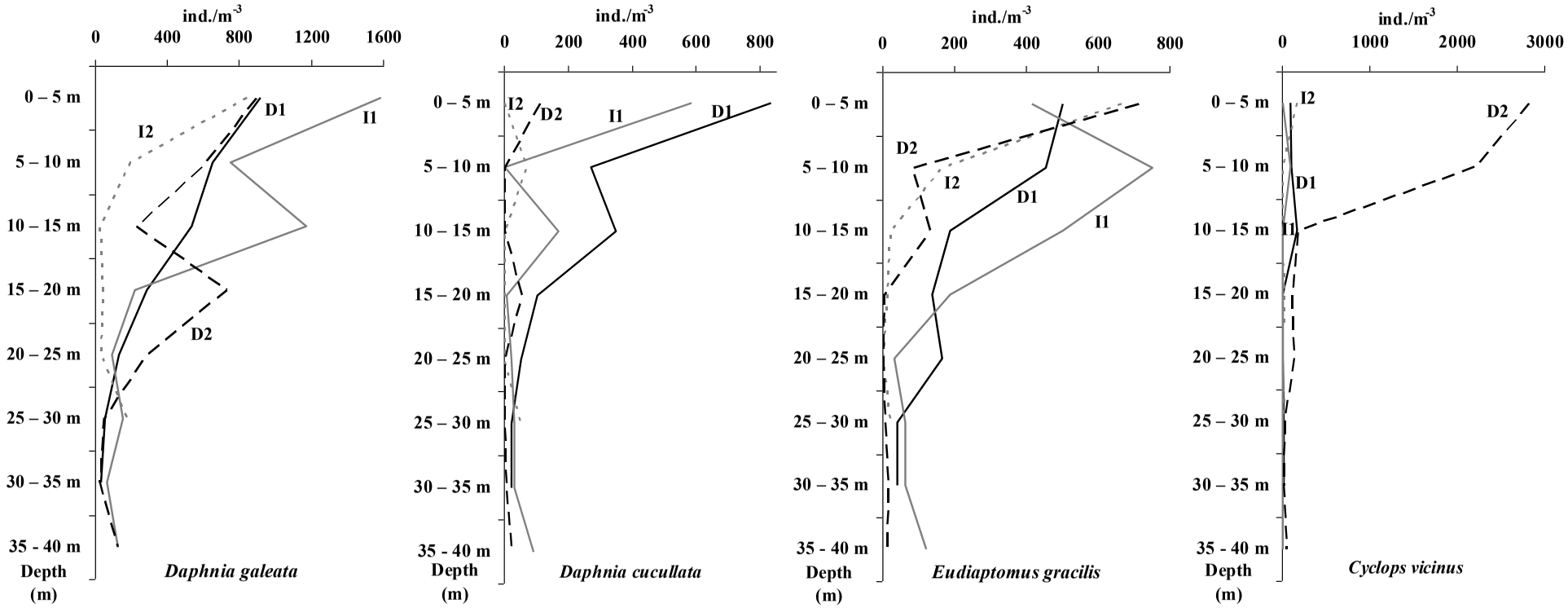

Figure 4. Detailed development of the dominant planktonic crustaceans in the water column

CONCLUSIONS

The direct and inverse thermal stratification phases analyzed in this paper captured in four different moments of winter and spring show the impact of the abiotic factors on the organisms living in the water column and how the species of the algae living in the water influence the development of the planktonic crustaceans.

The algal densities and their number of taxa are strongly correlated with the temperature and light intensity as the statistic analysis shows and, depending on the preferences, species like *Asterionella formosa* thrives under ice while other almost disappear.

The planktonic crustaceans develop poorly in extreme cold, especially under the ice layer and at high densities of *A. formosa*, while milder temperatures, correlated with more edible algal species (small, soft) lead to bigger populations.

REFERENCES

1. Aoncioaie, C., Erhan, M. – G., 2010. Evaluation of the Bicaz Reservoir trophic state using chlorophyll a as indicator. An. Şt. Univ. "Al. I. Cuza" Iasi, s. Biol. Anim., LVI, 237 – 247;
2. Bancilă, I., 1989. Geologia amenajărilor hidrotehnice / *The geology of hydrotechnical improvements* Ed. Tehnică, Bucureşti, 264 p.
3. Darabă, O.-M., 2008. Trophic level of the Izvoru Muntelui – Bicaz Reservoir using the chlorophyll-A concentration as indicator, according to the Romanian Standard S.R. ISO 10260/1997. An. Şt. Univ. "Al. I. Cuza" Iaşi, ser. Biol. Anim., LIV, 135 – 143;
4. Fietz, S., Kobanova, G., Izmest'eva, L., Nicklisch, A. 2005. Regional, vertical and seasonal distribution of phytoplankton and photosynthetic pigments in Lake Baikal. Journal of Plankton Research, 27, 8: 793 – 810.
5. Flint, E. A., 1949. An investigation of the distribution in time and space of the algae of a British water reservoir). Hydrobiologia 2 (3): 217 – 240
6. Goldman, C. R., Masom, D., T., Wood, B. J. B., 1963. Light injury and inhibition in Antarctic freshwater phytoplankton. Limol. Oceanogr. VIII (3), 313 – 322.
7. Hillebrand, H., Dürsellen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology. 35: 403 – 424.
8. Hutorowicz, A., 2005. Opracowanie standardowych objętości komórek do szacowania biomasy wybranych taksonów glonów planktonowych wraz z określeniem sposobu pomiarów i szacowania. Instytutu Rybactwa Śródlądowego. 42 p.
9. Karthick, B., Taylor, J. C., Mahesh, M. K., Ramachandra, I. V., 2010. Protocols for collection, preservation and enumeration of Diatoms from aquatic habitats for water quality monitoring in India. The Icfai University Journal of Soil and Water Sciences. III (1); 25 – 60.
10. Løvstad, Ø, 1983 Determination of growth-limiting nutrients of red species of *Oscillatoria* and two "oligotrophic" diatoms. Hydrobiologia 107: 221 – 230
11. Miron, I., Cărăuş, I., Măzăreanu, C., Apopei, V., Grasu, C., Ichim, I., Mihăilescu, F., Rujinschi, R.-I., Rujinschi, C., Simalcsik, F., Misăilă, C., Ţăruş T., Apetroaiei, N., 1983. Lacul de acumulare Izvoru-Muntelui Bicaz. Monografie Limnologică. / *Izvoru-Muntelui Bicaz Reservoir. Limnological monograph.* Ed. Acad. R.P.R. Bucureşti, 224 p.
12. Pasztaleniec, A. & Lenard T., 2008. Winter phytoplankton communities in different depths of three mesotrophic lakes (Łęczna-Włodawa Lakeland, Eastern Poland). Biologia (Section Botany) 63/3: 294 – 301
13. Ryding, S.-O.; Rast, W., 1994. Le contrôle de l'eutrophisation des lacs et des réservoirs. Masson Ed., Paris. 294 p.
14. Salmaso, N., 1996. Seasonal variation in the composition and rate of change of the phytoplankton community in a deep subalpine lake (Lake Garda, Northern Italy). An application of nonmetric multidimensional scaling and cluster analysis. Hydrobiologia 337 : 49 – 68.
15. Surugiu, V., 2008. Limnobiologie şi saprobiologie. Compendiu de lucrări practice./ *Limnobiology and saprobiology. Compendium tutorial*. Ed. Tehnopress, Iaşi. 331 p.
16. Wetzel, R., 2001. Limnology, 3rd edition. Lake and River ecosystems. Academic Press. 1006 p.

SUBPERIOSTEAL FLAP DIFFERENTIAL USE IN GINGIVAL RECESSION IN PERIODONTAL POCKETS AT DOGS

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Abstract:*Subperiosteal flap in gingival recession due to periodontal pockets represents an alternative in surgical therapeutic of periodontal pockets larger than 4 mm, which can not be treated by gingivectomy.*

The study was performed on 12 dogs of different breeds and genders, which at clinical examination presented periodontal pockets at different dental levels.

At the dogs which presented pocket at only one tooth the incision was vertical and at the ones with multiple pockets, incision was performed horizontally. The results were good to very good when the surgical debridement is adequate and the flaps are detached with the periostum.

Flaps suture is performed in simple interrupted pattern with nonresorbable thread and the healing is by first intention.

Keywords*: dog, flap, periodontal pockets*

INTRODUCTION

The evolution of periodontitis in dogs is related to many factors including age of the animals, nutrition, local hygiene, microorganisms pathogenic capacities, integrity and functionality of the local defense mechanisms in the mouth, etc.

The inflammatory process is usually chronic, tissue deterioration in various forms and locations, such as in chronic marginal periodontitis (2, 6), acute juvenile periodontitis, or in acute necrotic ulcerative gingivitis. Bacterial flora is complex and acts differently depending on the nature of germs and enzyme complex. Is appropriate to recall anaerobic bacteria that act through a complex mechanism, due to numerous enzymatic factors that have an aggressive role, endotoxin or destructive factors released by periodontal tissue (4, 10). Periodontal tissues remain intact as long as there is a balance between the body's resistance and bacterial pathogeny. Otherwise inflammatory processes appear with an necrotic nature (periodontal pockets), with tissue destruction, which are rejected gradually leaving the tissue exposed, healthy portion is reorganized different, gingival recession occurring this way.

MATERIAL AND METHOD

The study was performed on 12 dogs ,clinical cases, in a period of 2 years (2013-2015), of different breeds and sexes, which presented periodontal disease and periodontal pockets with the presence of gingival retraction, at the Surgery clinic of the Faculty of Veterinary Medicine in Cluj-Napoca. Evaluation was made by clinical examination to assess the severity and the seriousness of these injuries, their location, local changes, the extent of tissue destruction, size, depth and characteristics of periodontal pockets, and therefore subperiosteal flap treatment was performed.

In our study we used the modified Widmann technique, in which the flap aims to suppress the pockets that exists. In five cases, periodontal pockets were present just in one dental element, and in seven cases, periodontal pockets were extended to two or three dental elements. Gingival retraction of varying degrees and on single dental elements was present at eight cases. In four cases, gingival retraction was present at several dental elements. Anesthesia was performed by the administration of atropine sulphate (10.04 mg/kg intramuscularly), followed by Diazepam (0.2 mg/kg intramuscularly) and Ketamine 10% (3 mg/kg intramuscularly), and maintained with Isoflurane 2% after intubation. The dogs were restrained in lateral decubitus. Initially an incision is performed departing from the gingival margin at 0,5-1 mm of it in an 45^0 angle (fig.1), towards the alveolar crest, having an outline that seeks the gingival margin. If appropriate, at more pronounced periodontal pockets incisions can be made to discharge. After hemostasis is performed with an elevator the subperiosteal gingival tissue are raised. The flaps were hydrated with saline. An intra-crevicular incision is performed starting from the bottom of the periodontal pocket to the bone (fig.2), circumscribing the triangular area containing the internal wall of the pocket.

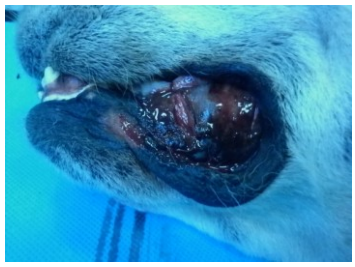

Fig.1. Gingival incision

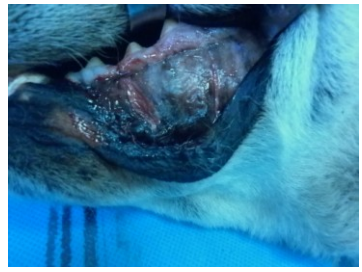

Fig.2. Intra-crevicular incision

A third incision is made in the interdental spaces with the scalpel interproximal, coronary as against the bone, then with a curette the excised portion is removed. In that way the access is appropriate for correcting the compromised portion. The granulation tissue is excised, tartar is removed root surfaces are smooth down with care not to damage the periodontal fibers which are attached. Citric acid (pH-1) was applied on root surfaces followed by lavage with saline. In our study, the seven cases with extended periodontal pockets the bone architecture had to be corrected for a better positioning of the flaps and adjust portions of osteolysis. After surgical debridement flaps are repositioned to cover all the bone portion (fig.3). Suture was performed in simple interrupted pattern with nonresorbable thread (fig.4). Stomodine Gel was applied local for 5 days.

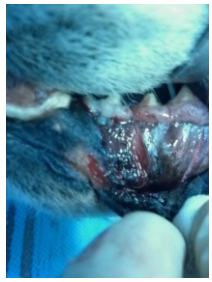

Fig.3. Parallel incision to more dental elements

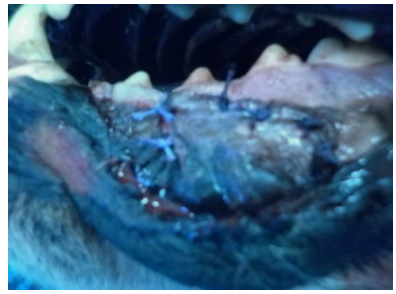

Fig.4. Subperiosteal flap suture

RESULTS AND DISCUSSIONS

Using subperiosteal flap in reducing periodontal pocket is a surgical technique that aims to suppress pockets with a minimum sacrifice according to the periodontal tissues correlated with postoperative tissue adaptation on the tooth surface. This is conditioned by a proper aseptisation of the affected part and a fast and efficient closing of healthy tissues. The technique of using subperiosteal flaps has the advantage that the apophysis of the alveolar bones acquires new characters, because attrition and defects, granulation tissue and the periodontal pockets are removed. This is directly correlated with the fact that in order to produce a regeneration of periodontium, periodontal ligament progenitor cells must migrate to denuded root surface, adhere to it and cause increased of the collagen fibers inserted functional ,view also shared by Zetu L. et al 1999. Another advantage of this technique is that by lifting the flap is created direct access to the apex of the tooth and can also be easily shaped the optimal gingival contour, aspect mentioned by authors such as Schluger S. et al., 1990. In case that we should intervene on the distal molars face, should be taken into account other aspects such as the height of the adherent gingiva, depth of periodontal pocket and the length from the distal retro molar tooth tuberosity, issues encountered by other authors such as Carranza FA et al 1996. Postoperatively, the most important issue is restoring dental-gingival junction, aspect which in our case studies we have improved by root demineralization with citric acid solution (pH -1) for 2.5 minutes, flushing with saline and application to the root of fibronectin derived from plasma of the subjects. In our study surgery proceeded in good conditions, with a moderate inflammatory process and the postoperative evolution were very good, except the three cases that initially presented periodontal pockets of fourth degree. Healing occurred in an interval of 14-18 days, and in three subjects healing was compromised by the appearance of granulation tissue which created abundant pressure on subperiosteal flap, which affected cure. In this context, due to the tension given by granulation tissue, the edge of flaps were sectioned leaving a denuded area which was subsequently treated by dental dressing , with the appearance of gingival retractions.

Postoperatively, subjects received Dexamethasone for 3 days at a dose of 2-4 mg / animal and Lincomycin 1.0 mg / kg.

Oral hygiene was maintained by oral lavage with chlorhexidine solution for 20 days. The diet administered was semiliquid. Postoperative controls were carried out weekly periodical which has appreciated incisions and sutures integrity.

CONCLUSIONS

The flaps can be used successfully in subperiosteal gingival recession, under conditions of a appropriate bone support and correct folding of the prepared surface.

Bone architecture should be adjusted as close to the natural shape of the zone, in such a manner that the flaps adhere to the bone bed support.

Widmann modified technique can be used with good results in dogs, but we must perform accurate flaps and the suture must not be in tension.

REFERENCES

1. Brook A. Niemiec and Robert Furman, 2013. *Osseous Surgery and Guided Tissue Regeneration*. Veterinary Periodontology, 254-288.
2. Frank R.M., 1980. *Bacterial penetration in the apical pocket wall of advanced human periodontitis*. J. Period. Res., vol. 15, 563.
3. Jan Gottlow, Sture Nyman and Thorkild Karring, 1992. *Maintenance of new attachment gained through guided tissue regeneration.* Journal of Clinical Periodontology19:10.1111/cpe.1992.19.issue-5, 315-317.
4. Listgarten M.A*., 1987. Nature of periodontal disease. Pathogenic mechanisms.J. Periodont.* Res.
5. Nyman S., Gottlow J., Karring T., et al, Wennstrom J., New attachement formation by guided tissue regeneration. J. Periodont. Res., vol. 22, 252.
6. Raul G. Caffesse, Carlos E. Nasjleti, Gissela B. Anderson, Dennis E. Lopatin, Billy A. Smith and and Edith C. Morrison, 1991. *Periodontal Healing Following Guided Tissue Regeneration With Citric Acid and Fibronectin Application*. Journal of Periodontology 62:1, 21-29.
7. Saglie R., Newman N.G., Carranza F.A. Ir., Pattison G.A., 1982. *Bacterial invasion of gingiva in advanced periodontitis in humans.* J. Periodont., vol. 53, 217.
8. Steve Garrett, Michael Martin and Jan Egelberg, 1990. *Treatment of periodontal furcation defects Coronally positioned flaps versus dura mater membranes in class II defects*. Journal of ClinicalPeriodontology 17:10.1111/cpe.1990.17.issue-3,179-185.
9. Steve Garrett, Bruno Loos, Durwin Chamberlain and Jan Egelberg, 1988. *Treatment of intraosseous periodontal defects with a combined adjunctive therapy of citric acid conditioning, bone grafting, and placement of collagenous membranes.* Journal of Clinical Periodontology 15:10.1111/cpe.1988.15.issue-6, 383-389.
10. Slots J., Genco R.J., 1984. *Black-pigmented Bacteroides species, Capnocytofaga species and Actinobacillus actinomycetemcomitans in human periodontal disease: virulence factors in colonization, survival and tissue destruction.* J. Dent. Res., vol. 63, 412-416
11. Zetu L., Popovici D., 1999. *Parodontologie. Tratament chirurgical*, Iaşi, Ed. Junimea, 223.

COMPARATIVE ASSESSMENT OF DIFFERENT SURGICAL TECHNIQUES IN COXOFEMORAL LUXATION IN DOGS

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Abstract:*Hip dislocation is a serious condition, most often traumatic, that occurs in dogs, of any age and can affect all breeds and it's characterized by the movement of the femoral head from the acetabular cavity. For our study we used 16 dogs of different breeds, ages, females but also males, diagnosed with hip luxation. Eleven (68,7%) out of the sixteen subjects were with dorso-cranial displacement of the femoral head and five (31%) of them were with dorso-caudal displacement. Surgery was performed within 72 hours for the first 10 cases and in the next 3-4 days for the other cases. The subjects were divided in two grups, for six of them we used two methods: the transposition of the greater trochanter, transarticular drilling and articular immobilization with a pin. The results were evaluated after 6 months. The results were 50% for the transarticular drilling and articular immobilization with a pin and 66% for the transposition of the greater trochanter. Postoperatively the subjects were monitored in terms of physical mobility through exercise and well known procedures as to obtain a high quality healing.*

Keyword: *Comparative methods, coxofemoral luxation, dog*

INTRODUCTION

Hip luxation is a common affection found in dogs, consecutive to trauma produced by car accidents, falling, other accidents or hip displasia (Trostel O.T.,2000). In this pathology takes place the rupture of bonds and anatomical structures of the coxofemural joint along with the displacement of the femoral head from the acetabulum. Although it is not a surgical emergency, the repositioning has to take place within 72 hours (Basher A.W.P.,and col., 1986; Bone D.L., and col., 1984) to prevent the pathological modifications of the femoral head and the acetabulum. If the 72 hours pass then the repositioning procedure becomes harder, aspect which has been described by other authors also (Basher A.W.P.,and col., 1986; Bone D.L., and col., 1984). For repositioning we can use both closed and open (surgical) procedures, both types being conditioned by certain factors.

Although closed repositioning is inefficient in most cases, if it is tried before the surgical approach, it does not affect the long term prognosis. Open repositioning is indicated when either the acetabulum or the femoral head are fractured, when the luxation is recurrent and is confirmed by radiography after closed repositioning, or in the case of chronic luxation and a visual assessment of the cartilage is needed. For the repositioning there are a series of methods, each having advantages and disadvantages.

METHODS AND MATHERIALS

Our research and observations have been taken on 16 dogs of different breeds and ages, clinical cases that came during a period of 4 years (2010-2014) which presented hip luxation without fracture of the great trochanter, femoral head or neck. From these cases, 11

subjects (68,7%) presented dorso-cranial luxation, and 5 subjents (31%) presented dorso-caudal luxation. The surgical procedure took place in the first 72 hours after the accident in 10 cases, and after 3-4 days after the accident in 6 cases. In all cases we chose the surgical approach, where we used two types of procedures: transpositioning of the great trochanter which was done in 8 cases, and transarticular pinning which was done in 8 cases.

Anesthesia was done using atropine sulfate 0,04 mg /kg i.m. (Nycomed Austria, Gmbh) fallowed by Diazepam 0,2 mg/kg i.m. (Diazepam, Terapia S.A. Cluj-Napoca) and Ketamin, Protulab Pharma b.v., Rdamsdonkszeer, The Neterlands), fallowed by orotracheal intubation with Isofluran 2% (Lunan Pharmaceutical group Corporation, Shandong, China) combined with oxigen. Considering that the surgical procedureis common for both procedures untill a certain point, it will be described only once in the following text. The pacient is placed on the surgery tabel in lateral recumbancy with the luxated limb up. After proper surgical preparation of the area we make a curved incision, over the coxofemural joint which involves the skin, subcutaneous conjunctive tissue and the superficial, medial and deep gluteal muscles. In the cases of craniodorsal luxation, the femoral head is pulled from the place of luxation trough grasp of the hock or knee, depending on the case, and making an external rotation movement. When the mobility is complete, the limb is pulled in a caudodistal direction for a right positioning of the femoral head against the acetabulum. For the cases of ventrocranial luxation, the femoral head was manipulated in a dorsocranial direction, and for the contrapressure created on the pelvis, the femoral head is brought next to the acetabulum. With this ocasion we put to view the acetabulum, and the damaged soft tissue, haemathomas and the remains of the round ligament from the femoral head are removed. Also, the possible damages of the femoral head are assesed allong with the acetabulum and the joint capsle. The integrity of the joint capsle is very important because it contributes to the stability of the joint if it can be sutured.

For the thehnique of transpositioning of the great trochanter, it is required the osteothomy of the great trochanter with mentenance of the insertion of the gluteal muscles. After the osteothomy of the great trochanter, we turn it to evidentiate the sectione surface. From this place we drill the femoral neck and head towards the fovea capitis using a single drill of the right dimension, or using guidance so that we can reach the very center. From the center of the acetabulum we make a second whole which penetrates the medial acetabulum wall also with the help of a drill, allowing the penetration of a homemade pin. We prepare a pin from Krischirer wire so that two ends go arround a central loop. Trough the center of the pin which is „8” shaped we pass a nylon wire which is tied so that the endsof the wire are 20-25 cm long (fig.1.). The pin prepaired thus is introduced with one end in the whole from the acetabulum so that it goes trough the created orifice completely (fig.2) (passing the medial acetabular wall). Trough moderate traction of the ends of the wire the pin is placed horizontaly without the possibility of returning trough the orifice. The tips of the wire are passed again trough the tunnel betweek the fovea capitis and the great trochanter. We position the femoral head in the correct position in the acetabulum and at this level we tie the wire. We reposition the great trochanter and fixate it with a screw (fig.3.) definetly after witch we suture the soft tissue withe resorbable suture material and the skin in separate stich points.

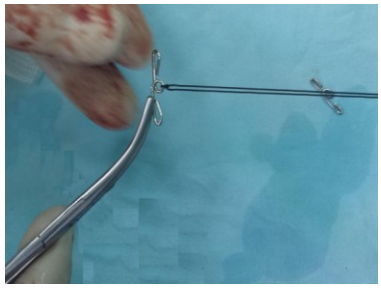

Fig.1. Prepared pin

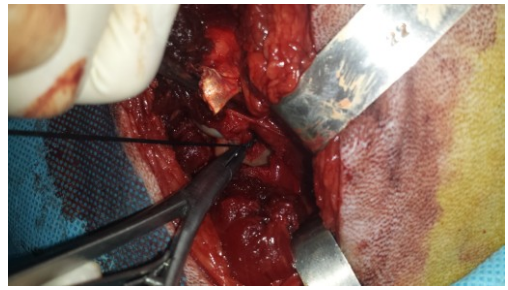

Fig. 2. Pin insertion in the pelvic cavity

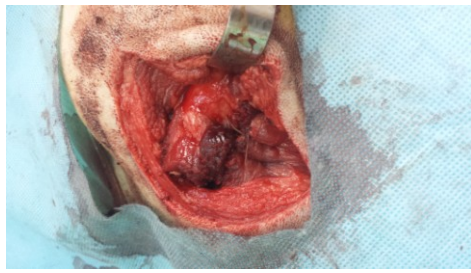

Fig.3. Reposition of the great trochanter with a screw

The second technique, theone of transarticular pinning involves the isolation of the luxated joint head, the cleanse of it, and we make a drill whole from the fovea capitis towards the great trochanter. Trough the orifice created (fig. 4.) we introduce the Steinmann pin after which we position correctly the joint, the limb is held in weight-bearing position and slight abduction while the pin is passed trough the acetabulum wall so that the end would go trough the pelvic channal. During both procedures a secong person assesses the penetration of the pin trough the medial wall of the acetabulum trough rectal examination or by radiologic exam (fig.5). We assess the mobility of the joint by anteroposterior movement, after which we suture the surrounding tissue with adequate suture material.

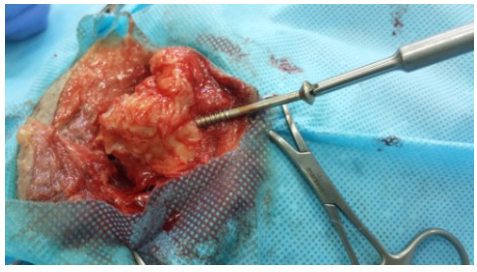

Fig.4. Steinmann pin (screw) positioning

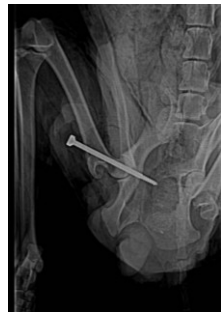

Fig.5. Radiological aspect of the screw

RESULTS AND DISCUSSIONS

Both methodes areused differently in current practice, according to different factors concerning height and weight, age, degree of tissue distruction, the health status of the bone tissue, surgical abilities for each methode. Central medular pinning in our observations is an operative methode, can be done in a relatively short period of time, tissue distruction is

reduced because acces to the great trochanter is relatively easy, without affecting the insertions of the gluteal muscles or other anatomical structures from this region. On the other hand the methode can be preffered because loss of bonetissue is minimal because the drills are of small diameter, a drill of 1,6 mm in diameter can be used for dogs weighing between 4-10 kg, and a 3-3,4 mm diameter drill can be used in dogs weighing over 30 kg. Besides this, we consider that for choosing this methode we have to take into consideration that certain complications can apear, like cartilage damage during the procedure, damageing the sciatic nerve, pin migration, rectal perforation, bending or breaking of the pin or osteochondritis.
The correct positioning of the femoral head in the acetabulum during the entire procedure of repositioning avoids the apparence of complicationd which would compromise the succes. This is the reason why this procedure is practiced less. In our caseload this methos was applied to 8 subjects, weighing between 10-20 kg. Althow the surgical procedure was fully respected, at 4 of them we noticed disconfort in the hip joint manifested trough mild pain and reduceing mobility in movement, by reduceing the lenght beetwen the steps.

The great trochanter transpositioning methode, is a wider used methode because on one hand it is easier to do, and on the other hand the new creater ligament offers the possibility of a relative elasticity in certain situations when it is repositioned in the acetabulum. We state that this mobility, concerning heavy breeds of dogs, creates problems in the such as those that in time and in the situation where the joint capsle can't be saved trough a adequate suture, at this level there can appear complications that can affect the good functioning of the joint. This apect is due to the fact that the weight of the animal forces the new created ligament and consequently the whole contrapment. In our caseload this methode was applied in 6 subjects of medium and heavy weight in which the application of the methode took place adequately. In 2 of the cases the pathology recurred so we were obligated to choose another methode. We mention that in the case of two dogs the trauma produced the rupture of the joint caple which could not be reconstructed, allong with a segnificant part of muscle tissue. Lezions manifested trough haemorrage, erozions and edema were evident at the level of the joint cartilage. In 6 cases (66%) with a weight between 20-30 kg results were good and after a 4 week pause the dogs started to use their limb more and more without recurring or complications.

Assessing the two methodes used trough the aspect of repositioning, pain management, the facility of the methode, postoperative evolution, we state the fact that the best results we obtained were trough the methode of transpositioning of the great trochanter.
At both methodes, postoperative patients were given antibyotic therapy (Gentavet 3 mg/kg) and antiinflamatory drugs (Dexafort 0,1 mg/kg) for three days to prevent the apparence of osteoarthritis or any local septic process. We have also made a series of radiographical assessments of the operated joints to confirm the good evolution.

CONCLUSIONS

1. The methods of repositioning of hip luxation by transpositioning of the great trochanter and the transarticular fixation with the Stainmann pin, are methods with good or very good results with minimal loss of (bone) tissue.
2. Concerning the caseload studied where we used the transpositioning of the great trochanter the results were 66% better than the ones with transarticular pinning.

3. The surgical procedure has to take place in the first 72 hours from the accident, because the delay would increase the probability of inflammatory or septic complications which are dangerous. Radiological assessment of the joint are necessary for confirming the repositioning.

4. For a fast and efficient recovery we recommend that after surgery and 4-6 week rest, the patients should fallow a physiotherapy program with adequate exercises, a well managed nutrition program or osteoarthritis modification agents.

BIBLIOGRAPHY

1. Ash K., Rosselli D., Danielski A., Farrell M., Hamilton M., Fitzpatrick N., 2011. Correction of craniodorsal coxofemoral luxation in cats and small breed dogs using a modified Knowles technique with the braided polyblend TightRope^{TM} systems, Veterinary and Comparative Orthopaedics and Traumatology, 25, 1, 54-60.
2. Basher A.W.P., Walter M.C., Newton C.D., 1986. Coxofemural luxation in the dog and cat. Vet. Surg., 15, 356-562.
3. Bone D.L., Walker M., Cantwell H.D., 1984. Traumatic coxofemural luxation in dogs, results of repair. Vet. Surg., 13, 263-270.
4. Flynn M.F., Edminston D.N., Roe S.C., et al., 1994. Biomechanical evaluation of a toggle pin technique for management of coxofemoral luxation. Vet. Surg., 23:311.
5. Fox S.M., 1991. Coxofemoral luxation in dogs. Comp Contin Educ., 13:381.
6. Harari J., Smith C.W., Rauch L.S., 1984. Caudoventral hip luxation in two dogs. J. Am. Vet. Med. Assoc., 185:312.
7. Hunt C.A., Henry W.B., 1985. Transarticular pinning for repair of hip dislocation in the dog: a retrospective study of 40 cases. J.Am. Med. Vet. Assoc., 187:828.
8. Lewis D.D., Beale B.S., Dean P.W., et al., 1990. Ischio-ilial pinning for stabilization of coxofemoral luxation: an anatomical study. Vet. Comp. Orthop. Traumatol., 1:31.
9. McLaughlin R.M., 1995. Traumatic joint luxation in small animals. Vet. Clin North Am Small Anim Pract., 25:1175.
10. Meij H.A., Hazewinkel W., Nap R.C., 1992. Results of extra-articular stabilization following open reduction of coxofemoral in dogs and cats. Journal of Small Animal Practice, 33, 7, 320-326.
11. Rochat M.C., 2001. Managing coxofemoral luxation in dogs. Vet. Med., 5:385.
12. Thacker C., Schrader S.C., 1985. Caudal ventral hip luxation in the dog: a review of 14 cases. J. Am Anim Hosp Assos., 21 (Suppl 2): 167.
13. Trostel C.T., Peck J.N., deHaan J.J., 2000. Spontaneous bilateral coxofemoral luxation in four dogs. J. Am Anim Hosp Assoc, 36:268.

ASPECTS ON THE EPIDEMIOLOGY OF *BARTONELLA* INFECTION IN CATS AND ZOONOTIC RISK

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Abstract*: Bartonella infections and their associated pathology have been studied predominantly in the XX century. In recent decades, studies are providing important data on biological aspects and their immuno-pathogenesis. Domestic cats are the main reservoir of Bartonella henselae, the causative agent of Cat Scratch Disease (CSD) in humans. Other species, such as Bartonella clarridgeiae and Bartonella koehlerae have been also reported to be causative agents in certain pathological conditions in humans (i.e. endocarditis, CSD). Hematophagous arthropods are involved in the transmission of Bartonella species of mammals and humans, of them fleas playing a major role. Bacteriological and sero-epidemiological studies conducted worldwide show that the number of potentially zoonotic Bartonella species has increased considerably. With regard to B. henselae infection in cats, antibody prevalence varies from 3% - 80%, and the prevalence of bacteraemia varies from a few percent to more than half of the population tested, depending on the geographical distribution and life category of cats (owned or stray cats). Also, B. clarridgeiae is distributed unequally among the population of cats worldwide, with variable prevalence (from 10% to up 36%). Considering the both animal and public health and the environmental factors that influence the spread, distribution and transmission of these bacteria, it is considered necessary to approach Bartonella infections from the "One Health" perspective. Therefore, by this approach it is aimed to reduce the zoonotic risk and to promote a better understanding of the epidemiology and clinical signs of the disease known as bartonellosis.*
Key words: *Bartonella* spp., epidemiology, cats, zoonotic risk

INTRODUCTION

Bartonella infections and their associated pathology have been studied predominantly in the XX century, contributing to a better understanding and knowledge of the emerging zoonotic diseases. Within the last decades, the studies have shown that the genus *Bartonella* is a highly adapted parasitic microorganism, capable of inducing a wide variety of persistent infections of animals, including human beings (Breitschwerdt and Kordick, 2000).

The present name of the genus - *Bartonella* was proposed in 1993 by Brenner et al., to replace the old name - *Rochalimaea* (*Rickettsiaceae* family) (Brenner et al., 1993). Consequently, it is proposed that the species previously associated with the genus *Rochalimaea* to be united with the genus *Bartonella* and renamed as follows: *B. henselae, B. quintana, B. vinsonii* and *B. elizabethae*. This new classification had the effect of transferring of these species from the family *Rickettsiaceae* into the family *Bartonellaceae* (Brenner et al., 1993), which included *B. bacilliformis*, and moved the family *Bartonellaceae* from the order *Rickettsiales* (Breitschwerdt and Kordick, 2000) in order *Rhizobiales*. Two years later, it was proposed the unification of both genus *Grahamella* with *Bartonella*, which resulted in the addition several species of *Bartonella*: *B. grahamii, B. peromysci, B. talpae, B. doshiae* and *B. taylorii* (Brenner et al., 1993; Breitschwerdt and Kordick, 2000).

All the *Bartonella* species belong to the group of the small intracellular bacteria, Gram-negative organisms (Sander et al., 1998); they are immobile, with lengths of 1-2 μ, negative tests for oxidase, catalase, urease and oxidation or fermentation of glucose (Palmer et al., 2005). *Bartonella* spp. infects and persists in mammalian erythrocytes and endothelial cells and is found in a wide range of domestic and wild mammals worldwide (Mietze et al., 2011).

In this paper it will be detailed the epidemiology of infections caused by *Bartonella* spp. in cats, the impact on their physiological status as a consequence of exposure to the causative agent, and the potential zoonotic risk.

I. Cats, the main reservoir for *Bartonella* spp. infections

Among of the many species of mammals, carnivores are an important reservoir for *Bartonella* spp.; in addition, most species of *Bartonella* which have been identified in carnivores have a zoonotic potential. Of the 11 species or subspecies of *Bartonella* known or suspected to be pathogenic to humans, six were isolated from pets (Chomel et al., 2006).

Domestic cats are the main reservoir for *B. henselae* (Staggemeier et al., 2010), the causative agent of Cat Scratch Disease (CSD) in humans. Other species, such as *B. clarridgeiae* and *B. koehlerae* have been also reported to be involved in certain pathological conditions in humans (i.e. CSD, endocarditis) (Chomel et al., 2004; Avidor et al., 2004).

Bartonella infections in cats are classified as vector-borne diseases, and the vector preference for a particular host can influence the transmission and spread of these organisms (Breitschwerdt and Kordick, 2000). The transmission of *Bartonella* species involves blood-sucking arthropods, amongst them fleas playing a major role. However, other potential vectors (ticks, haematophagous insects) were identified as carriers of *Bartonella* spp. (Breitschwerdt and Kordick, 2000).

Due to the ability of *Bartonella* spp. to infect the red blood cells, there is the possibility that various species of blood-sucking arthropods take bacteria during feeding. After inoculation of bacteria (e.g. bacteria in arthropods feces that are superficial scratched into the skin), these locate and persist at the site of inoculation (Pulliainen and Dehio, 2012). Bacteremia is initiated several days post-inoculation (Dehio, 2001) with the rapid emergence of a large number of bacteria into the bloodstream. The bacteria adhere and invade the erythrocytes and subsequent intense intracellular replicating within a "compartment" delimited by the membrane, until reaching a critical density, time to install an equilibrum, which is maintained throughout the life of infected erythrocytes (Schulein et al., 2001).

It is considered that, at regular intervals of about five days, new episodes of synchronous bacteria release in the bloodstream, this probably, as a result of the cycle of "infection type-five days", this reinfection are triggered each time from the site of inoculation (primary niche) by bacteria issued at the end of each cycle (Chomel et al., 2009).

Bacteremia can persist up to several weeks without cause in most cases an associated pathology, aspect encountered in most species of *Bartonella*. Major characteristic of *Bartonella* infections in animals is long lasting intraerythrocytic bacteremia, which represents a specific adaptation to the mode of transmission through blood-sucking arthropods (Chomel et al., 2009). The installation of a chronic intraerythrocytic bacteremia occurs in animal reservoir hosts, exclusively (Dehio, 2001).

II. Etiological and epidemiological aspects of *Bartonella* spp. infections in cat

Domestic cats are the main reservoir for *B. henselae*, and its fleas (*Ctenocephalides felis*) are a competent vector. Cats infected with *Bartonella* spp. are usually asymptomatic, but can still present recurrent bacteremia, which may last from months to years (Bouhsira et al., 2015).

The fleas are vectors responsible for the transmission of *Bartonella* species such as *B. henselae, B. clarridgeiae* and possible *B. koehlerae* (Chomel et al., 1996).

Regarding the tick species demonstrated carrying *Bartonella*, *Ixodes scapularis* in the United States and *Ixodes ricinus* in the Netherlands were reported (Schouls et al., 1999; Breitschwerdt and Kordick, 2000).

The main aspects of the epidemiology of *B. henselae* and *B. clarridgeiae* will be discussed, as follows bellow.

- ***B. henselae:*** is a small bacterium, red blood cells, wide spread on cats in all temperate regions of the world (Pennisi et al., 2010). The invasion of erythrocytes is accomplished through cell-mediated mechanisms, actino-dependent (in vitro study, using endothelial cells from umbilical vein, in humans) (Dehio et al., 1997).

B. henselae was first identified in the early 1990s in a domestic cat; that fact then leaded to numerous studies worldwide on the importance of cat as reservoir of bacteria (Rolain et al., 2003).

With regard to *B. henselae* infection, antibody prevalence varies from 3% to 80%, and the prevalence of bacteraemia varies from a few percent to more than half of the population tested, depending on the geographical distribution and life category of cats (owned or stray cats), such as in: Portugal, 2.9% (Maia et al., 2014), Brazil – San Luis, 3% (Braga et al., 2012), Germany, 16.0% (Mietze et al., 2011), Spain, 21.1% (Gil et al., 2013), Turkey, 27.9% (Guzel et al., 2011), Korea, 33.3% (Kim et al., 2009), Italy, 83.5% (Pennisi et al., 2010) (**Table 1, Table 2**).

Cats are usually bacteraemic for a few weeks, in the experimental infections, or for more than a year, in natural infections (Kordick and Breitschwerdt, 1995, 1998). Also, the prevalence and level of bacteraemia (the number of colony forming units per ml of blood) are usually higher in young cats (<1 year) than in adult cats (Chomel et al., 1995). However, it is claims that wide spread infection among cats occurs more frequently through flea feces than through their bites (Finkelstein et al., 2002).

The genetic characterization (genotyping) is useful particularly for epidemiological studies. For *B. henselae* have been described two 16SrRNA genotypes: 16S type I (called Houston - I) and 16S type II (called Marseille II) (Bergmans et al., 1996, Ebani et al., 2012). Studies have demonstrated that different types may induce varying pathologic features in human bartonellosis; it seems that type I is more pathogenic than type II. The cat and man can be infected with *B. henselae* type I or II, but occasionally, mixed infections with both types may occur (Ebani et al., 2012).

- ***B. clarridgeiae****:* was first isolated in the United States from a domestic cat, its owner being diagnosed as HIV-positive (Clarridge et al., 1995). This species of *Bartonella* was less frequent found in domestic cats than *B. henselae*, and distributed unequally in cat populations worldwide (Chomel et al., 2006).

On the basis of serologic testing, several recent publications have reported detection of *B. clarridgeiae* in cat populations in Europe, and also, on other continents. *B. clarridgeiae* infections prevalence were reported in a percentage ranging between 10%-36% in studies conducted in France (Boulouis et al., 2005), Spain (Gil et al., 2013), and Iraq (Switzer et al., 2013); variations with ≤ 10% in domestic cats were found in the northern Italy, 1.5% (Brunetti et al., 2013), China, 1.1% (Yuan et al., 2011), Germany and United States, 0.6% (Mietze et al., 2011; Namekata et al., 2010), and has not been isolated in studies from Korea (Kim et al., 2009) (**Table 1, Table 2)**.

Tabel 1. Sero-epidemiological data on *Bartonella* spp. infections in cats, in Europe

Location	No. of tested animals	*Bartonella* spp.	*B. henselae*	*B. clarridgeiae*	References
Portugal	649	2.9% (19/649)	-	-	Maia et al., 2014
Italy (Pavia, Varese, Bologna)	1,334	17.0% (229/1,334)	15.5% (208/1,334)	1.5% (21/1,334)	Brunetti et al., 2013
Spain	147	27.2% (40/147)	21.1% (31/147)	10.9% (16/147)	Gil et al., 2013
Italy (Pisa)	234	11.1% (26/234)	11.1% (26/234)	-	Ebani et al., 2012
Germany	169	16.6% (28/169)	16.0% (27/169)	0.6% (1/169)	Mietze et al., 2011
Southern Italy	85	83.5% (71/85)	83.5% (71/85)	-	Pennisi et al., 2010
Italy (Sardinia)	55	10.9% (6/55)	10.9% (6/55)	-	Zobba et al., 2009
Italy (Sassari)	79	21.5% (17/79)	21.5% (17/79)	-	Pinna Parpaglia et al., 2007

Tabel 2. Sero-epidemiological data on *Bartonella* spp. infections in cats, on other continents

Location	No. of tested animals	*Bartonella* spp.	*B. henselae*	*B. clarridgeiae*	References
Brazil (San Paulo)	47	25.5% (12/47)	17.02% (8/47)	12.76% (6/47)	Staggemeier et al., 2014
Brazil (Mato Grosso)	178	2.2% (4/178)	1.7% (3/178)	0.5% (1/178)	Miceli et al., 2013
Iraq	207	27.5% (57/207)	15.0% (31/207)	12.5% (26/207)	Switzer et al., 2013
Tailand (Bangkok)	153	17% (26/153)	7.8% (12/153)	3.9% (6/153)	Assarasakorn et al., 2012
Brazil (San Luis)	200	4.5% (9/200)	3.0% (6/200)	1.5% (3/200)	Braga et al., 2012
Algeria	211	17.0% (36/211)	17.0% (36/211)	-	Azzag et al., 2012
Turkey	298	27.9% (83/298)	27.9% (83/298)	-	Guzel et al., 2011
China	361	12.7% (46/361)	12.7% (46/361)	1.1% (4/361)	Yuan et al., 2011
USA (California, Michigan)	180	25.0% (45/180)	24.4% (44/180)	0.6% (1/180)	Namekata et al., 2010
Brazil (Rio Grande do Sul)	47	17.02% (8/47)	10.63% (5/47)	6.38% (3/47)	Staggemeier et al., 2010
Korea	48	33.3% (16/48)	33.3% (16/48)	0 %	Kim et al., 2009

III. Potentially zoonotic *Bartonella* species and risk factors

Considering the rapid expansion of knowledge on infections in reservoir mammals and the large number of arthropods that were involved in the transmission of *Bartonella* species, both animal and human exposure to these pathogens can be more important than thought until now (Breitschwerdt and Kordick, 2000).

The main route of transmission to humans is through cat scratch (Cat scratch disease – CSD, atypical manifested by encephalitis, osteolysis, granulomatous splenitis and/or hepatitis, pneumonia, thrombocytopenic purpura, and pleural effusion) (Breitschwerdt, 2014), while the transmission of infection by bite is rare (Zobba et al., 2009). In 1992, Regnery et al. identified *B. henselae* antigens seroactivity in 88% of 41 human patients with suspected CSD (Regnery et al., 1992). Recently, microbiological data show that the most common genotypes of *B. henselae* found in bacteremic cats are not the same genotypes found in patients with CSD, which suggests that only a subset of the more virulent strains of *B. henselae* found in cats causes acute form of CSD (Chaloner et al., 2011; Breitschwerdt, 2014). A typical CSD, indicate a self-limiting disease characterized by fever and lymphadenopathy, associated with cat scratch or bite (Breitschwerdt, 2014). Although it is known that CSD is a self-limiting illness, the *B. henselae* infection can cause intermittently symptomatic, minimally symptomatic, asymptomatic or chronic manifestations, accompanied by prolonged bacterenia, in humans (Breitschwerdt, 2014).

Considering the both animal and public health and the environmental factors that influence the spread, distribution and transmission of these bacteria, it is considered necessary to approach *Bartonella* infections from the "One Health" perspective (Breitschwerdt, 2014). The "One Health" concept involves cooperation between specialists in human health, animal health, bio-ecology and other related areas to develop complex integrated projects for development of knowledge of ecology, biology and vector competence for various species of *Bartonella*, and developing sustainable strategies of prevention (Mascarelli et al., 2013).

REFERENCES

1. Assarasakorn, S., Veir, J.K., Hawley, J.R., Brewer, M.M., Morris, A.K., Hill, A.E., Lappin, M.R., 2012. Prevalence of *Bartonella* species, hemoplasmas, and *Rickettsia felis* DNA in blood and fleas of cats in Bangkok, Thailand. Research in Veterinary Science, 93 (3): 1213-1216.
2. Avidor, B., Graidy, M., Efrat, G., Leibowitz, C., Shapira, G., Schattner, A., Zimhony, O., Giladi, M., 2004. *Bartonella koehlerae*, a new cat-associated agent of culture-negative human endocarditis. Journal of Clinical Microbiology, 42 (8): 3462-3468.
3. Azzag, N., Haddad, N., Durand, B., Petit, E., Ammouche, A., Chomel, B., Boulouis, H. J., 2012. Population structure of *Bartonella henselae* in Algerian urban stray cats. PLOS One, 7 (8): e43621.
4. Bergmans, A.M.C., Schellekens, J.F.P., van Embden, J.D.A., Schouls, L.M., 1996. Predominance of two *Bartonella henselae* variants among cat-scratch disease patients in The Netherlands. Journal of Clinical Microbiology, 34 (2): 254-260.
5. Bonilla, D.L., Kabeya, H., Henn, J., Kramer, V.L., Kosoy, M.Y., 2009. *Bartonella quintana* in body lice and head lice from homeless persons, San Francisco, California, USA. Emerging Infectious Diseases, 15 (6): 912-915.
6. Boulouis, H.J., Chang, C.C., Henn, J.B., Kasten, R.W., Chomel, B.B., 2005. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Veterinary Research, 36 (3): 383-410.
7. Boushira, E., Franc, M., Lienard, E., Boullin, C., Gandoin, C., Geurden, T., Becskei, C., Jacquiet, P., Thomas, A., Boulouis, H.J., 2015. The efficacy of a selamectin (Stronghold) spot on treatment in the prevention of *Bartonella henselae* transmission by *Ctenocephalides felis* in cats, using a new high-challenge model. Parasitology Research, 114 (3): 1045-1050.
8. Braga, Mdo.S.,Diniz, P.P., André, M.R., Bortoli, C.P., Machado, R.Z., 2012. Molecular characterization of *Bartonella* species in cats from São Luís, state of Maranhão, north-eastern Brazil. Memorias do Instituto Oswaldo Cruz, 107 (6): 772-777.

9. Breitschwerdt, E.B., 2014. Bartonellosis: One Health Perspectives for an Emerging Infectious Disease. ILAR Journal, 55 (1): 46-58.
10. Breitschwerdt, E.B., Kordick, D.L., 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clinical Microbiology Reviews, 13 (3): 428-438.
11. Brenner, D.J., O'Connor, S.P., Winkler, H.H., Steigerwalt, A.G. 1993. Proposals to unify the genera *Bartonella* and *Rochalimaea*, with descriptions of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb.nov., *Bartonella henselae* comb.nov., and *Bartonella elizabethae* comb.nov., and to remove the family *Bartonellaceae* from the order *Rickettsiales*. International Journal of Systematic Bacteriology, 43 (4): 777-786.
12. Brunetti, E., Fabbi, M., Ferraioli, G., Prati, P., Filice, C., Sassera, D., Dalla Valle, C., Bandi, C., Vicari, N., Marone, P., 2013. Cat-scratch disease in Northern Italy: atypical clinical manifestations in humans and prevalence of *Bartonella* infection in cats. European Journal of Clinical Microbiology & Infectious Diseases, 32: 531-534.
13. Chaloner, G.L., Harrison, T.G., Coyne, K.P., Aanensen, D.M., Birtles, R.J., 2011.Multilocus sequence typing of *Bartonella henselae* in the United Kingdom indicates that only a few, uncommon sequence types are associated with zoonotic disease. Journal of Clinical Microbiology, 49 (6): 2132–2137.
14. Chomel, B. B., Boulouis, H. I., Maruyama, S., Breitschwerdt, E. B., 2006. *Bartonella* spp. in pets and effect on human health. Emerging Infectious Diseases, 12 (3): 389-394.
15. Chomel, B. B., Boulouis, H. J., Breitschwerdt, E. B., 2004. Cat scratch disease and other zoonotic *Bartonella* infections. Journal of the American Veterinary Medical Association, 224 (8): 1270-1279.
16. Chomel, B.B., Abbott, R.C., Kasten, R.W., Floyd-Hawkins, K.A., Kass, P.H., Glaser, C.A., Pederson, N.C., Koehler, J.E., 1995. *Bartonella henselae* prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. Journal of Clinical Microbiology, 33 (9): 2445-2450.
17. Chomel, B.B., Boulouis, H.J., Breitschwerdt, E.B., Kasten, R.W., Vayssier-Taussat, M., Birtles, R.J., Koehler, J.E., Dehio, C., 2009. Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors.Veterinary Research, 40 (2): 29.
18. Chomel, B.B., Kasten, R.W., Floyd-Hawkins, K., Chi, B., Yamamoto, K., Roberts-Wilson, J., Gurfield, A.N., Abbott, R.C., Pedersen, N.C., Koehler, J., 1996. Experimental transmission of *Bartonella henselae* by the cat flea. Journal of Clinical Microbiology, 34 (8): 1952-1956.
19. Clarridge J.E. 3rd, Raich T.J., Pirwani, D., Simon, B., Tsai, L., Rodriguez-Barradas, M.C., Regnery, R., Zollo, A., Jones, D.C., Rambo, C., 1995. Strategy to detect and identify *Bartonella* species in routine clinical laboratory yields *Bartonella henselae* from human immunodeficiency virus-positive patient and unique *Bartonella* strain from his cat. Journal of Clinical Microbiology, 33 (8): 2107-2113.
20. Dehio, C., 2001. *Bartonella* interactions with endothelial cells and erythrocytes. Trends in Microbiology, 9 (6): 279-285.
21. Dehio, C., Meyer, M., Berger, J., Schwarz, H., Lanz, C., 1997. Interaction of *Bartonella henselae* with endothelial cells results in bacterial aggregation on the cell surface and the subsequent engulfment and internalization of the bacterial aggregate by a unique structure, the invasome. Journal of Cell Science, 110: 2141-2154.
22. Drancourt, M., Tran-Hung, L., Courtin, J., Lumley, Hd., Raoult, D., 2005. *Bartonella quintana* in a 4000-year-old human tooth. The Journal of Infectious Diseases, 191 (4): 607-611.
23. Ebani, V.V., Bertelloni, F., Fratini, F., 2012. Occurrence of *Bartonella henselae* types I and II in Central Italian domestic cat. Research in Veterinary Science, 93 (1): 63-66.
24. Finkelstein, J.L., Brown, T.P., O'Reilly, K.L., Wedincamp, J. Jr., Foil, L.D., 2002. Studies on the growth of *Bartonella henselae*in the cat flea (Siphonaptera: Pulicidae). Journal Medical Entomology, 39 (6): 915–919.
25. Gil, H., Escudero, R., Pons, I., Vargas, M. R., Esteban, C. G., Moreno, I. R., Amil, C. G., Lobo, B., Valcárel, F., Pérez, A., Jiménez, S., Jado, I., Juste, R., Segura, F., Anda, P., 2013. Distribution of *Bartonella henselae* variants in patients, reservoir hosts and vectors in Spain, PLOS One, 8 (7): e68248.
26. Guzel, M., Celebi, B., Yalcin, E., Koenhemsi, L., Mamak, N., Pasa, S., Aslan, O., 2011. A serological investigation of *Bartonella henselae* infection in cats in Turkey. The Journal of veterinary medical science, 73 (11): 1513-1516.
27. Kim,Y.S., Seo, K.W., Lee, J.H., Choi, E.W., Lee, H.W., Hwang, C.Y., Shin, N.S., Youn, H.J., Youn, H.Y., 2009. Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in cats and dogs in Korea. Journal of Veterinary Science, 10 (1): 85-87.
28. Kordick D.L., Breitschwerdt, E.B., 1998. Persistent infection of pets within a household with three *Bartonella* species. Emerging Infectious Diseases, 4 (2): 325-328.
29. Kordick, D.L., Breitschwerdt, E.B., 1995. Intraerythrocytic presence of *Bartonella henselae*. The Journal of Clinical Microbiology, 33 (6): 1655–1656.

30. Maia, C., Ramos, C., Coimbra, M., Bastos, F., Martins, Â., Pinto, P., Nunes, M., Vieira, M.L., Cardoso, L., Campino, L., 2014. Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal. Parasites & Vectors, 7: 115.
31. Mascarelli, P.E., Maggi, R.G., Hopkins, S., Mozayeni, B.R., Trull, C.L., Bradley, J.M., Hegardy, B.C., Breitschwerdt, E., 2013. *Bartonella henselae* infection in a family experiencing neurological and neurocognitive abnormalities after woodlouse hunter spider bites. Parasites & Vectors, 6: 98.
32. Miceli, N.G., Gavioli, F.A., Gonçalves, L.R., André, M.R., Sousa, V.R.F., Marques de Sousa, K.C., Machado, R.Z., 2013. Molecular detection of feline arthropod-borne pathogens in cats in Cuiabá, state of Mato Grosso, central-western region of Brazil. RevistaBrasiliera de ParasitologiaVeterinaria, 22 (3): 385-390.
33. Mietze, A., Morick, D., Köhler, H., Harrus, S., Dehio, C., Nolte, I., Goethe, R., 2011. Combined MLST and AFLP typing of *Bartonella henselae* isolated from cats reveals new sequence types and suggests clonal evolution. Veterinary Microbiology, 148: 238-245.
34. Namekata, D.Y., Kasten, R.W., Boman, D.A., Straub, M.H., Siperstein-Cook, L., Couvelaire, K., Chomel, B.B., 2010. Oral shedding of *Bartonella* in cats: correlation with bacteremia and seropositivity. Veterinary Microbiology, 146 (3-4): 371-375.
35. Palmer, S.R., Soulsby, L., Simpson, D.I.H., 2005. Zoonoze. Editura Ştiinţelor Medicale, Bucharest, 58-62.
36. Pennisi, M. G., La Camera, E., Giacobbe, L., Orlandella, B. M., Lentini, V., Zummo, S., Fera, M. T., 2010. Molecular detection of *Bartonella henselae* and *Bartonella clarridgeiae* in clinical samples of pet cats from Southern Italy. Research in Veterinary Science, 88: 379-384.
37. Pinna Parpaglia, M.L., Masu, G., Masala, G., Porcu, R., Zobba, R., Pintori, G., Cocco, R., 2007. Seroprevalence of *Bartonella henselae* in dogs and cats in Sassari. Veterinary Research Communications, 31 (1): 317-320.
38. Pulliainen, A.T., Dehio, C., 2012. Persistence of *Bartonella* spp. stealth pathogens: from subclinical infections to vasoproliferative tumor formation. FEMS Microbiology Reviews, 36 (3): 563-599.
39. Regnery, R.L., Olson, J., Perkins, B.A., Bibb, W., 1992. Serological response to *Rochalimaea henselae* antigen in suspected cat-scratch disease. Lancet, 339 (8807): 1443–1445.
40. Rolain, J.M., Franc, M., Davoust B., Raoult, D., 2003. Molecular detection of *Bartonella quintana, B. koehlerae, B. henselae, B. clarridgeiae, Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. Emerging Infectious Diseases, 9 (3): 338-342.
41. Sanchez Clemente, N., Ugarte-Gil, C.A., Solórzano, N., Maguiña, C., Pachas, P., Blazes, D., Bailey, R., Mabey, D., Moore, D., 2012. *Bartonella bacilliformis*: A systematic review of the literature to guide the research agenda for elimination. PLoS Neglected Tropical Diseases, 6 (10): e1819.
42. Sander, A., Ruess, M., Bereswill, S., Schuppler, M., Steinbrueckner, B., 1998. Comparison of different DNA fingerprinting techniques for molecular typing of *Bartonella henselae* isolates. Journal of Clinical Microbiology, 36 (10): 2973-2981.
43. Schouls, L. M., Van de Pol, I., Rijpkema, S. G. T., Schot, C. S., 1999. Detection and identification of *Ehrlichia, Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. Journal of Clinical Microbiology, 37 (7): 2215-2222.
44. Schülein, R., Seubert, A., Gille, C., Lanz, C., Hansmann, Y., Piémont, Y., Dehio, C., 2001. Invasion and persistent intracellular colonization of erythrocytes. A unique parasitic strategy of the emerging pathogen *Bartonella*. The Journal of Experimental Medicine, 193 (9): 1077–1086.
45. Staggemeier, R., Pilger, D.A., Spilki, F.R., Cantarelli, V.V., 2014. Multiplex SYBR Green-Real Time PCR (qPCR) assay for the detection and differentiation of *Bartonella henselae* and *Bartonela clarridgeiae* in cats. Revista do Instituto de Medicina Tropical de Sao Paulo, 56 (2): 93-95.
46. Staggemeier, R., Venker, C.A., Klein, D.H., Petry, M., Spilki, F.R., Cantarelli, V.V., 2010. Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in cats in the south of Brazil: a molecular study. Memorias do Instituto Oswaldo Cruz, 105 (7): 873-878.
47. Switzer, A.D., McMillan-Cole, A.C., Kasten, R.W., Stuckey, M.J., Kass, P.H., Chomel, B.B., 2013.*Bartonella* and *Toxoplasma* infections in stray cats from Iraq. The American Journal of Tropical Medicine and Hygiene, 86 (6): 1219-1224.
48. Yuan, C., Zhu, C., Wu, Y., Pan, X., Hua, X., 2011. Bacteriological and molecular identification of *Bartonella* species in cats from different regions of China. PLOS Neglected Tropical Diseases, 5 (9): e1301.
49. Zobba, R., Chessa, G., Mastrandrea, S., Pinna Parpaglia, M.L., Patta, C., Masala, G., 2009.Serological and molecular detection of *Bartonella* spp. in humans, cats and dogs from northern Sardinia, Italy. European Society of Clinical Microbiology and Infectious Diseases, 15 (2): 134-135.

RESEARCH REGARDING PARAMETERS THAT CHARACTERIZE THE FERTILITY OF COWS EXPLOITED IN N-E OF ROMANIA

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Abstract: *In the zootechnical domain, artificial inseminations represent a modern biotechnical method of controlling and managing the reproductive function, with the purpose of optimizing the process of multiplication and amelioration of animals, at their level of reproduction capacity.*
The gestation indicator, in 2014, recorded a minimum value of 47.5 % between December and February and a maximum value of 57.4 % between June and August.
In 2013, a bigger percentage of the total fecundity was recorded in young cows, of 67.2 %, in comparison with the one obtained in mature cows, of 42.8 %.
The artificial inseminations made between 2013 and 2014 using the Spolor bull's sperm led to obtaining the highest percentage, of 60.3 % (in 2013) and 61.0 % (in 2014).

Key words*: cow, seminal material, artificial insemination.*

Artificial insemination represents a biotechnical method for reproduction and amelioration of the domestic animals performances, which contribute substantially to obtaining a genetically progress in the populations of animals (1,4).

By artificial insemination, we understand the elimination of the contact between a male and a female, the sperm being yielded from the reproductive male by an authorized person and inserted into the genital tract of a female in estrus using proper instruments.

Artificial insemination permits using the seminal material yielded from the most representative bulls, having qualities used to genetically improve the cow populations.

The sperm being frozen it offers the possibility of international exchange of valuable seminal material, and by importing and exporting of frozen seminal material, the inconvenience caused by live animal quarantines is eliminated (3,5,7).

By adopting this biotechnology, the possibility of genital transmitted diseases is considerably reduced. At the same time, it is desired to obtain a fecundity percentage as high as possible, the usage of qualitatively tested and verified seminal material, selecting by type of production (milk, meat), lowering the costs and rising the security regarding the usage of bulls in farms (2).

MATERIAL AND METHOD

The seminal material used at the veterinary clinic from the NE of Romania, for artificially inseminating bovines, is of superior quality and comes by import from firms of great importance, such as: **Semex – S.C. Kanata Farm Genetics S.R.L.** –the seminal

material can be ordered using the following adress **: World Wide Sires – LTD. U.S.A. – S.C. Schntal – Schul Impex S.R.L., ABS – Genus.**

At the clinic, the adopted method for artificial inseminations is the tactility rectum-cervical method. The proper preparation before making a artificial insemination has the following steps: identifying the female that has to be inseminated and verifying it in the insemination register's data and parturitions, if existed (eutocic or distocic), the data from the gynecological file; the necessary instruments are prepared: sowing sparkles container, sowing pipettes, sowing torch, thermos, cutter, gynecological glove and, last but not least, a clean napkin.

The gynecological research is one of the basic methods through we can know each animal in the clinic and fitting it into a proper physiological or pathological state of reproduction.

The anamnesis contains data about: female's age, if it is at its first parturition or it had another one before, gestations number, how did anterior parturitions evolved, sexual manifestations, the regularity and intensification of the estrus, numbers and dates of the preceding inseminations, lactation state, alimentation, maintenance hygiene, quality and quantity of vaginal secretions in general and during the estrus, them being: translucent and leaking, opalescent-catarrhal, purulent striations or floaters.

Taking into account the reduced of the estrus manifestations (18-24 h, with variable limits within 6-36 h), the careful observing of the signals that notify their apparel is mandatory, in order for the artificial inseminations to be made in time.

One of the essential factors is represented by the noticing of the estrus, that influences directly the reproduction efficiency and the economic efficiency of the dairy cows.

Not noticing correctly the females in estrus is a major factor of the decreased gestation rates. It is very important to know with certitude the primary and secondary signs of estrus manifestations in order to establish the optimum moment of fertilization. In order to notice the females in estrus, a carefully and continuous observing of the cows is necessary, especially in the favorable moments such as early in the morning, in the afternoon and evening, and in certain places.

The mucus in the estrus is produced by the uterus and cervix, and it is accumulated in the vagina during the estrus and a short time afterwards. In the beginning, especially during the first hours of estrus, the mucus is transparent, abundant and of liquid consistency, and while the estrus advances, the consistency of the mucus grows, it becomes thicker.

The decrease of milk production and food consumption also represent secondary signs in the initial phase of the estrus, sometimes before other signs to appear.

The seminal material used in the clinic is kept into containers with liquid nitrogen, assuring the longevity of the seminal material by preserving it on very low temperatures of -196 C.

Not respecting certain rules to defreeze the seminal material, as well as wrong handling of the sperm sparkles and preparing the sowing torch, all can lead to reducing the fecundity after artificial insemination.

RESULTS AND DISCUSSIONS

The researches have been done between the years 2013 and 2014, in a veterinary clinic in the NE of Romania, on a number of 961 females (young and mature cows).

In the clinic, the doses containing frozen material used for artificial insemination come mostly from genetically valorous bulls from Holstein and Frize breed.

The frozen bull seminal material doses used between the years 2013 and 2014 are written in table 1.

Table 1.

Numbers and identification codes of seminal material used in artificial inseminations in 2013-2014

Nr. crt.	Bull			Semen quality		
	Name	Code	Breed	M2 (%)	Total number of sperm/dose ($x10^6$)	Motile sperm/dose ($x10^6$)
1	Spolor	73011	HF	35	25	8.75
2	Vitalis	51231	HF	35	25	8.75
3	Goliath	52111	HF	35	25	8.75
4	Muller	52001	HF	35	25	8.75
5	Eternal	51812	HF	35	25	8.75
6	Mannsberg	50234	HF	35	25	8.75
7	Manso	51234	HF	35	25	8.75
8	Honda	52280	HF	35	25	8.75
9	Kelch	52753	HF	35	25	8.75
10	Osman	52285	HF	35	25	8.75

The gestation indicator represents the ratio between the number of gestated females and the number of females artificially inseminated or fertilized (several times until they got pregnant).

Due to our research, in the observation period, we noticed that this indicator is influenced by the weather conditions, by the biotechnology used in the growing system, by exploitation and by the way in which the artificial insemination has been done. In these cases, the heritage has high value.

In normal growing, feeding and exploitation conditions, the indicators values should be between 85 and 95%.

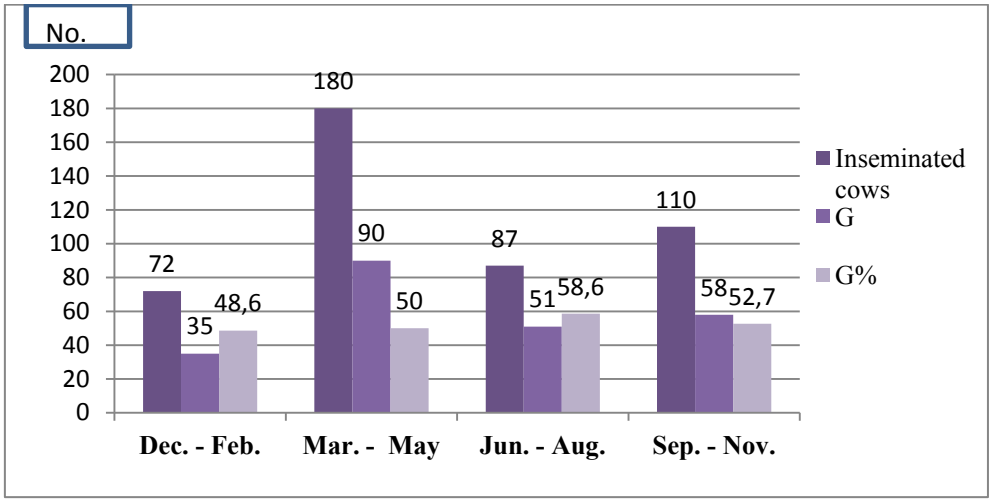

Diagram 1. Value of fecundity by seasons in 2013

Looking to the table 1 and the diagram 1, we can observe the rising of the total number of cows (I.A.n) and of the total number of gestated cows (Gn) in the seasons March to May and September to November, and in this year, the indicator of gestation G% has a high value in September to November (52.7 %) and a maximum value in the summertime between June and August.

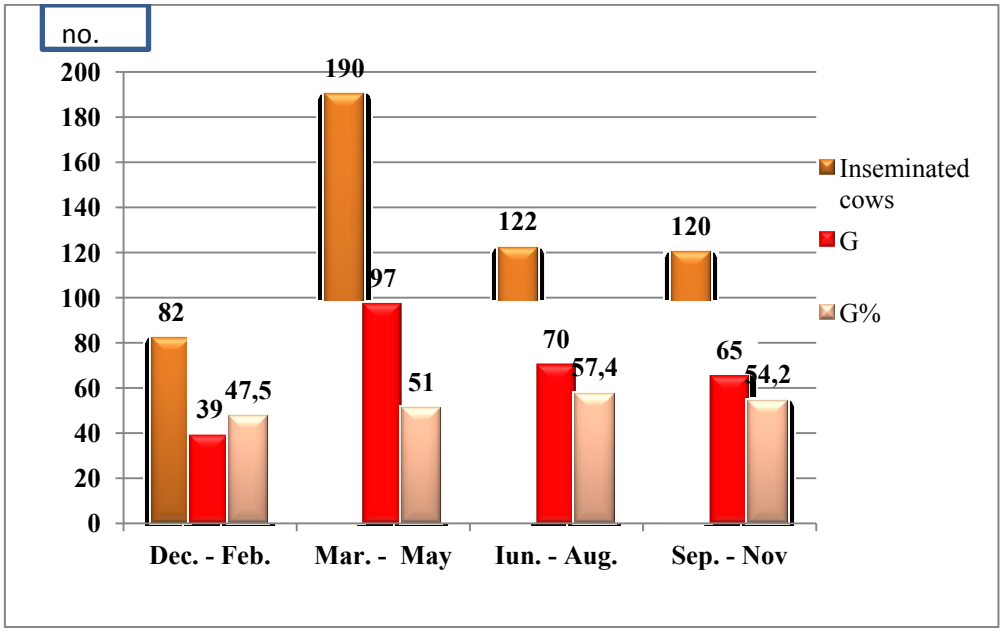

Diagram 2. Value of fecundity by seasons in 2014

In 2014 it can be observed a higher value of the gestation indicator (G%), by seasons, compared to 2013, even if during this year, the total number of artificially inseminated females is higher than in the past year. The gestation indicator in 2014 has recorded the

minimum value of 47.5 %, between December and February, and the maximum value of 57.4 % between June and August.

From the diagram 2, we can understand that total fecundity value of the number of artificial inseminated cows in 2014, of 512 females, is of 52.5%. According to the data in the specialty literature, the gestation indicator must be between 60 and 80 %. Therefore, at the clinic that has participated to this study, we must give our permanent attention to optimize this value.

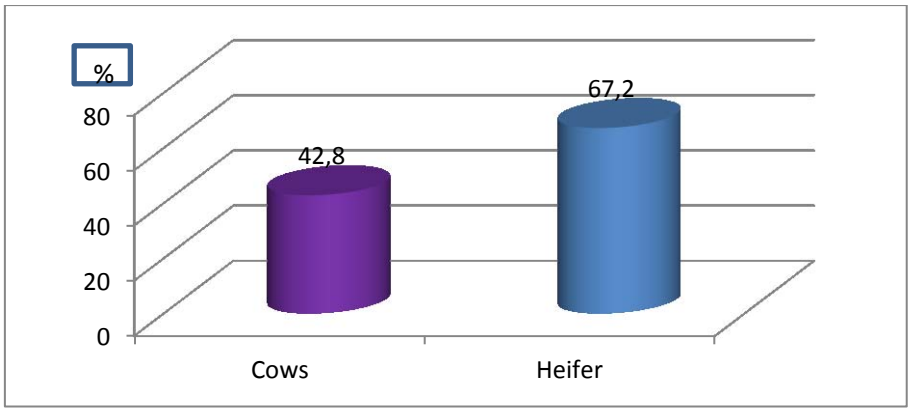

Diagram 3. Value of total fecundity of young and mature cows in the year 2013

In 2013 we can observe a higher percentage in young cows, of 67.2 %, in comparison with the fecundity percentage of 42.8 % in mature cows.

In table 4 we can observe a rise of the fecundity total value in 2014, with approximately 2 % in comparison with the total fecundity value of the past year.

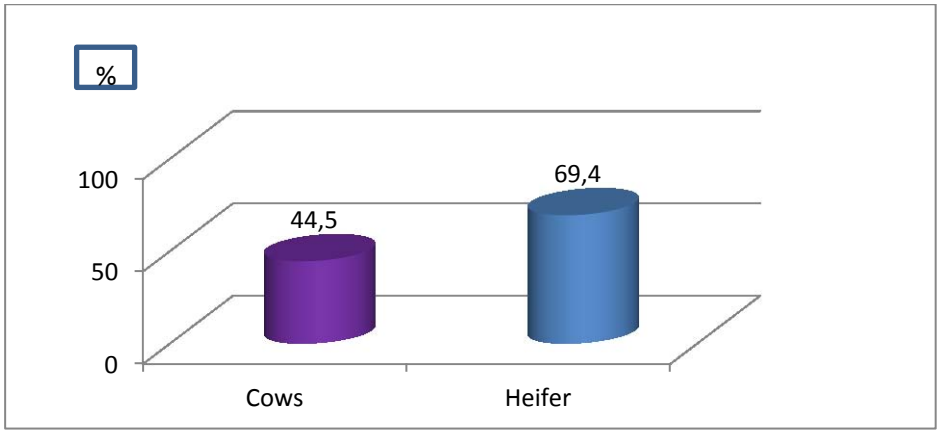

Diagram 4. Values of fecundity in young and mature cows in 2014

In conformity with the research done, a slight increase of the total fecundity value in 2014 is observed, in young and mature cows, in comparison with 2013. In mature cows as well as in the young cows, it is observed an increase of approximately 5 % in both years taken into study (diagram 4).

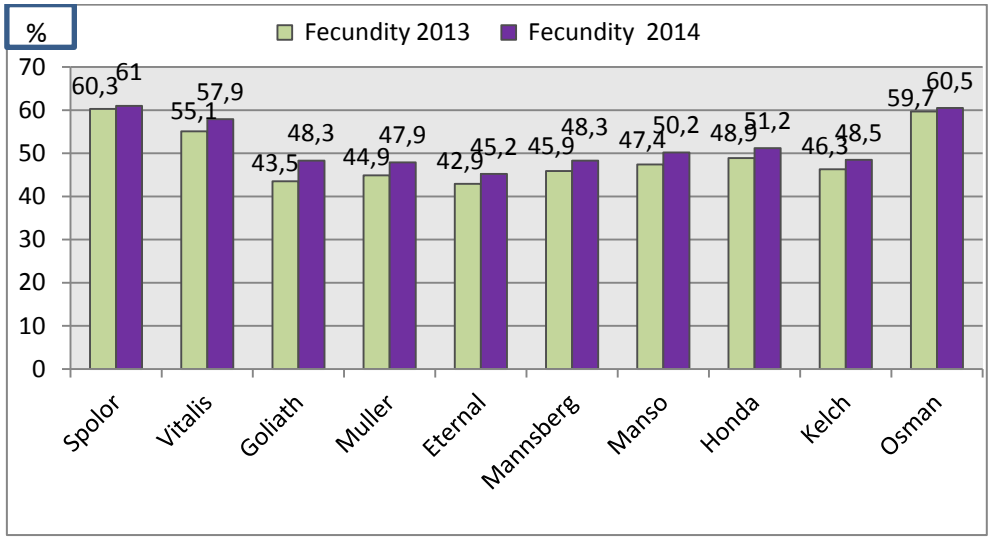

Diagram 5. Fecundity values according to the used seminal material in 2013-2014

The artificial inseminations made between 2013 and 2014 with the Spolor bull's sperm led to the highest percentage, of 60.3 % (in 2013) and 61.0 % (in 2014).

Through the artificial inseminations made using the Eternal bull's sperm was obtained the lowest percentage values of 42.9 % in 2013 and 45.2% in 2014.

By comparison, during the two years in which the study has been done, among the best and most utilized bulls, there was recorded a visible increase of the fecundity of Spolor, Osman, Vitalis, Honda and Manso, though the most valuable according to statistics are Spolor and Osman.

Analyzing the dynamics in 2013, 2014, we observe that the seminal material fecundity was higher in 2014 in most reproductive males.

The percentage and the difference between the fecundity in 2013 and in 2014, is rising to different values depending on the bull, Vitalis 55.1 and 57.9 (an increase of 2.8%). Continuing to analyze the fecundity, we can see that other bulls, such as Goliath have a variety in their fecundity of 2.8%; the Muller bull has a fecundity percentage higher with 3%; Eternal presented an increase of 2.3% and Manso bull recorded an increase of 2.8% (diagram 5).

The past puerperal periods, such as other factors: diseases of the mammary gland, of the genital apparatus, of the limbs and parasites infestations, have a negative influence on cows' fecundity, in comparison with young cows that are not affected by this. Therefore, to prevent these diseases and to assure the highest fecundity percent possible, there must be assured, all year long, a well-organized management and correlated in matters of alimentation, parturition hygiene, shelter hygiene, different disinfestations treatments, and last but not least, a proper hygiene during milking.

The overlooking analysis of the fecundity of 10 studied bulls showed a difference of 2.4%, which means, to a number of 961 cows, a significant increase regarding the number of products and the production level.

CONCLUSIONS

1. The artificial inseminations made between 2013 and 2014 using the Spolor bull's sperm led to obtaining the highest percentage, of 60.3 % (in 2013) and 61.0 % (in 2014).
2. Using the sperm of the bull Eternal, there was obtained the smallest percentage value of 42.9 % in 2013 and of 45.2 % in 2014.

REFERENCES

1. Ball P. J. H., Peters A. R. (2007)- Reproduction in Cattle, Third Edition, Blackwell Publishing. pp. 216-230
2. David E. și col. (2001) -Veterinary Reproduction and Obstetrics, Eight Edition pp.86-95.
3. Drugociu D.,(2001)- Reproducţie, însămânţări artificiale şi ginecologie veterinară. Ed. Venus, Iaşi.
4. Mircu C. (2001) - Biotehnici de reproducere la bovine. Ed. Mirton Timișoara pp. 136-162.
5. Nechifor F. (2015) – Fiziologia perioadei puerperale și monitorizarea acesteia la vacă, Ed. Ion Ionescu de la Brad, Iași pp.100-116
6. Păcală N.,(2004) - Biotehnologia însămânţării artificiale la vacă. Ed. Waldpress, Timişoara pp. 68-110.
7. Runceanu L., Drugociu D., Roșca P., Ciornei Șt., Nechifor F. (2014) - Fiziopatologie materno-fetală veterinară, Ed. Ion Ionescu de la Brad Iași, pp. 48-65

RESEARCH ON THE INFLUENCE OF THE PUERPERAL PERIOD ON COW'S LACTATION

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Abstract:*The research has been done on a number of 310 cows from the Holstein breed, raised and exploited in an intensive system. The research shows the correlation between the puerperal period and its influence on the level and curve of lactation.*
The results show that for females with puerperal disorders, the medium production is of 1 liter of milk per day, between 25 and 30 days post-partum. It is also observed a research dynamics between the years 2014-2015.
As for the level of milk production, studies highlight a strike level in the III to IV puerperal months, and a decrease in the X month, this phenomenon is more likely to appear in cows with puerperal disorders.

Key words: *reproduction, cow, milk*

During the gestation period, the mother's organism suffers a series of morphological changes, of the biochemical, hematological and hormonal status. These changes represent adaptation elements of the mother's system to the gestation status.

After parturition, the readapting consists in a gradual return to the morphological and physiological values from before giving birth, so that at the specific term, every steps of the reproduction cycle would be resumed: appearance of the estrus, ovulation, first I.A., fecundation and gestation (4,8).These elements are the more important as, through the puerperal period, the protective and adaptation capacity of the mother is smaller. It can determine the reducing of fecundity.On the other hand, the evolution of the puerperal period depends on some genital lesions caused by parturition, as well as on the uterine cavity inflammations (metritis, perimetritis, parametritis). An overwhelming lactation is another factor that weakens the mother's organism, mainly through the homeostatic changes (1,3).

For preventing gynecological diseases during the puerperium it is necessary to monitor these phenomena and the systematic and active intervention (5).The animal productions are depending on the reproductive activity and organizing the growth and exploiting of cows, for a maximum economic efficiency.Evaluating the reproduction efficiency gives the opportunity of permanent appreciation of the situation regarding the development of genital processes (2,8).

MATERIAL AND METHOD

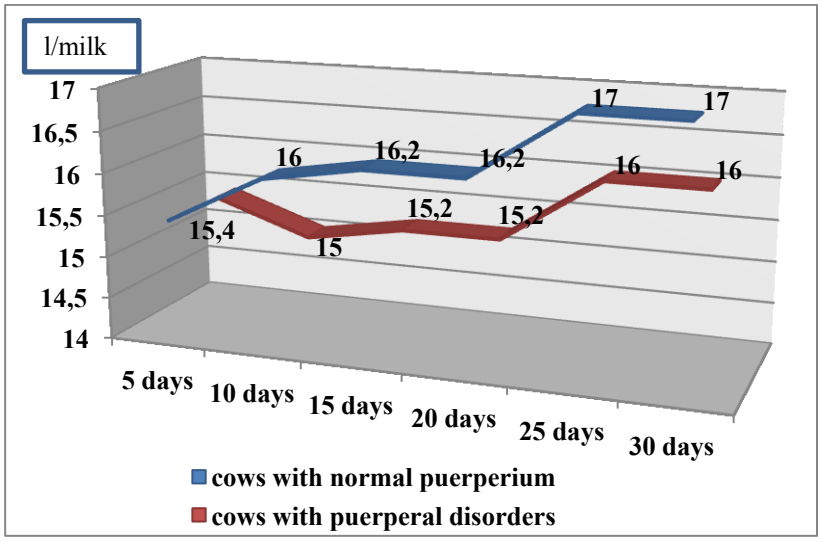
Research has been done on 310 cows between the years 2014-2015, exploited for milk production, belonging to the Holstein bread. In order to appreciate the reproduction state, there have been used several synthetic indicators, which reflect through stages, the reproduction biotechnology, such as: parturition, stages of puerperal period, display of the physiological and pathological phenomena in the puerperium, biotechnology of artificial sowing, and the biological value of the seminal material.

In this context, there were also taken into consideration: the feeding diet of the cows, their maintenance state, the presence or the absence of other disorders, apart from the reproductive ones.In order to establish the reproductive state of cows at the farm, there have been selected several synthetic indicators, that later helped with the calculation of the economic efficiency. The most important synthetic indicators are: the sowing indicator, the gestation indicator, the conception rate, the service-period and the calving-interval.Some data has been taken from the earlier documents of the farm. There has been added the ones obtained by clinical examination done in the production unit.The display of this data shows the main stages of the biotechnology of exploiting cows from the reproduction unit and offers indicators in order to make this data closer to the normal ones and the possibility of prophylactic or medicinal intervention.On the basis of these synthetic indicators, it has been established the economic efficiency of cow exploiting, taking into consideration the reproduction organization schedule, obtaining calves and the rhythm of milk production in dynamics.Based on the obtained results, the applying of zootechny, veterinary methods, is recommended, their purpose being the reducing of loss and increasing of profitableness.

RESULTS AND DISCUSSIONS

The influence of puerperal disorders on the lactation curve

The medium values per day in the first month of lactation in 2014 show that for females that had a normal puerperium, the highest monthly production was observed between 25-30 days post-partum (17l/day). For females that had puerperal disorders, the medium production values were lowered with 1 liter per day, in the same period and in the same interval (diagram 1).



Digram 1. Mean daily milk production in the first month of lactation in 2014

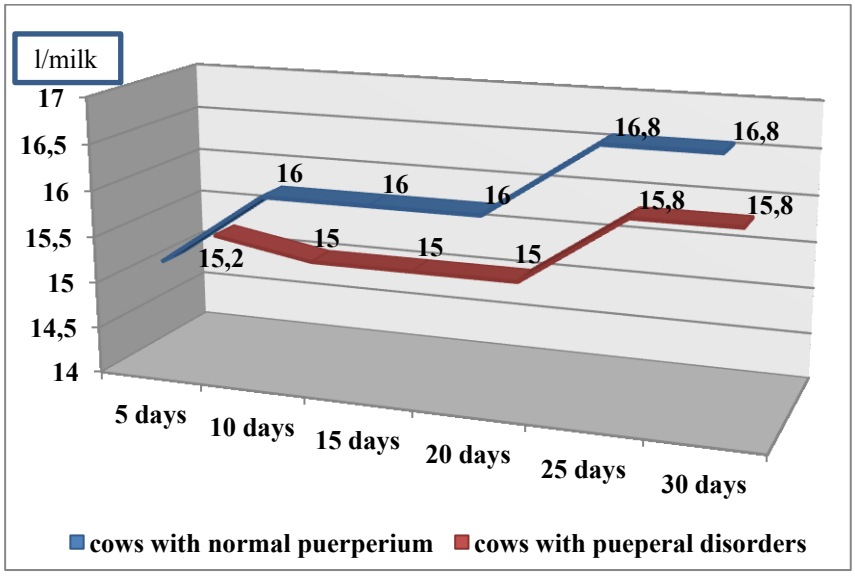


Diagram 2. Mean daily milk production in the first month of lactation in 2015

The appreciated values in the dynamics of the years 2014-2015, show that, in comparison with the lactation curves in 2015, in 2015 there has been recorded a similar aspect (a decrease of about a liter per day for cows between 25-30 days post-partum). This shows that inside the farm there are similar conditions during the two years in which the studies have been made (diagram 2, 3).

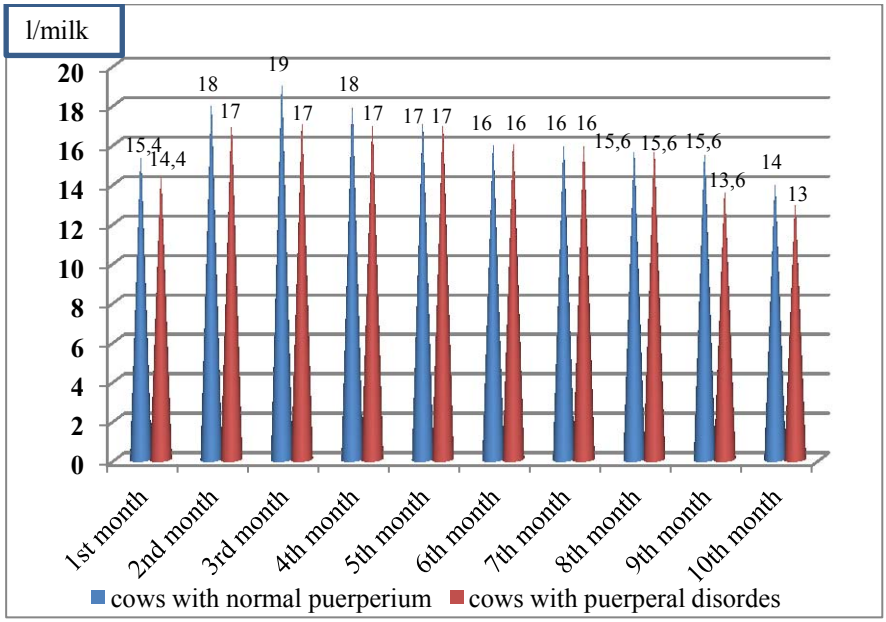


Diagram 3. Monthly averages of milk production in 2014

The analysis of the medium values of milk production of the year between 2014 and 2015, shows that the strike level of lactation is recorded in the fourth and third months, with a decrease until the X month. From the data shown in the diagram 3 and 4, results a descending

curve of lactation starting with the third month, which is similar, standing out with a stressed decrease for cows with puerperal disorders at the end of lactation.

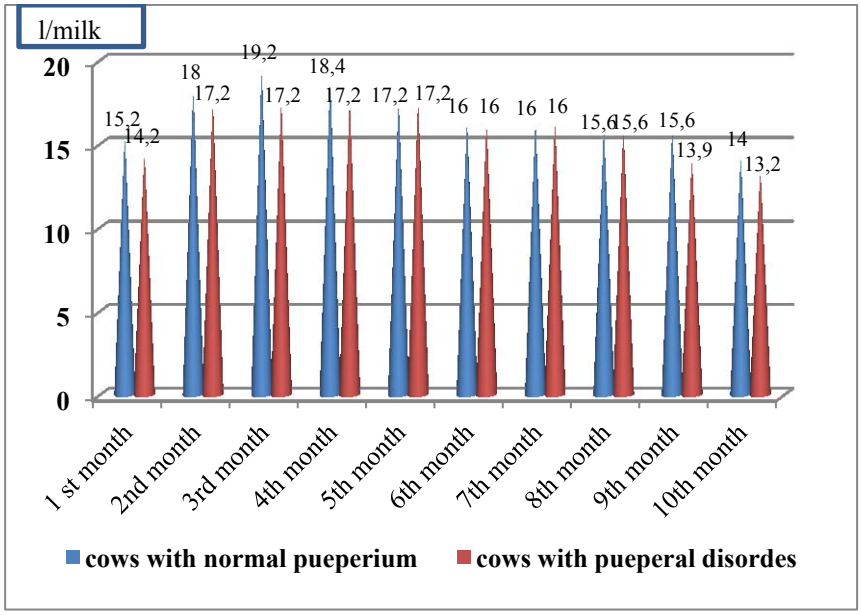


Diagram 4. Monthly averages of milk production in 2015

In these conditions, we find that the period of lactation may influence the milk production directly, meaning that if the milk production is higher and the descending curve is longer, the overtaking of the 305 days value diminishes productions in further lactations and causes the loss of a calf. By this regard, our researches correspond to the ones made by Maciuc, V. 2006.

The analysis of the lactations curves made between 2014 and 2015 shows that, when speaking about quantity, in 2014 there is a loss of 140 l milk/lactation and in 2015, 150 l milk/lactation.

We can remark that in the recovery of females with puerperal disorders we must take into consideration the complications that follow the pathological puerperium, the most important being the affections of the acropodium.

CONCLUSIONS

1. In the interval of 5 to 30 days post-partum, there can be noticed a diminishing of the lactation curves, especially in cases of puerperal affections.
2. The level of milk productions that record a spike level of lactation in the third and the forth month, mark a diminishing until the X month. This decrease is more stressed for females with puerperal disorders, manifested by the decrease of productive level with 1 liter of milk/day (16 l of milk/ day), in comparison with a production in this interval of 17 l milk/day for females without puerperal disorders.

REFERENCES

1. Acatincăi Stelian (2010) - *Tehnologia creșterii bovinelor*. Ed. Agroprint. Timișoara. pp.115-123
2. Ardelean V. (2003) - *Fiziologia și patologia gestației și parturiției la animale*. Ed. Mirton, Timișoara. pp. 146-170
3. Bârțoiu A., Seiciu Fl., (2006) - *Tratat de patologia reproducției la animale*. Ediția a II-a, Ed. Bic ALL, București. pp. 200-210
4. Bogdan A.T., Bogdan Dorina, Vasile Maria (2000) - *Zootehnie speciala*, vol., „*Tehnologia de creștere, alimentație, reproducție și ameliorare pe specii*". Ed. Bioterra, București.
5. Bogdan L.M., Groza I. ȘT. (2009) - *Obstetrică veterinară*, Ed. Academic Press, Cluj-Napoca pp. 144-152.
6. Drugociu D. (2009) - *Bolile obstetrical ginecologice la animale*. Ed. Ion Ionescu de la Brad, Iași pp.67-82.
7. Manciuc Vasile (2006) - *Managementul creșterii bovinelor* Ed. Alfa, Iași
8. Runceanu L., Drugociu D., Roșca P., Ciornei Șt., Nechifor F. (2014) - *Fiziopatologie materno-fetală veterinară,* Ed. Ion Ionescu de la Brad Iași pp.112-146

ARTHROSCOPIC TREATMENT OF A 2-YEARS-OLD AMERICAN BULLDOG WITH UNILATERAL FRAGMENTED CORONOID PROCESS OF THE ELBOW

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Abstract:*A 2-years-old intact American Bulldog male was presented at our clinic because of obvious clinical signs of lameness at right forelimb. No clinical signs were observed at left forelimb. Fragmented coronoid process (FCP) of medial compartment disease (MCP) was suspected based on thickened joint, pain response elicited during maximal flexion of the right elbow joint, combined with lateral rotation of the forelimb. Elbow radiography, computed tomography and arthroscopy confirmed the diagnosis. Arthroscopic treatment was performed and follow-up period was 3 months. The follow-up consisted of telephones with the owner and his satisfaction rate was 100%. According to his informations, the dog did not reveal any signs of pain or lameness after three months postoperative. This report describes the use of arthroscopy for surgical management of FCP in an American Bulldog, resulting in rapid return of full function. Arthroscopic fragments removal was demonstrated to be a valuable treatment option and provided a very good outcome. To our knowledge this is the first report of an arthroscopic management of a FCP in an American Bulldog in Romania.*

Keywords: *Arthroscopy, dog, FCP, fragmented coronoid process*

INTRODUCTION

Elbow dysplasia is a common debilitating condition of large and giant breed dogs. The syndrome includes several conditions that result in incongruence of the joint, eventually leading to degenerative joint disease. In 1993, the International Elbow Working Group (IEWG) agreed that "elbow arthrosis caused by fragmented coronoid process (FCP), osteochondrosis (OC[D]), ununited anconeal process (UAP), articular cartilage anomaly, and/or joint incongruity is the manifestation of inherited canine elbow dysplasia (Tobias and Johnston, 2012).

Medial compartment disease is most commonly diagnosed in young, large- to giant-breed dogs and males are about twice as frequently affected as female dogs. Osteochondrosis/ osteochondritis dissecans and medial coronoid disease share similar breed predisposition in Labrador Retrievers, German Shepherd Dogs, and Rottweilers (Beale et al., 2003; Jannuta et al., 2006).

Although odds ratios have not been specifically reported for elbow joint incongruity, this condition is seen primarily as a component of elbow dysplasia in large-breed dogs, such as Labrador Retrievers, German Shepherd Dogs, Golden Retrievers, Rott- weilers, and Bernese Mountain Dogs. Environmental factors and a complex genetic heritability play a role in predisposing dogs to elbow dysplasia (Michelsen J, 2013).

The etiopathogenesis of medial coronoid disease has been particularly well studied but remains poorly understood. Olsson proposed fragmentation of the medial portion of the coronoid process as a manifestation of osteochondrosis, along with ununited anconeal process (Olsson, 1974; Olsson S,1987; Olsson, 1983). Bilateral disease is diagnosed in 37% to 50%

of patients, supporting routine imaging studies of both elbows (Trostel et al., 2003). Proposed advantages of arthroscopy over arthrotomy include reduction in patient morbidity, ease of treatment of multiple joints in single sessions, improved viewing of intra-articular structures, and use of a minimally invasive approach (Capaldo et al., 2005). This report describes the use of arthroscopy for surgical management of FCP in an American Bulldog, with rapid return to function following surgery..

MATERIAL AND METHODS

A 2-years-old American Bulldog was presented for evaluation approximately 4 months following onset of moderate lameness in the right forelimb. Thickened joint, pain response elicited during maximal flexion of the right elbow joint, combined with lateral rotation of the forelimb suggested a medial compartment disease.

Radiographs of craniocaudal, 90 degrees lateral, and flexed lateral were obtained. No evidence of ununited anconeal process was observed at the flexed lateral projection on both forelimbs. Abnormal contour, poor definition, of the cranial margin of the medial coronoid process on the lateral view were observed (Fig. 1A).

Radiographs of the contralateral elbow showed no changes associated with medial compartment disease. Both elbows joint were evaluated by CT examination in a standardized position, with the elbow joint in neutral and standing position. Fragmentation located at the apex of the medial coronoid process can be seen in computed tomographic appearance (Fig. 1B şi C).

Under general anesthesia, the dog was placed in dorsal recumbency. Medial aspect of the elbow was surgically prepared (Fig. 2). The joint was infused with a hypodermic needle (20 gauge). The needle was inserted distal and slightly caudal to the medial epicondyle (Fig 3, 4). Joint arthroscopy was performed using medial portals (Fig. 4, 5). A 1.9 mm short 30 degree arthroscope was used (Fig 5). The egress portal consisted of an 20 gauge needle directed in the joint pouch just proximal to the anconeal process (Fig 5).

The caudal aspect of the humeral condyle and the anconeal process were used as landmarks to introduce the egress portal in a proximodistal and slightly mediolateral direction. Joint fluid was aspirated, and the joint was distended with 15 mL of saline solution. A stab incision allowed placement of the cannula and trocar, with the joint firmly abducted. A pointed trocar was introduced into the joint.

The scope was placed in the cannula, which was connected to a fluid line (Fig. 5). Fluid irrigation was provided by the use of a fluid pump. An instrument portal located about 1 cm cranial to the scope portal, just caudal to the medial collateral ligament, was placed using triangulation of a needle as a guide. A 5 mm long stab incision was performed through the skin and was extended into the joint, to allow placement of an instrument without a cannula.

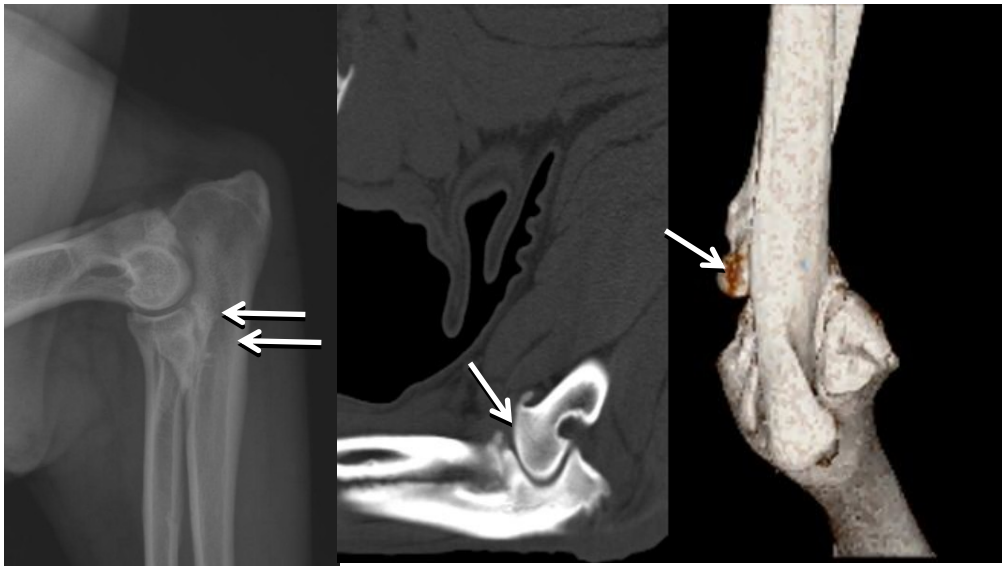

Fig. 1 A. Lateral radiographic view of a fragmented medial coronoid process; abnormal contour, poor definition of the cranial margin of the medial coronoid process.
B. Computed tomography image of the elbow with a fragmented medial coronoid process and degenerative joint disease. The fragmented coronoid process (white arrow) is observed
C. Computed tomography image of the elbow with three-dimensional reconstruction aid in surgical planning

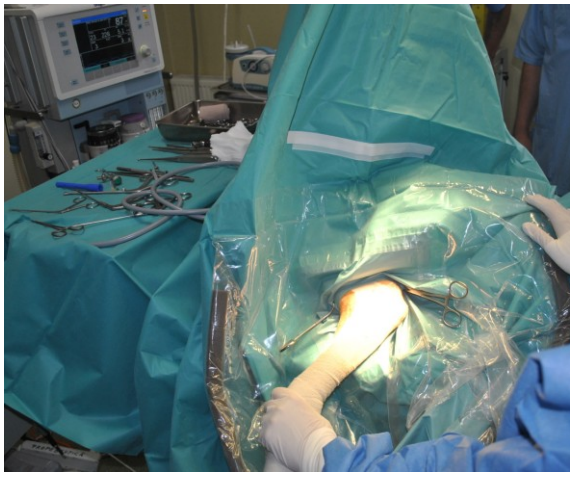

Fig. 2. Positioning of the dog and surgical preparation for elbow joint arthroscopy

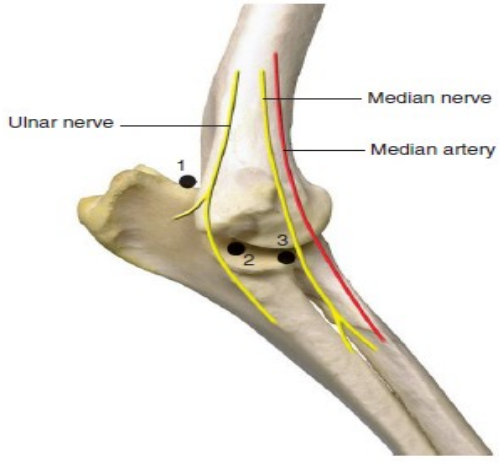

Fig. 3. Medial view of portal locations and pertinent anatomy for canine elbow joint arthroscopy. 1, Infusion and egress portal; 2, arthroscope portal; and 3, instrument portal. (From Beale et al: 2003)

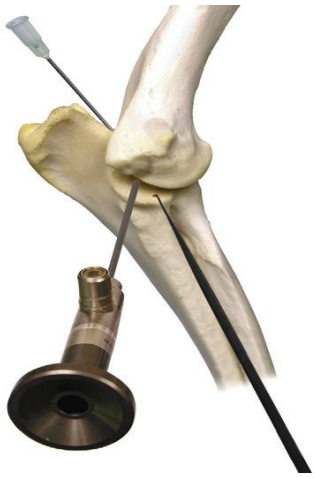

Fig. 4. A. Bone model showing a medial view of the positioning of instruments for arthroscopy of the canine elbow joint. (From Beale BS, Hulse DA, Schulz KS, et al: Small animal arthroscopy, Philadelphia, 2003, Saunders/Elsevier.)

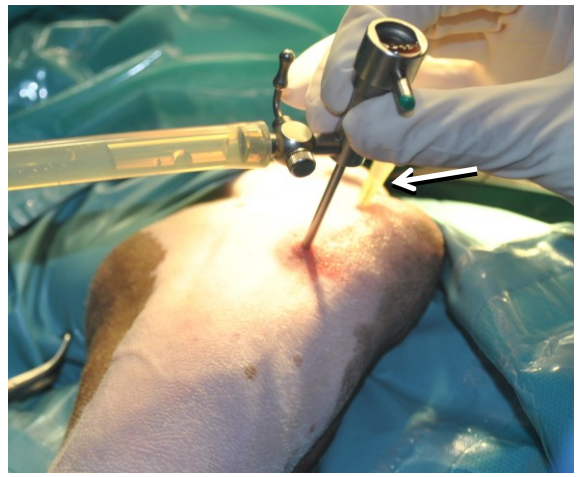

Fig. 5. Medial positioning of the needle (white arrow) and the scope for arthroscopy of the right elbow joint in our case

Arthroscopy tower used for the arthroscopies is a Karl Storz tower package (Fig. 6). Evaluation started with the scope directed toward the caudal and proximal compartment of the joint, while viewing the anconeal process. All intra-articular structures were evaluated (Fig. 7). The scope was advanced into the humeroulnar space to view the lateral coronoid process and joint capsule. The scope was retracted medially to evaluate the articular surfaces of the humeral condyle and the medial portion of the coronoid process. Arthroscopy has allowed the identification of cartilage damage and fragmentation of the medial portion of the coronoid process (Fig. 8). Treatment of fragmentation of the medial portion of the coronoid process included removal of the fragments using forceps (Fig. 9), combined with excision and abrasion of surrounding structures (Fig. 10) based on their appearance via arthroscopy. The underlying bone (Fig. 11) and joint capsule was viewed to confirm the absence of smaller bone fragments.

Postoperative management of the patient included pain management, rehabilitation, and medical management of degenerative joint disease. A soft-padded bandage was applied for 24 hours to minimize extravasation of fluid.

Fig. 6. Arthroscopy tower, including monitor, camera box, light source and fluid pump

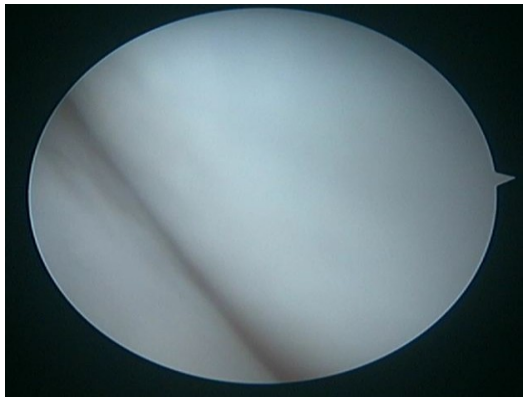

Fig. 7. Arthroscopic findings of the normal cartilage showing the humerus,and radial head

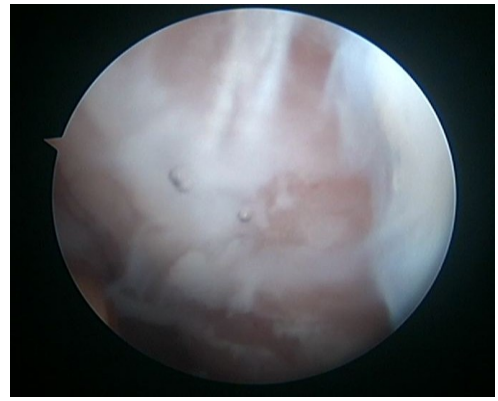

Fig. 8. Full-thickness cartilage defect of the medial portion of the coronoid process.

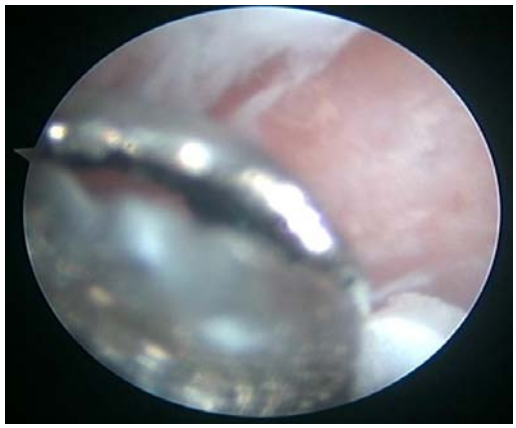

Fig. 9. Removal of the fragments of the medial portion of the coronoid process using forceps

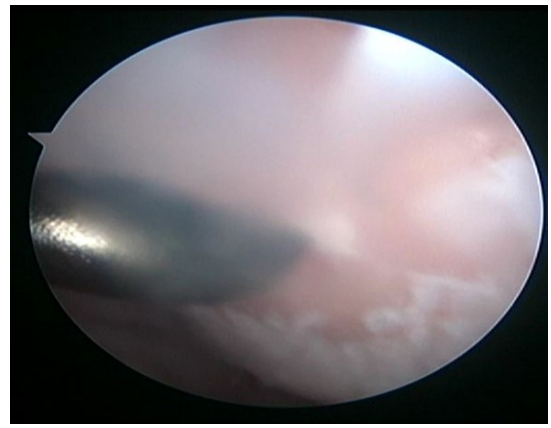

Fig. 10. Excision and abrasion of surrounding structures

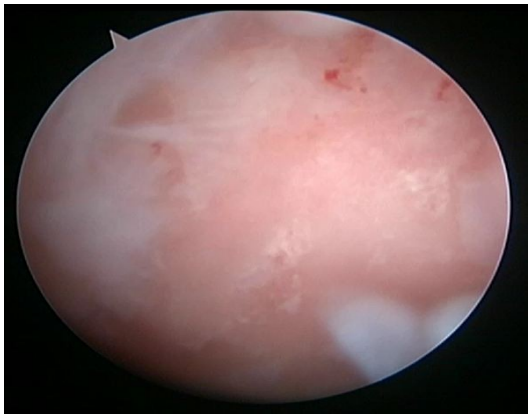

Fig. 11. The underlying bone after the abrasion

RESULTS AND DISCUSSIONS

Arthroscopic evaluation of the elbow joint provides unparalleled visibility of joint surfaces and allows simultaneous minimally invasive treatment of medial compartment disease (Van Ryssen, 2001). Van Ryssen developed the technique for arthroscopic examination of the elbow joint in normal dogs in 1993. The original report describes the use of a 2.7 mm diameter, 25 degree scope. In all joints, the humeral trochlea, medial collateral ligament. Although the principles remain identical, the technique has been improved by the use of smaller arthroscopes (Beale et al., 2003; Griffon, 2006).

Arthroscopy has been instrumental in differentiating the variety of diseases previously grouped under the term "fragmentation of the (medial) coronoid process," or fragmentation of the medial portion of the coronoid process (Berson and Quick, 1980). The level of visibility achieved by arthroscopic examination provided evidence that fragmentation of the medial portion of the coronoid process represents one of several types of lesions in elbows with medial compartment disease, and that medial coronoid disease is a better term to reflect that variety (Tobias and Johnston, 2012). In our case, FCP was not associated with other lesions of intraarticular structures.

Although, the age at presentation varies between reports and components of elbow dysplasia, with onset typically seen in immature dogs between 6 and 18 months of age, in our case the dog was presented at 2 years old age, with episodes of right forelimb lameness started with few months earlier.

Although our patient did not present signs of obvious lameness on left forelimb, the incidence of bilateral disease reported from 25% to 80% of dogs (Cook, 2001; Puccio et al., 2003; Van Ryssen and Bree, 1997), justified evaluation of the contralateral elbow. Advanced imaging (computed tomography and/ or arthroscopy) is therefore recommended in dogs with lameness localized to the elbow, even in the absence of radiographic signs of elbow dysplasia. Fragmentation of the medial portion of the coronoid process in the contralateral limb was not observed in our case after CT scan.

Whereas fragmentation tends to occur near the tip (or apex) of the medial portion of the coronoid process, fissures develop more commonly along and parallel to the radial incisures (Griffon et al., 2009; Grondalen and Grondalen,1981). In our case, fissures appeared near the radial incisures.

Under general anesthesia arthroscopic evaluation of the elbow joint, provided unparalleled visibility of joint surfaces and allowed simultaneous minimally invasive treatment of medial compartment disease (Van Ryssen, 2001). According to the owner informations, the recovery was very quick and the the dog did not reveal any signs of pain or lameness after three months postoperative.

ACKNOWLEDGEMENT

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REFERENCES

1. Beale BS, Hulse DA, Schultz KS, et al: Small animal arthroscopy, ed 1, Philadelphia, Saunders, 2003.
2. Berson JL, Quick CB: Fragmented coronoid process: anatomical, clinical, and radiographic consideration with case analyses. *J Am Anim Hosp Assoc* 16:241, 1980.
3. Capaldo F, Kapatkin A, Gilley R: Diagnostic and surgical applications of arthroscopy in dogs: general applications and forelimb joint diseases. *Compend Cont Educ Pract* 27:580, 2005.
4. Cook JL: Forelimb lameness in the young patient. *Vet Clin North Am* 31:55, 2001.
5. Griffon DJ: Controversies in the arthroscopic treatment of fragmented coronoid process. Presented at: World Small Animal Veterinary Association, October 11–14, 2006, Prague, Czech Republic.
6. Griffon DJ, Blond L, Schaeffer D: Elbow incongruity: arthroscopy or CT. Presented at: 4th Annual Veterinary Advanced Arthrology Association Symposium and Laboratory, August 20–21, 2009, Naples, FL.
7. Grondalen J, Grondalen T: Arthrosis in the elbow joint of young rapidly growing dogs: a pathoanatomical inves- tigation. *Nord Vet Med* 33:1, 1981.
8. Janutta V, Hamman H, Klein SB, et al: Genetic analysis of 3 different classification protocols for the evaluation of elbow dysplasia in German shepherd dogs. *J Small Anim Pract* 47:75, 2006.
9. Michelsen J: Canine elbow dysplasia: aetiopathogenesis and current treatment recommendations. *Vet J.*196 (1):12-9, 2013.
10. Olsson S: En ny typ av armbagsledsdysplasi ho hund? En preliminary rapport. *Svensk Vet Tidn* 26:152, 1974.
11. Olsson S: General and local etiologic factors in canine osteochondrosis. *Vet Q* 1987:268, 1987.
12. Olsson S: The early diagnosis of fragmented coronoid process and osteochondritis dissecans of the canine elbow joint. *J Am Anim Hosp Assoc* 19:616, 1983.
13. Puccio M, Marino DJ, Stefanacci JD, et al: Clinical evaluation and long-term follow-up of dogs having coronoidectomy for elbow incongruity. *J Am Anim Hosp Assoc* 39:473, 2003.
14. Tobias KM, Johnston SA. *Small Animal Surgery Textbook*, Saunders, pp. 733, 2011.
15. Trostel C, McLaughlin R, Pool R: Canine lameness caused by developmental orthopedic diseases: FCP and UAP. *Compend Cont Educ Pract* 25:112, 2003.
16. Van Ryssen B, van Bree H: Arthroscopic findings in 100 dogs with elbow lameness. *Vet Rec* 140:360, 1997.
17. Van Ryssen B: Role of arthroscopy in elbow diseases in dog. Proceedings 12th International Small Animal Arthroscopy Workshop, Refresher Course, January 2001, Ghent, Belgium.

RESEARCH ON THE USE OF A QUALITATIVE TEST FOR DETERMINING THE LEVEL OF PROGESTERONE IN ORDER TO IMPROVE THE MANAGEMENT OF DAIRY COWS FARMS

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Abstract:*The present research aims to determine the suitability of a qualitative test for determining the levels of progesterone (P_4) in order to establish the diagnosis of pregnancy and to establish a correct gynecological diagnosis. The study was conducted in three cattle farms on a number of 49 multiparous cows belonging to several breeds. The qualitative test for observing the level of P_4 was determined in milk and consisted in reading a color reaction consecutively to submerging a test strip in milk. The gestation diagnosis of pregnancy (n= 6) as well as that of the gestation absence (n= 5) was established within 21-24 days post insemination and the results were compared with the ultrasound examination performed within 35-42 days post insemination. The ultrasound exam results have confirmed both the pregnancy diagnosis and the gestation absence in 100% of the cases. In order to assess the advisability of this test use to establish a correct gynecological diagnosis, ovarian inactivity diagnosis, established in an anamnestic,clinical and ultrasound way (n=11) was compared with the results obtained determining the level of P_4 serum by ELISA test and the qualitative test for determining the level of P_4. All females diagnosed with ovarian inactivity through the test for determining the progesterone levels and ultrasound scanning, through lack of luteal tissue had values of P_4 of 0,28 ± 0, 16ng/ml. Out of 49 cows, 5 were diagnosed, clinical and by ultrasonography, with cystic ovarian disease. The qualitative test was abel to make the diffrence between follicular cyst and luteal cyst. The rest of the females (n= 22) were investigated in an anamnestic, clinical and through echography and the results were compared with the rapid test for determining the level of P_4.The data obtained indicate that this test can be used to improve the reproductive performance in dairy cattle farms.*

Key words: *progesterone, gynecological diagnosis, cow*

The lack of attaining the appropriate reproductive parameters is the main cause of economic loss incurred by cattle farmers. The most effective method of monitoring the reproductive activity and CL is the determination of the levels of P4 in blood or milk.

Determining the P4 levels in milk or blood in order to establish the pregnancy diagnosis, both for dairy cattle and meat cattle represents an important method of the reproductive management. Establishing an early diagnosis of pregnancy by P4 dosing, can anticipate and prevent some managerial problems encountered in the farm, by identifying, treating and inseminating yet again the non-pregnant females.

MATERIALS AND METHODS

The present study was conducted on a number of 49 multiparous cows belonging to several breeds. In order to assess the usefulness of such a test to improve the farm management, the product has been tested in two ways. On the one hand, an attempt was made to carry out its assessment in order to establis an early diagnosis of pregnancy (21-24 days after insemination). On the other hand, it has been tried a cross-check of several types of

investigation (clinical, immunological and ultrasound) with the results obtained by means of the qualitative test for determining the P4 level.

The qualitative test for determining the P4 level has been conducted in accordance with the producing company as follows: after an initial washing of the female udder and the removal of the first jets an amount of 10-20 ml of milk has been harvested in a clean recipient. The sample thus obtained was stirred vigorously for a few seconds. Using a pipette that is provided with the kit a certain amount of milk is taken out from the sample. The contents of the pipette filled up to a sign indicated by the manufacturer is emptied into a test tube that is provided with the kit. In this tube a test strip is inserted, provided with the kit as well. The color reaction is interpreted after the strip stays in contact with the milk for 10 minutes.

The interpretation of color is done in the following way: If on the strip one intense line appears in the control area and in the test area the second (II) line is poorly highlighted or missing, the P4 level is high. If two lines appear on the strip, an intense one in the control area and in the test area the line is least intense compared to the test line, then the P4 level is an average one. If two intensely highlighted lines appear on the strip then P4 level is low.

For the first stage of study the, pregnancy diagnosis, a number of 11 females within the range of 21-24 days postinsemination were identified. The qualitative test for determining the P4 level was conducted according to the methodology described above. The results were compared with the ultrasound examination performed at 28-35 days postinsemination. The ultrasound examination was performed with ESAOTE Linears Tringa Piemedical, with the linear transrectal probe with dual frequency 5-8 MHz.

The gestation diagnosis in cows through this indirect method is based on the principle that, in the case of pregnancy, by releasing factors that prevent luteolysis, the foetus maintains the progesterone to increased levels during the entire pregnancy period. [7]. Thus, low levels of progesterone between 18th and 23rd day of insemination indicate the absence of pregnancy and the returning to the follicular stage of the sexual cycle.

Data in the scientific literature on the optimal timing to harvest the samples in order to establish the gestation diagnosis are different. Seida et al. [17] carry out the gestation diagnosis in days 18, 22 and 24 post insemination for a number of 91 by dosing the P4 in milk using the immunological-enzymatic method. The results indicate a higher accuracy of the test on days 22 and 24 compared to day 18. Thus, the accuracy of the test for the gestation diagnosis was 56%, 78% and 79% for the three days investigated, and the accuracy of the non-gestation diagnosis was 90% 93% 93% respectively.

The comparative study conducted by Pennington et al.[13] on the pregnancy diagnosis performed at 21 and 24 days after the insemination revealed a higher accuracy of this review at 24 days (88,4%) compared to day 21 post insemination (77,4%).

To reduce costs, Rhodes [15] is of the opinion that the sampling of milk or blood can be performed at 23-25 days post insemination. This is because a good system for the estrus detection would make unnecessary to determine the P4 levels before the 23rd day post insemination and most of the unfecundated females will experience estrus up to that day.

In the second stage of study, several types of investigation (clinical, immunological and ultrasound) were linked to the results obtained with the help of the qualitative test for determining the P4 level. Thus, 38 cows were taken in the study being at more than 60 days

after calving and presenting different breeding disorders (anestrus, repeated copulations etc.). Following the gynaecological examination, the ultrasoundexamination of the females was carried out, using the device ESAOTE Linears Tringa Piemedical, with transrectal probe with dual frequency 5 to 8 MHz.

Each female has been established clinically and by the ultrasound method a gynaecological diagnosis. Females that were proven clinically and by the ultrosound method to lack the luteal tissue and the presence of follicles less than 9 mm were diagnosed with ovarian inactivity (n = 11). In order to etasblish the gynecological diagnosis of blocked ovarian activity the clinical examination and the ultrasound method were condeidered in conjunction. Thus, the females presenting one or more formations larger than 2.5 cm, together with shortening the interval between the cycles of oestrus or continuance of oestrus were diagnosed with foliculinic cyst (n = 4). Females showing an anaecogenous formation larger than 2.5 cm, with a thickened membrane while insidethe cyst structures were partially affected by luteosis, expressed through the areas of increased echogenity, in conjunction with lengthening the interval between estrus cycles, were established the diagnosis of luteinic cyst. (n = 1). Females diagnosed with cyclic ovarian activity (n = 22) presented on one of the ovaries a luteum formation or follicles larger than 1 cm.

The diagnoses were compared with results obtained from the use of the qualitative test for establishing the P4 level, and 10 of females, randomly chosen, with the P4 levels determined in the laboratory by ELISA method as well.

The blood sampling to establish the P4 level was made in a test tube with activator gel labelled with the identification information of the cow by puncturing the artery or the coccyx vein. After using the serum, it was moved using a disposable syringe (pipette) in an Eppendorf tube labelled with the identification information of the female. The samples were preserved at -30 ° C until establishing the P4 level by immunoenzymatic method was done. The P4 level in serum was established using the immunoenzymatic method (SensioScreen kit)and it was performed within the Horia Cernescu Research Laboratory,The Laboratory for Functional and Methabolical Explorations, using the automatic analyzer ELISA, model Crocodile, Titertek-Bethold, Germany.

RESULTS AND DISCUSSIONS

Of the 11 cows considered for the study in order to establish the gestation diagnosis in 21-24 days post insemination, 6 of them have exhibited increased levels of the P4 hormone (one line per strep) and 5 presented low levels of P4 (two lines per strep). The gestation diagnosis ultrasonographically performed in 28-35 days post insemination has determined that 100% (5 out of 5) of the 5 females with low levels of P4 were not pregnant and 100% (6 out of 6) of females with the high levels of the P4 were pregnant.

The data in the scientific literature concerning the accuracy of this test on the identification of pregnant and non-pregnant females are relatively different. According to Rhodes [15] and Shearer [18], the accurate identification of the non-pregnant cows on the basis of the P4 levels in serum or milk in 19-24 days is done up to 95%-100%. High levels of P4 are only 80% accuratein identifying the pregnant females according to the above mentioned researchers. Drake et al.. [6], citing different authors, assert that the accuracy of

the pregnancy diagnosis by dosing the P4 is 70%-80% and for the non- pregnancy diagnosis is 100%.

As a result of studies carried out, Kaul et al. [10] estimate the test accuracy for the gestation diagnosis at 90.9% and for the non-gestation at 100%. The gestation diagnosis by determining the values of P4 is correct to the ratio of 98% for non-pregnant females and 90% for pregnant ones, according to other authors (12).

The accuracy of the gestation diagnosis by P4 dosing in milk, using the immunoenzymatic method and performed by Romagnolo et al. [16] in 19 and 21 days post insemination was 68,4% and 83,8%, respectively and for the non-gesation diagnosis was 84.6 and 100%, respectively.

Chang et al. [5] consider that the appropriate identification of pregnant cows by determining the levels of P4 is done 67.2% and 87.5% and correct identification of non-pregnant females is done in 95% 98.3% of the cases. The gestation diagnosis by establishing the P4 level in milk using the immune enzymatic method performed by Inaudi et al. [8] has been proven correct in 93, 5% of cases to identify the pregnant females, and the non-pregnant females were correctly identified in 100% of the cases. Instead, the positive gestation diagnosis proves to be correct only in 75% of the cases [11].

Of the 49 cows considered for the study, 11 were diagnosed by the ultrasound method with ovarian inactivity. This diagnosis was established due to the lack of image for the corpus luteum and the presence of follicles less than 7-8 mm. We consider,subsequently to the results obtained, that the diagnosis of ovarian inactivity has been carried out correctly in 100% of the cases using the qualitative test for establishing the P4 levels.. 4 of the females diagnosed with ovarian inactivity were harvested blood samples in order to determine the P4 levels using the immune enzematyc method. These 4 females had a low level of P4 between 0.2 and 0.4 ng/ml ng.

Table 1

The relationship between the gynaecological diagnosis and the level of P4 hormone determined qualitatively and quantitatively

	Diagnosis type	**Result of the qualitative test for establishing the P4**	**The result of P4 level**
	Ovarian inactivity (n = 11)	Low level (n=11)	0,3 ± 1 ng/ml (n = 4)
	Ovarian activity blocked (n = 5)	Low level (n=4)	0,2 ng/ml (n = 2)
		High level (n=1)	
	Cyclic ovarian activity (n = 22)	Low level (n=7)	0,2 ng/ml and 0,4 ng/ml (n = 2)
		Medium level (n=12)	0,2 ng/ml and 0,4 ng/ml (n = 2)
		High level (n=3)	

Of the 49 cows considered for the study, 5 were diagnosed clinically and by the ultrasound method with ovarian activity. 4 of the 5 females were established the diagnosis of foliculinic cyst. In all thesecows,the level of p4 hormone,established using the test under study, was a lowone (two lines intesively highlighted). 2 of the females diagnosed with ovarian inactivity were harvested blood samples in order to determine the levels of P4 using the immune enzematyc method. Both females had low levels of P4 (0.2 ng/ml). One of the females was diagnosed with a luteinic cyst. In this female, the level of P4 hormone, established using the test under study, was ahigh one (one line intensely expressed). Subsequently to the results obtained, we consider that the diagnosis of blocked ovarian activity was correct in 100% of the cases by the qualitative assay for establishing the levels of P4.

As far as the clinical examination is concerned, the follicular cysts are indistinguishable from the luteinic ones, even for an experienced examiner. This differentiation is made on account of the cyst membrane that is thickerin the luteic cyst compared to the follicular one. The differential diagnosis accuracy increases as a result of the ultrasound investigation concerning the two formations, but this method of investigation is not infallible either according to Ribadu(14). The author findsin some follicular cysts some areas of echogenity characteristic to the luteinic cysts, and anecogene areas specific to the follicular cysts were also identified in the luteinic ones. The ultrasound diagnosis of follicular cyst is correct in 90% of the cases and for the luteinic one in 75% of the cases (14). Thus, the most accurate differential diagnosis is done by establishing the level of P4. If the P4 level is low the formation is follicular cyst and if it is high it is a luteinic cyst. (15)

Of the 22 females diagnosed with cyclic ovarian activity 7 had a low level of P4 (two lines well expressed on strip)in accordance with the test, 12 had an average level of P4 (a line well expressed and the secon II weakly highlighted) and 3 females had an increased level of P4 (one line well expressed on strip). The data obtained using the semiquantitative test for establishing the P4 levels are slightly inconsistent with those obtained consecutively to establishing the P4 level the immune enzematyc method. Of the 7 females with lowp4 levels in the semiqualitative test, for 2 females, this hormone level, drawn by the ELISA method, was 0.2 ng/ml ng/ml, 0,4 respectively. On the contrary, two of the females that were established average levels of P4 (n = 12) had values of P4 of 0.2 ng/ml or 0.4 ng/ml, respectively.

As a result of this study, it was noticed that the presence of a corpus luteum (CL) on the ovary is not associated with increased values of P4 hormone. It is very difficult to establish if a CL is growing or is in regression, using the ultrasound examination. Even in the case of an undeveloped CL which theoretically secretes large amounts of P4, low values of this hormone were obtained by ELISA test.

Pancarci (12), as wll as Kayacik et al. (9), points out that CL regression is less evident during an ultrasound examination, accepting that its full morphological regression requires several weeks.

In his study published in1999, Pancarci (12) underlines that the resulting general correlation has not been satisfactory as between 15 and 17 days of the oestrus the CL sizes decreased slowlier than the P4 levels (the correlation between the dimensions of the CL and

the P4 was r = 0.08 on days 15 and 17), and the ultrasound examination was characterized low accuracy, particulaly for the detection of fully developed CL.

Ribadu et al. (14) highlight a strong correlation (r = 0.85) between the diameter of the CL and P4 plasma, and 2-3 days before oestrus, the diameter was the same as in the mid-luteal phase, only that the CL did not presented any activity (P4 level was below 0.5 mg/ml).

Comparing the results obtained in summer and winter, Borges et al. (4) report the maximum surface of CL registered in the day 8,9 ± 1.4 of oestrus (summer) and 9.2 ± 1.1 in winter. The daily growth rate was 0.3 ± 0.1 cm2 in winter and 0.5 ± 0.1 cm2 in summer, while the regression of CL was 0.2 ± 0.1 cm2 in winter and 0.4 ± 0.1 cm2 in summer.

The undeveloped CL are difficult to be differentiated from the ovarian stroma due to the small size and irregular structure. Generally, they look grey and ecogenically spotted. The fully developed CL is separated from the ovary, having obvious edges,with a granular structure a hypoecogenous structure.It can exceed the edge of the ovary. CL in the regression is difficult to be differentiated due to the decrease in echogenity difference of structures in time and the loss of differentiation between the luteal tissue and the ovarian stroma. (14).

The P4 level rise occurs in steps similar to that of the CL dimensions. On the contrary, the CL sizes decrease differently from that of P4 level. Battochio et al. (2) presented a classification system applicable to the CL position connected to the P4 level changes and showed a 95% correlation.

The correlation between the CL diameters and the P4 level was assessed as being significant for its development and luteal regression in heifers and in cows, only at the stage of CL development (1).

Lowering P4 has been observed starting with day 16 (in the case of sexual cycles with 2 follicular stages) or with the 23rd day (in the case of sexual cycles with 3 follicular stages), and the CL started to reduce dimensionally from day 17th, 24th respectively. Ribadu et al. (14) highlight that the P4 levels were 6,13 ± 0.68 mg/ml at mid-cycle and 0.22 ± 0.11 mg/ml during regression.

CONCLUSIONS

The qualitative test for establishing the progesterone levels can be used with good results, in 21-24 days post insemination, in order to establish the diagnosis of pregnancy.

The qualitative test for establishing the progesterone levels can be used with good results in order to establish the diagnosis of ovarian inactivity

The qualitative test for establishing the progesterone level represents a proper method to differentiate between the follicular and luteinic cyst.

Although situatios were sometimes encountered when the color reaction interpreting was performed difficulty, the test for the qualitative measuring the progesterone levels represents an efficient way to improve the management in dairy farms.

REFERENCES

1. ASSEY, R. J., PURWANTARA, B., GREVE, T., HYTTEL, P., SCHMIDT, M. H. (1993) - *Corpus luteum size and plasma progesterone levels in cattle after cloprostenol-induced luteolysis,* Theriogenology, 39, 1321-1330;
2. BATTOCHIO, M., MOLLO, A., VERONESI, M. C., SOLDANO, F., BONO, G., CAIROLI, F. (1999) - *Agreement between ultrasonographic classification of the corpus luteum and plasma progesterone concentration in dairy cows,* Theriogenology, 51, 1059-1069;
3. Beckers, J.F., *Signaux embryonnaires de la gestation*. SFB-Paris, 29, 30, 31 p. 95 Oct. (2002).
4. BORGES, A.M. ET AL (2003) - *Follicular Dynamic and Luteal Regression in Gir and Nelore Cows after Treatment with Cloprostenol Sodic*, R.Bras.Zootec, 32, 2, 276-283;
5. Chang, C.F., Estergreen V.L., Development of a direct enzyme immunoassay of milk progesterone and its application to pregnancy diagnosis in cows. Steroids Feb;41(2):173-95. (1983).
6. Drake, T., O'Connor, M., *Milk Progesterone as an Aid in Bovine Reproductive Management.* May edition of Herd Health Memo, (2001).
7. Dunlap, L.M., Hein, M.A., Dean, C.L., Way, M.T., *The accuracy of an expensive computer system in detecting estrus in dairy cow.*Governors School of Agriculture, Virginia Tech Univercity, Blacksburk VA, (1997).
8. Inaudi P., Bacigalupo, M., Monittola, C., Lugaro, G., Genezzani, A.R., *Pregnancy diagnosis in cattle by a rapid and highly reliable method for progesterone determination in milk.* J. Reprod. Fertil. Jul; 65(2):265-73, (1982).
9. KAYACIK, V., SALMANOGLU, M. R., POLAT, B., OZLUER, A. (2005) - *Evaluation of the Corpus Luteum Size Throughout the Cycle by Ultrasonography and Progestrone Assay in Cows,* Turk J. Vet. Anim. Sci, 29, 1311-1316;
10. Kaul, V., Prakash, B.S., *Accuracy of pregnancy/non pregnancy diagnosis in zebu and crossbred cattle and Murrah buffaloes by milk progesterone determination post insemination.* Trop. Anim. Health Prod. Aug.;26(3):187-92, (1994).
11. O'Connor, M., *Milk progesterone analysis for determining reproductive status.* DAS-5: Dairy cattle (replaces 98-3, 91-3, 92-3) (1998).
12. PANCARCI, S.M. (1999) - *Monitoring and Comparing Follicular and Luteal Function Between Genetically High- and Low-Producing Dairy Cows by Ultrasonography*, Tr.J of Veterinary and Animal Sciences, 23, 141-147;
13. Pennington, J.A., Schults, L.H., Hoffman, W.F., - Comparison of pregnancy diagnosis by milk progesterone on day 21 and day 24 postbreeding: field study in dairy cattle. J Dairy Sci Oct; 68(10):270-5, (1985).
14. RIBADU, A. Y., WARD, W. R., DOBSON, H. (1994) - *Comparative evaluation of ovarian structures in cattle structures in cattle by palpation per rectum, ultrasonography and plasma progesterone concentration,* Vet. Rec., 135, 452-457;
15. Rhodes, R.C., *Use for milk progesterone assay for reproductive management.* Dairy integrated reproductive management IRM-9.
16. Romagnolo, D., Nebel, R.L., *The accuracy of Enzime-Linked Immunosorbent assay and latex agglutination progesterone test for the validation of estrus and early pregnancy diagnosis.* Theriogenology 23 576-595, (1993).
17. Seida, A.A., Bretzlaff, K.N., Elmore, R.G., *Pregnancy diagnosis by milk progesterone on days 18, 22, and 24 postbreeding in dairy cows.* Arch. Exp. Veterinarmed.; 44(3):489-91, (1990).
18. Shearer, J.K., *The milk progesterone test and its application in dairy cattle reproduction*. Dairy Production Guide. Fact Seet DS 60 sept. (1992).

INFLUENCE OF SURGICAL PROCEDURES IN MINIPIG MODELS' UPON CELL BLOOD COUNTS

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Abstract:*Minipig model with neuromodulation in spinal cord injuries (SCI) is often used in research for its physiological similarities with human. In SCI minipig study were collected 64 blood samples from 4 study groups, according to the surgical intervention applied: i.) 7 healthy animals (control group), animals with ii.) 6 with laparoscopic surgery (LAP), iii) 4 with classical surgical intervention (SCI) and i.v.) 4 minipigs with both procedures SCI and LAP. Monitoring the average physiological vital functions, body weight and fat thickness and the evaluation of blood parameters in minipig model was made during the entire period of the experiment, at the moment of surgical interventions and then monthly to check the changes in cell blood count (CBC). Considering that surgical interventions represent the major factor of influence, by monitoring the fluctuations in CBC and through statistical processing, were obtained values for this study. The red blood count (RBC) ranged between 7,35±0,15x1012/L, which can be considered normal (p<0.060); same was observed for HCT, which had an average value of 40,50±0,93% - between limits; Statistics performed Kruskal Wallis revealed p<0.682. Total haemoglobin registered a normal value of 13.0±0.28 g/dL, with a statistical significance of p<0.807. Among all parameters associated with red cells, only mean cell volume (MCV) was influenced by the type of surgical intervention (according to p<0.033). It's value of 55.20±0.74 is slightly raised above normal. CBC registered a low white blood count (WBC) of 9.22±0.42x10^9/L in all four groups (p<0.008) proving the impact of procedures upon animals. The normal average value 5.41±0.40 x 10^9/L and 55.01±0.40% of WBC in neutrophils emphasized the influence of surgeries (p<0.004). Significance values were also registered in %LYM, %MONO, %EOS, %BASO and indices as MPV, PDW and PCT. As a conclusion, the surgical procedure which had the biggest influence on CBC's animals was SCI +LAP, followed by LAP and SCI.*

Keywords: *minipig, CBC, spinal cord injuries*

Swine breeds as Göttingen Minipig are used more frequently in research as an alternative to dogs and primates, main reason being the social acceptance. Another motivation is given by the existence of similarities (1,2) to human anatomy and physiology characters, including cardiovascular, urinary, tegumentary and digestive apparatus and systems (3).

Ethical considerations, as well as the existence of a significant amount of data provide additional support in using this breed in research studies. In the last two decades, the swine have replaced dogs (4), mainly in research fields as toxicology, absorption, distribution, metabolism and excretion of pharmacological constituents.

In recent experiments (5), Göttingen Minipigs are considered a suitable model for preliminary studies in innovative therapeutic strategies which aimed the regeneration of spinal cord in paraplegic patients.

In the study conducted in the Experimental Units of Horia Cernescu Research Unit[1] there collected 64 blood samples from 4 study groups, classified in accordance to the surgical intervention applied: i.) 7 healthy animals (control group), animals with no surgical intervention ii.) 6 swine which had laparoscopic surgery (LAP), iii) 4 animals upon which it was made classical surgical intervention – spinal cord injuries (SCI) and iv.) 4 minipigs with a double procedure – classical surgery and laparoscopic intervention (SCI and LAP).

MATERIALS AND METHODS

Own research and practical activities involved the pursuit, interpretation and monitoring variations in haematological and biochemical parameters of Göttingen swine, imported from Denmark, housing in the Experimental unit and analysed in Laboratory of Metabolic and functional explorations of Horia Cernescu Research Unit.

Blood sampling was performed monthly, starting from the surgeries, in order to monitor the changes in sanguine parameters. There were also carried out analyses to determine the animals' biochemical array. Blood count and biochemical results were corroborated with medical history and clinical signs to check the health status of animals within the experiment.

Blood sampling in minipigs was performed *à jeun* or postprandial. The preferred sites for the procedures depend on the volume and frequency of collection: jugular, auricular or saphenous veins (6); within the study it was used mainly the jugular. Materials used were the height-adjustable table (Fig. no. 1) and medical disposables – vacutainer (with and without anticoagulant), 20-21 x 1½ G collection needles, needle holder and sanitary alcohol, compresses and cotton wool, gloves.

Figure no. 1: **Preparing minipig model for blood sampling**

For the post-sampling preparation and analysis of samples, the content is mixed by slight inversion of the tube approx. 10 times to homogenize blood. If the sample is not sent immediately to the laboratory, it must be refrigerated. The stability of the sample is 36 to 48

[1]Experimental Units is a part of *Horia Cernescu Research Unit from Banat University of Agricultural Science and Veterinary Medicine* established by POSCCE, Project no. 18 - *Development of research, education and services infrastructure in the fields of veterinary medicine and innovative technologies for West Region* - 2669 SMIS code.

hours if kept at 2–8°C, but for determining the haemoglobin and cell count is recommended analyse to be performed in the first 6 hours from collection. It is not recommended to delay the time interval to determine haematocrit and red blood cell indices. If the sample was refrigerated, it must be balanced to room temperature before being analysed.

In order to obtain cell blood counts it was used an Automatic haematology analyser with veterinary dedicated software IDEXX Procyte Dx™ that uses fluorescence flow cytometry. It was predefined Minipig breed in order to obtain highly accurate results. Biochemical profiling was done using an automatic biochemistry Analyser RX Daytona Plus. Both equipment are part of Laboratory of metabolic and functional explorations. Analyses were preceded by blood serum separation by centrifugation to 4000 rpm for 5 minutes (Fig. no. 2), using a centrifuge Hettich model EBA 21[2]. Biochemical results are not subject of this paper.

The measurements were included in data bases using Excel and statistically analysed using MINITAB and SPSS; the nonparametric Kruskal Wallis test procedures were considered.

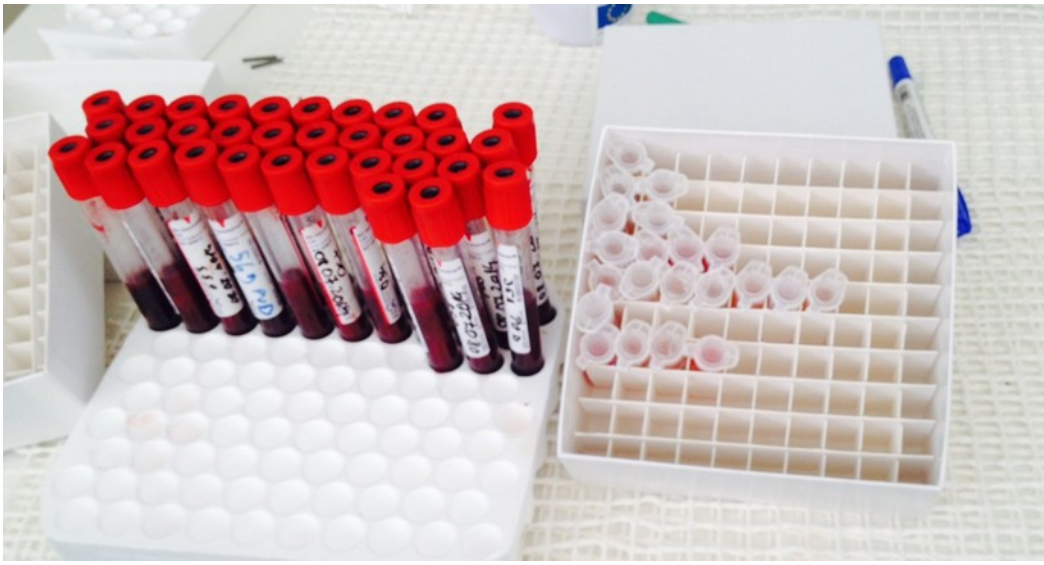

Figure no. 2: **Samples centrifuged to obtain blood serum**

RESULTS AND DISCUSSION

For conducting the study, there were performed 64 CBC on 21 female Göttingen minipigs. Considering that surgical interventions represent the major factor of influence, by monitoring the fluctuations in CBC and through statistical processing, were obtained values for this study. All the parameters analysed were taken into consideration (Fig. no.3), as listed below:

- Red blood cells - RBC
- White blood cells - WBC

[2] Equipment used was purchased through project nr. 18 POSCCE, Development of research, education and services infrastructure in the fields of veterinary medicine and innovative technologies for West Region - 2669 SMIS code and belong to endowments of in Laboratory of Metabolic and functional explorations of Horia Cernescu Research Unit.

- Total amount of haemoglobin in the blood - HGB
- Percentage of red blood cells (haematocrit) - HCT
- Mean cell volume - MCV
- Mean cell haemoglobin - MCH
- Mean cell haemoglobin - MCHC
- Red blood cell distribution width – RDW
- Reticulocyte count RETIC
- Platelets PLT
- Mean platelets volume - MPV
- Leucocyte percentage: neutrophils (NEUT%), eosinophils (EOS%), basophiles (BASO%), lymphocytes (LYMPH %), monocytes (MONO %)
- Leucocyte count: neutrophils (NEUT#, eosinophils (EOS#), basophiles (BASO#), lymphocytes (LYMPH #), monocyte (MONO#)
- Platelet distribution width (PDW)
- Platelet count (PCT).

Regarding RBC, for the samples analysed, the mean value was 7.35±0.15 x 10^{12}/L and it is considered between normal ranges of 5.00 – 8.00 x 10^{12}/L. The differences between mean values registered in the four groups cannot sustain the hypothesis that the intervention applied to animals had a significant effect on RBC; on Kruskal Wallis test it was obtained *p=0.060.*

The mean value of haematocrit in the four groups was 40.50±0.93%; in our results the *p=0.682* suggest that also the surgical interventions impact hypothesis cannot be associated with this parameter.

The hemoleucograms showed a mean value of 13.0±0.28 g/dL haemoglobin which ranges between normal (10.7 – 16.7 g/dL) and the statistical interpretation of differences between means indicates that also the hypothesis of surgeries impact cannot be accepted on this parameter at usually probability - *p<0.807.*

Blood analyses indicates a mean MCV of 55.20±0.74, which is considered slightly raised (normal value is 44.49 – 51.27 fL) and which, if correlated with other modification it can suggest a macrocytic anaemia. Among all red blood cell associated parameters, MCV is the only one who denotes that treatments had an influence upon animals; on Kruskal Wallis test, the differences between means had significance at *p<0.033*.

The Mean cell haemoglobin in all four groups had a normal value of 17.74±0.22. Statistical processing indicates that hypothesis of impacting of surgical treatments cannot be accepted for MCH - *p<0.106*.

MCHC represents the haemoglobin quantity found in 100 ml of erythrocytes mass. The mean concentration obtained in the four groups was 32.20±0.17 g/dl and ranges between physiological limits of 30.00 – 34.00 g/dl. After the statistical interpretation, it was found that there the *p* value for means difference of MCHC was p<*0.059*, threshold still unaccepted in medical biological studies.

RDW represents the variation's coefficient of erythrocytes dimensions; it is always considered in association with MCV to differentiate anaemias. RDW parameter varied in the four experimental classes from 18.10 % in the LAP group to a maximum of 30.80% in

animals with double intervention. The mean value of RDW is 64.00±0.35 K/μ L and the p value of difference between means test threshold was *p<0.145*.

Reticulocytes represent immature red blood cells and their number provides information about bone marrow's ability to synthesize in response to an overload (anaemia). In minipig experimental model, it was registered a normal mean value of reticulocytes of 64.00±0.35 K/μL. It is observed that values associated to reticulocytes cannot support the overall effect of surgery; by using Kruskal Wallis test there were obtained *p<0.781* for % reticulocytes and *p<0.632* for total number of reticulocytes. So, we cannot accept the hypothesis of treatment influences.

For the minipigs used in the experiment, the CBC resulted with a mean number of leucocytes of 9.22±0.42 $x10^9$/L. According to analysis software dedicated to veterinary medicine, the limits of reference values for miniature swine is between 11.00 – 22.00 $x10^9$/L, which classifies the amount as decreased. Statistical analysis and *p<0.008* proves that surgical intervention have influenced the animals' blood parameters.

The mean amount of neutrophils in the four groups is 5.41±0.40 $x10^9$/L and the percentage in WBC is 55.01±0.40%. The limits of benchmarks of 4.48 – 7.52 x 10^9/L classifies the value of neutrophils as being physiological. The differences between means values were analysed using nonparametric test; for the % of neutrophils, the threshold the value of *p <0.118* cannot sustain the hypothesis that interventions have influenced the WBC, but in number of neutrophils, *p<0.004* demonstrates the impact of surgeries treatments upon WBC.

Analysing the 64 samples, it was obtained an average number of lymphocytes of 2.85±0.19 $x10^9$ /L, which denotes a percentage of 33.75±2.50% of total white cells. The benchmarks of the software – 6.60 ÷ 18.70 x 10^9 /L used classifies the values obtained as low. The percentage of LYMPH was influenced by treatments (*p<0.002*). This not apply to total amount of lymphocytes - *p<0.110.*

Regarding monocytes, it was registered a mean value in the groups of 0.56±0.03 $x10^9$ /L and a % of 6.65±0.38. The value exceeds normal value of 0.30 ÷ 1.25 $x10^9$/L. The differences between groups demonstrates that surgical procedures have influenced the %MONO due to *p<0.020*, but not the number of monocytes - *p=0.838.*

Determination concerning the eosinophils have emphasized an average number of 0.37±0.14 $x10^9$/L, which can be classified between regular limits of 0.20 ÷ 1.10 $x10^9$/L. Significance thresholds in % *p<0.452* and # *p<0.326* proves that those cells were not affected by the procedures.

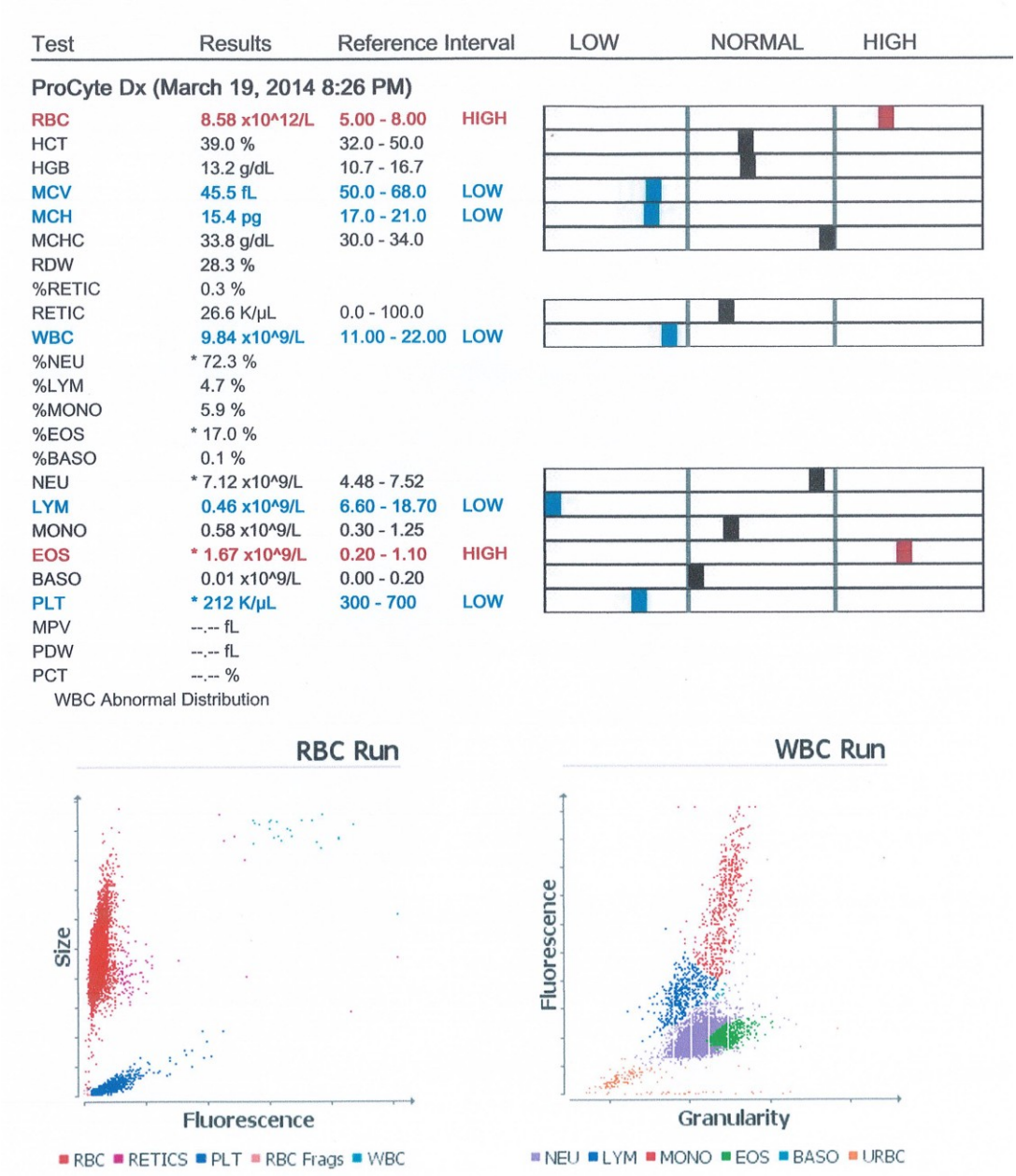

Figure no. 3: **Output of** *IDEXX Procyte Dx*™ **- complete blood count in minipig model**

Basophils granulocytes in Göttingen Minipig obtained in CBC made within the study had a mean value in percentage distribution of 0.21±0.03 and a 0.02 x10^9/L of #BASO. Average so obtained can fetch between reference thresholds of 0.00 ÷ 0.20 x 10^9/L. After the statistical interpretation it resulted that surgical interventions influenced the number ($p<0.040$) and percentage of blood basophils ($p<0.008$)

Regardless of the group subject to study in samples analysed, platelets have an average value of 377.23±12.88, that fall between normal thresholds of 300 – 700 K/µL.

As with other blood cells, the hypothesis regarding impacting of platelet count by surgical procedures cannot be accepted because, in this study, the p was *0.098*.

The average platelet volume value recorded from four groups has an average of 9.90±0.20 fL. The device used to perform blood counts does not provide benchmarks for platelet indices. The average platelet volume as platelets index was influenced by surgical operations, as illustrated statistically by $p< 0.026$. Platelet distribution width has an average value recorded in the four experimental groups of 14.19±0.39 fL. As in the previous case, the surgical procedures had a marked effect on platelet distribution width ($p<0.049$).

Plateletcrit is a parameter used to determine the average platelet volume and has a value of 0.39±0.02% in the four lots. Haematology analyser does not provide reference range for this index. From statistical processing with nonparametric test cannot be argued that hypothesis that surgical procedures have influenced the value of trombocrite ($p=0.345$).

DISCUSSIONS

Hemoleucograms obtained are comparable with the ones made available by CiToxLab Denmark (7) on healthy adult females Gottingen minipig. Comparing the values communicated but not published, it can be suggested that red blood cells are more numerous; however haemoglobin and haematocrit are framed within comparable and considered normal. In terms of total number of white blood cells, it is low in comparison with reference ranges used in our study; among white blood cells, there were low percentages of neutrophils, lymphocytes and monocytes, while eosinophils and basophils are within normal parameters. The number of platelets may be considered normal and to the same levels in both studies.

CONCLUSIONS

- The surgical procedure which had the biggest influence on Cell blood counts in Göttingen minipigs were spinal cord injuries (SCI) together with laparoscopy (LAP).
- The second impact of surgical procedure was laparoscopy (LAP).
- The least surgical procedure with impacted the haematology of treated models were spinal cord injuries (SCI).

REFERENCES

1. Thorn C., (2010) - *Hematology of the Pig*. In Schalm 'S Veterinary Hematology, Edit. Weiss, D.J. Wardrop K.J., Blackwell Publishing Ltd, USA, p. 843–850
2. Moroni Maria, Eric Lombardini, Rudolph Salber, Mehdi Kazemzedeh, Vitaly Nagy, Cara Olsen, Mark H. Whitnall (2011) - Hematological Changes as Prognostic Indicators of Survival: Similarities Between Gottingen Minipigs, Humans, and Other Large Animal Models, PLoS Onev.6(9); 2011
3. Braun W.F. Jr., Casteel S.W. (1993) - *Potbellied pigs. Miniature porcine pets*, Veterinary Clinics of North America: Small Animal Practice, 23, pg. 1149–1177
4. Swindle M. M., Makin A., Herron A. J., Clubb F. J. Jr, Frazier K. S., (2012) - *Swine as Models in Biomedical Research and Toxicology Testing,* Veterinary Pathology, 49(2), 344-356
5. Mercedes Zurita, Concepción Aguayo, Celia Bonilla, Laura Otero, Miguel Rico, Alicia Rodríguez, Jesús Vaquero (2012) – *The pig model of chronic paraplegia: A challenge for experimental studies in spinal cord injury*, Progress in Neurobiology, Progress in Neurobiology, 97, 3, pg. 288–303
6. Adrian Zeltner - Handling, Dosing and Training of the Göttingen Minipig, http://minipigs.dk/the-goettingen-minipig/handling-dosing
7. Andrew Makin, Jes Tovborg Jensen and Signe Klastrup, CiToxLAB Scantox A/S, Ejby - http://minipigs.dk/uploads/media/Age_related_changes_in_clinical_pathology_of_minipigs.pdf

THE INCIDENCE OF GMO SOY AND CORN OF FEED AND FOOD

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Abstract:*Romania's EU accession, amended presence of GMOs issue-level public debate, complying with European legal regulations. L.S.V.S.A. Iaşi screening for GMO analysis performed by PCR samples classic soybeans, corn, feed and food (containing soy or corn). The test procedure is based on the isolation and purification of DNA, followed by PCR to identify specific DNA plant (soybean lectin gene and the invertase gene of maize) and specific DNA soybean line 40-3-2 GTS and p35S detection of genetic elements and/or corn t-nos. The share of positive results on the presence of GMO soybean samples analyzed in the period 2011-2014 were: 19.65% in 2011; 10.00% in 2012; 4.26% in 2013 and 9,10% in 2014. The incidence of GMO soy established for each of the counties surrounding was variable: 38.47% in Ialomita, in Vrancea 16.67%, 12.50% in Vaslui , 5.56% Iasi, Prahova 3.39% and the county. Buzau and Galati were not identified. Final results for the 187 samples soya (flour, grains, textured soy protein, etc.) examined, showed that 11.23% of the samples contained GM soya. The results for GM maize to the 179 samples analyzed in the years 2013-2014 reveals that not identified GMOs in feed and food examined.*

Keywords-*OMG,PCR CLASIC, soy, maize;*

INTRODUCTION

In recent decades it has become possible not only hybridization between related species, and other species distant taxonomic whose cross under natural conditions is not possible or if performed, hybrids are sterile.

We mention here the techniques used for this purpose are: the technique of embryo isolation, *in vivo* and *in vitro* culture of embryos or of ovaries, pollination and *in vitro* fertilization. Despite the use and improving the methods of hybridization and natural selection, thes presents a number of weaknesses. Often, a major one is that growers want rather to introduce a single characteristic than to transfer and recombine the entire genomes. The selection and choosing a stable phenotypic variety is a slow process.

These shortcomings seem to be removed by the application of recombinant DNA and genetic transformation techniques. The term "genetically modified organism" (GMO) was introduced to describe organisms whose genetic material has been altered in a way that does not occur in nature under natural conditions by crossing or recombination.

Genetically modified organism itself should be a biological unit, capable to allow the introduction, recombination and replication of genetic information new or foreign.

The term OMG refers to plants with one or more genes from different species have been stably introduced, into a host genome, using techniques of gene transfer. In most cases, these genes are expressed (transcribed and translated), creating a new heterologous protein. The process of introducing genes into an unrelated species to the body which "provided" genetic material of interest and their operation, is known as genetic transformation.

For detection and quantification of genetically modified organisms at all levels of the chain foods production of plant origin, is compulsory to use optimized and standardized analytical methods. These methods are target on the identification of DNA, mRNA of the foreign

genes, the proteins or the new biological molecules produced by the living modified organism, as a direct or indirect consequence of transgenic proteins function. Currently, analytical methods for the detection of GMOs is based almost exclusively on identifying transgenic DNA or transgenic proteins. For a long period of time was possible only for qualitative tests based on PCR or semi-quantitative, based on the ELISA technique. These were only able to state if a sample contains or not a genetically modified organism, instead quantitative tests provides information on the quantities of these organisms.

MATERIAL AND METHOD

The test procedure for comprises the following steps soybean samples (10):

- isolation and purification of DNA and quality control of isolated nucleic acids ;
- identify the presence of specific plant DNA (lectin gene);
- identification of specific DNA line GTS 40-3-2 (Roundup Ready) in food and feed containing soybean (identifying a DNA fragment which includes a sequence of Ca MV 35S promoter derived from cauliflower mosaic virus and sequence, " chloroplast transit signal " derived from *Petunia hybrida*) (9).

The test procedure comprises the following steps maize samples (10):

- isolation and purification of DNA and quality control of isolated nucleic acids ;
- identify the presence of specific plant DNA (invertase gene);
- identification of genetically modified material by conventional PCR method for detecting genetic elements that can be found in most GMOs (35S Promotor and NOS Terminator). Identification of these genetic elements have the disadvantage that they can be naturally present in the host organism, because of the presence of viruses (*Cauliflower Mosaic Virus*), bacteria (*Agrobacterium tumefaciens)*.

The study was conducted over a period of four years, from 2011 to 2014 on the presence of GMOs in products containing soya or consisting or corn. DNA was extracted using "GeneSpin" - Eurofins, GeneScan in order to detect GMO soybean and corn CEs Protocol classic PCR using primers specific for soy (gene lectin- forward primer 5'-GCC GMO3 CTC TCC TAC ACC CCC ATC C-3 ', reverse primer 5'-GCC GMO4 CAT CAA CTG TTG GCC TTT TG-3'; 35S-2- RRS- forward primer 5'-TGA TGA CAC CTC TGT ATC GAT C C-3 'primer petu reverse-r1 5 '- TGA CCT GCC ATC ATG TGT TTG T - 3'); primers specific for maize (invertaza- gene IVR1F forward primer 5'-CCG CAC CTG TAT TGG AAG GGC TACC-3 ', reverse primer 5'-GGA IVR1R CGT GTA GAG GCC GAC CAT GATC-3', 35S promoter forward primer 35S- CF3 5'-CCA CAA AGC CGT AAG CTT TGC-3 'reverse primer CR4 35S 5' - CCA TCT TCC ATG GAA AAT AAC TTCC - 3 '; terminator NOS- forward primer 5'-118f HA nos GCA TAT TGA TTA CGT TGA GGG GAT-3 ', reverse primer 118r HA nos 5' - GAT GAC AAT ACC TTA GCG CGC TCC- 3 '). As reagents for amplification were used Taq DNA polymerase. 5 IU/ml, dNTP, 25 mM MgCl2 (Qiagen). As PCR equipment was used termociclorul YCycler (BioRad). The necessary reagents for electrophoresis used, were: agarose, "Molecular biology grade", TAE/TBE (Tris acetic acid/acidboric, EDTA), EtBr 10 mg/ml, 100 bp migration marker (Biorad).

RESULTS AND DISCUSSIONS

Between 2014 01.01.2011-31.12 research intended to follow the presence of GMOs in soybean samples establishing a dynamic for each of the counties surrounding LSVSA Iaşi. The number of samples analyzed were localized mainly in the region of Moldavia.

Table no. 1

The incidence of GMO soy in the period 2011-2014

Year	Number	Positive samples OMG		Negative Positive samples OMG	
		number	%	number	%
2011	56	11	19,65	45	80,35
2012	40	4	10,00	36	90,00
2013	47	2	4,26	45	95,74
2014	44	4	9,09	40	90,91
ADDED	187	21	11,23	166	88,77

Out of 187 samples soy (soy flour, soy granules, textured soy schnitzels, etc.) analyzed in 2011-2014, 166 samples (88.77 %) were negative and in 21 samples we identified the presence of GMOs, which represents 11.23 % of the total samples analyzed. The presence of GMO soy analyzed in the period 2011-2014 was: 19.65 % in 2011; 10.00 % in 2012; 4.26 % 2013; 9.09 % and 2014.

Table no. 2

GMO soy incidence established by counties, during 01.01.2011-31.12.2014

County	Number	Positive samples OMG		Negative samples OMG	
		number	%	number	%
Buzău	13	0	0,00	13	100,00
Galați	4	0	0,00	3	100,00
Ialomița	13	5	38,47	8	61,53
Iași	36	2	5,56	34	94,44
Prahova	59	2	3,39	57	96,61
Vaslui	40	5	12,50	35	87,50
Vrancea	18	3	16,67	15	83,33
Other counties	4	4	100,00	0	0,00
ADDED	187	21	11,23	166	88,77

Table no. 3

Samples of feed, food and soybeans analyzed during 01.01.2011-31.12.2014

YEAR	NUMBER SAMPLES	Of which				
		FEED	FOODS	SOYBEANS	Negative samples	Pozitive samples
2011	56	7	47	2	45	11
2012	40	0	35	5	36	4
2013	47	0	45	2	45	2
2014	44	3	40	1	40	4
ADDED	187	10	167	10	166	21

Table no. 4

Samples of feed, food, maize, Identification GMO during 01.01.2013-31.12.2014

YEAR	NUMBER SAMPLES	Of which:				
		FEED	FOODS	SOYBEANS	Negative samples	Pozitive samples
2013	94	3	55	36	94	0
2014	85	2	66	17	85	0
ADDED	179	5	121	53	179	0

The incidence GMO soy, established based on the number of samples, examined for each county was: 38.47 %; in Ialomița, 16.67 %, in Vrancea; 12.50 % in Vaslui; 5.56 % in Iasi end 3.39 % in Prahova. In Buzau and Galati counties no positive samples were identified for GMO soybeans.

Table no. 5

Identification GMO maize
samples of feed, food, maize, during 01.01.2013-31.12.2014

YEAR	NUMBER SAMPLES	Of which:				
		FEED	FOODS	SOYBEANS	Negative samples	Pozitive samples
2013	94	3	55	36	94	0
2014	85	2	66	17	85	0
ADDED	179	5	121	53	179	0

The results for the maize GMO in the samples containing 179 Corn analyzed years 2013-2014, show that GM has not been identified in the feed and food examined.

In 2007, the Laboratory of Molecular Biology and GMO Unit within the National Sanitary Veterinary and Food Safety Authority, was accepted on the list of European reference laboratories for GMO field. Romania has therefore also technical and logistical means to test market products to detect GMO-labeled food products.

In Romania, the first GM crops were introduced in 1998. Romania's EU accession has amended the GMOs issue on a wide public debate to obligate to comply with European legal regulations.

Although the law provides for labeling GM products as binding (Law 106/2002 - provisions relating to GMOs being completed by GD 173/2006), is not implemented.

Currently there is no product on the Romanian market labeled as containing GMOs the results of any laboratory tests conducted by authorities on products on the market, are not made public. Even today there is even a food or feed marketed in Romania, to be labeled as genetically modified or having genetically modified ingredients.

Consumers in Romania are denied the right to choose products on the shelves in our shops GM can not identify those genetically modified products by simply consulting the label (6).

CONCLUSIONS

1. GMOs were first obtained in 1973, demonstrating that transfer of genetic information can far exceed the boundaries of the species, subsequently the transgenic plants in many regions of the world;
2. In the 80's were first technology of genetic modification of crops applied using bacteria that provided the host plant gene of interest;
3. Opponents of new technologies and promoting plant breeding challenges the negative impact that GMOs may have on human and animal health, economic situation, ecosystems, possible long-term effects;
4. Legislation U.E. for raising consumer confidence has adopted transparent policies of GMOs usage, introducing new concepts concerning the labeling of foods containing GMOs, traceability and monitoring them. EU regulations stipulate labeling of foods containing genetically modified organisms (GMOs), unless the GMO content is due to "contamination" accidental and unintentional and not exceed 0.9% of the basic ingredients. The consequence of this legislation is the need to quantify the presence of material derived from GMOs in food and food ingredients.

REFERENCES

1. Codex Alimentarius – 2003-2004;
2. Maria Duca, Angela Lozan, 2003 – "Aspecte metodologice în testarea plantelor modificate genetic", Chișinău;
3. Maria Duca, Aliona Glijin, Lupașcu V., 2008 – "Aspecte metodologice în testarea plantelor modificate genetic USM";
4. Milică C.I., 1999 – "Biotehnologiile viitorului", Ed. Ion Ionescu de la Brad, Iași;
5. Moldoveanu D., Militaru C., Moldoveanu I., 2001 – "Microbiologie și inginerie", Ed. Fiat Lux București;

6. Programe A.N.S.V.S.A. – Funcționare laboratoare de biologie moleculară. Politici privind monitorizarea OMG.
7. Raicu P., 1997 – “Genetică Moleculară și inginerie genetică”, partea II, București
8. Solcan Carmen, Sisea R., 2012- “Biologie moleculară”, Ed. Ion Ionescu de la Brad, Iași;
9. User Manual, EC, JRC, 2004- “The Analysis of Food Samples for the Presence of GMO “;
10. SR ISO 21569:2005 Foodstuffs – “Methods of analysis for the detection of genetically modified organisms and derived products – Qualitative nucleic acid based methods.”
11. www.info.omg.ro;
12. www.biosaftey.eu;

THE ROLE AND CONTRIBUTION OF DIGITAL RADIOGRAPHY TECHNIQUE IN EVALUATION OF BONE TUMORS IN DOG

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Abstract:*Changes in bone cortex and periosteum can take various forms, and these changes have diagnostic value in identifying the etiology of localized bone lesions. Radiological identifying the affected bone is of great importance helping to establish the etiology of the condition.*

The aim of this work is to compare radiographic images obtained by conventional (analogue) X-ray radiography and images obtained by digital radiographic technique to establish the relevance and contribution of digital radiography to the veterinary imaging diagnostic. Using digital radiography has primarily technical advantages, allowing post-processing of the obtained images for a better quality. The comparative assessment of the two modes of radiographic capture will analyze changes in the periosteum and bone cortex in bones localized tumors in dogs.

Keywords: *direct digital radiography, analogue radiography, bone tumors, dog*

BACKGROUND

The relevance of diagnostic in veterinary medical imaging is given by the quality of the imaging obtained after the X-ray beam is transmitted through patient's body. In radiography the X-ray beam signal could be captured on a film, which is the case of analogue radiography, or could be detected and processed by a digital detector (Korner et al., 2007). The digital radiography varies depending of the digital system used (Oborska-Kumaszyńska and Wiśniewska-Kubka, 2010). The digital signal can be detected using Flat Panel Detectors (FPD) or High-density Line-scan Detectors (HLD) (Adachi et al., 2000). The digital radiography brings new technological solution that increase the quality of the images obtained. The possibility of post-processing the acquired image help put in evidence details that usually are not evident on an analog radiography (Mattoon, 2006, Widmer, 2008).

MATERIAL AND METHODS

For assessing the relevance of digital radiographic technique, we have taken in study 8 images of bone tumors obtained by analog radiography techniques, and 8 images of bone tumor obtained by digital radiography. The analog images were stored on AGFA radiologic film, after that the images were put on negatoscope and were photographed, using the same condition of light and same parameters, to obtain a digital image.

The digital radiographies were acquired and processed using a FTD with Csl : Tl / Gd2O2S:Tb scintilator and TFT (thin film transistors) for signal reading and conversion. The active pixels for the detector were 3,328 x 3,328px and the energy range between 40-150 kV. The digital images were processed using VetView VXV software.

All the radiographies were taken using a Temco GRX roentgen diagnostic device. For the study were examined 16 dogs with different type of bones neoplasm. A number of 8 dogs were examined using the analog procedure and the other 8 dogs were examined usxing the digital X-Ray procedure. For each examined region the same parameters and exposure positions were used for both analog and digital radiography. The dogs were of different breads and sex with age between 6 and 12 years.

Radiographic examination aimed at assessing the compact bone, the periosteum and adjacent tissues, position of the lesion on both analog and digital radiography.

RESULT AND DISCUSSION

Changes in bone compact and periosteum can take various forms, and these changes have diagnostic value in identifying the etiology of localized bone lesions. The most common radiologic changes evident in the bone compact and the periosteum are:

- parallel aspect of new bone blades that characterize a progressive slow process, being found in non-invasively lesions and have a non-aggressive character: trauma, infections, benign tumors;
- smooth, well-defined periosteum, in chronic lesions, invasive, the most frequently encountered in the healing of fractures or chronic infections (fig. 8);
- irregular appearance is characteristic in aggressive lesions, active proliferative osteomyelitis, neoplasia, hypertrophic osteopathy (fig. 1, fig. 4, fig. 5);
- radiant appearance is found in malignant lesions, invasive nature, being a relative aspect often seen in the case osteosarcoma (Sullivan and Dale, 2000) (fig. 2, fig. 3).

Analog radiography is influenced by the quality of the film used, the quality of the developing substances and by the characteristics of the film casete. Also there is no possibility to post process the image after acquisition. All of this aspect could produce loss of details on the radiographic image (fig. 1).

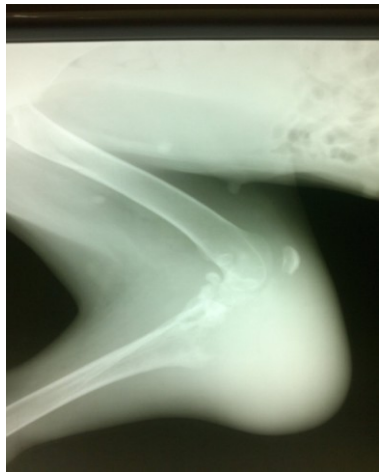

Fig. 1 Knee radiograph, bone lysis and increase of soft tissue opacity, 77kV, 25 mAs, analog

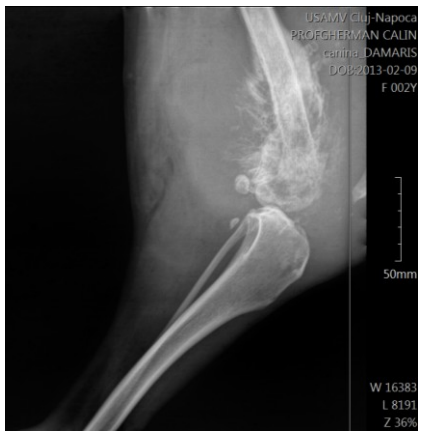

Fig. 2 Radiant periosteal reaction and demineralization of the femoral epiphysis and diaphysis, 77 kV, 25 mAs, digital

Digital radiography allows post processing of acquired images so the radiologist can adjust the intensity and contrast of images to highlight certain changes localized to the soft tissue or to the bone. Digital radiography decreases the risk of overexposure and underexposure of images (Cruz, 2008) (fig. 3, fig. 4, fig. 5).

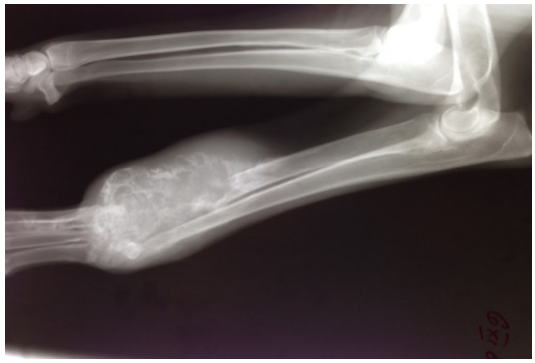

Fig. 3 Tumor process localized to radius and carpal region, 70 kV, 25 mAs, analog

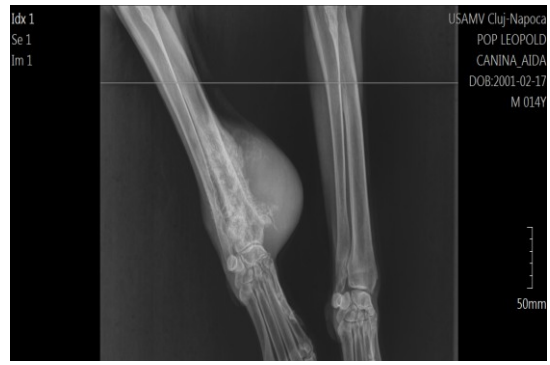

Fig. 4 Proliferative formation on radial epiphysis, 70 kV, 25 mAs, digital

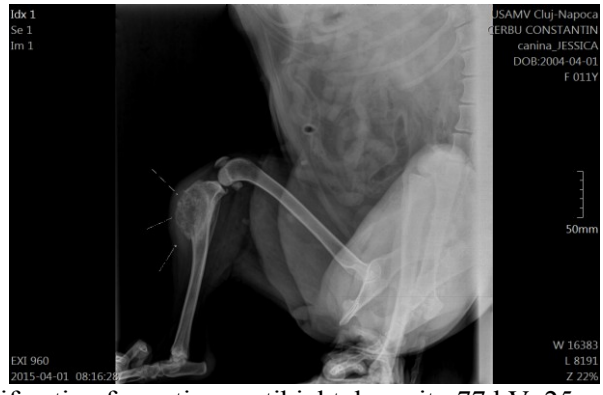

Fig. 5 Proliferative formation on tibial tuberosity 77 kV, 25 mAs, digital

The possibility to obtain a greater number of images in a short time without the need of replacement of exposed film as if conventional radiographs does.

For digital radiography software enables applying digital image filters which increases the quality of images obtained by removing artifacts (fig. 6, fig. 7, fig. 8, fig. 9).

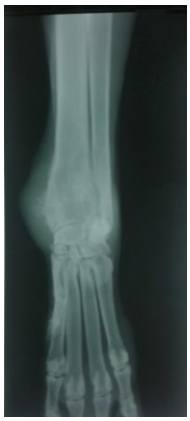

Fig. 6 Periosteal reaction with radiant appearance, 70 kV, 25 mAs, analog

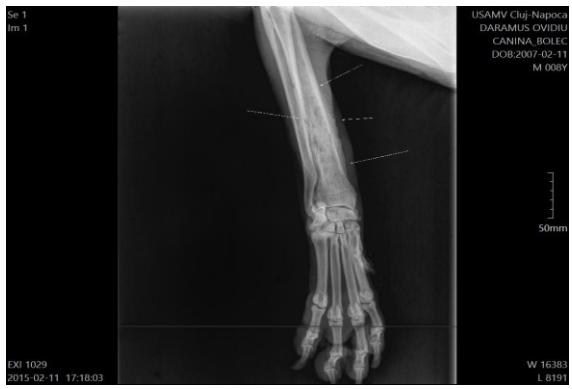

Fig. 7 Demineralization and bone degeneration at diaphyseal radius at 70 kV, 20 mAs. digital

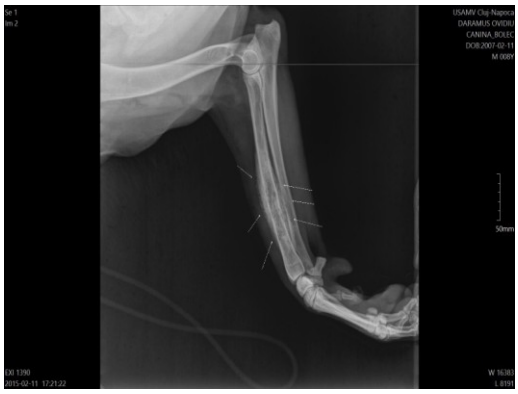

Fig. 8 Proliferation of periosteal bone and demineralization of radius 74 kV, 25 mAs, digital

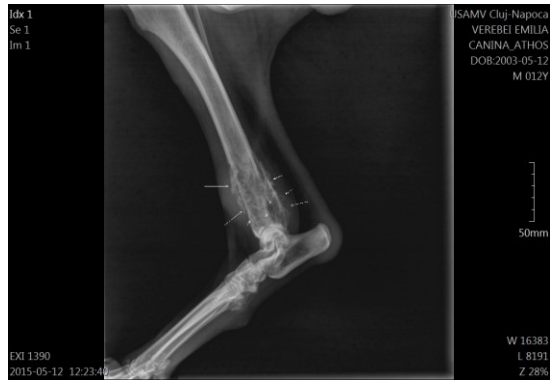

Fig. 9 Lithic proliferative process tibia, 74 kV, 20 mAs, digital

CONCLUSION

The digital radiography examination technique present certain advantages, it reduce the time of radiography acquisition and permit image processing, and the image is output in electronic format. The possibility to acquire a greater number of images in a short time without the need of replacement of exposed film as if conventional radiographs.

Digital radiography decreases the risk of overexposure and underexposure of images. If conventional radiography, image quality is influenced by both the parameters used, and quality of the solutions used in the process of developing images. This impediment is removed if digital radiography.

Using the same exposure parameters for conventional radiography and digital radiography for the same regions, digital radiographic image quality will be superior to radiographic images obtained by conventional radiography. To play back images high-resolution monitors are used and it is able to faithfully reproduce over 4000 shades of gray, which increases the images quality highlighting aspects that on conventional radiography could not be identified.

Reducing the storage space of radiological films, digital images are stored on magnetic media that reduces the risk of depreciation of images while being extremely easy to

reviewing the acquired images. Dissemination of images to the third parties is easy, the pictures can be stored CD or sent via internet networks.

Also decreases image processing cost, the cost of digital radiography is the same as the cost of a conventional radiography.

Digital radiography increase quality of care and medical research.

REFERENCES

1. Oborska-Kumaszyńska D and Wiśniewska-Kubka S, 2010, Analog and digital systems of imaging in roentgenodiagnostics, Pol J Radiol. 75(2): 73–81.
2. Adachi S., Hori N., Sato K., Tokuda S., Sato T., Uehara K., Izumi Y., Nagata H., Yoshimura Y., Yamada S., 2000, Experimental evaluation of a-Se and CdTe flat-panel x-ray detectors for digital radiography and fluoroscopy, Proc. SPIE 3977, Medical Imaging 2000: Physics of Medical Imaging, 38
3. Sullivan M, Dale Vicki, 2000, Small animal diagnostic imaging to the Manual of small animal diagnostic imaging, BSAVA.
4. Cruz R., 2008, Digital radiography, image archiving and image display: Practical tips, Can Vet J. Nov; 49(11): 1122–1123.
5. Mattoon JS. 2006, Digital radiography. Vet Comp Orthop Traumatol., 19:123–132.
6. Widmer W. 2008,Acquisition hardware for digital imaging. Vet Radiol Ultrasound., 49:S2–8.
7. Korner M, Weber CH, Wirth S, Pfeifer K, Reiser M, Treitl M. 2007, Advances in digital radiography: Physical principles and system overview. Radiographics, 27:675–686

URINARY INFECTION PRODUCED BY ESBL -POSITIVE *ESCHERICHIA COLI* IN MALE CATS. CASE REPORT

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Abstract:*Infections with Extended-Spectrum betaLactamase-positive microorganism can be serious and life threatening giving to the fact that they are very antibioresistant. The most common germs with this kind of drug resistance are E. coli and Klebsiella ssp. This study followed not only the clinical side effects of ESBL-positive E. coli cystitis, but also the possibility of spreading this germ to people and other inhabiting pets.*

Two Domestic Shorthair male cat was presented at the Internal Medicine Clinic of the Faculty of Veterinary Medicine Iasi,with recurrent cystitis. Urine was collected through sterile catheterization for culture, macroscopic, microscopic and cytological examination. The direct bacteriological examination revealed the presence of Gram negative bacteria. Cultivation of the urinary sediment on selective media and then on Oxoid Briliance ESBL medium, used on the screening of ESBL producing microorganism and the phenotypic confirmation using the combined disc method, revealed a multidrug resistance of the E. coli strain. Furthermore stool samples were analyzed from the inhabiting cat and the owners of the first patient, and revealed the presence of ESBL-positive E. coli in the cat, while the owner's samples were negative.

ESBL-positive Enterobacteriaceae and the pathways of transmission between pets and owners and vice versa, represent a subject of interest for upcoming microbiological research.

Key words: *cystitis, male cat, EBSL-positive Escherichia Coli*

INTRODUCTION

Antibiotics in veterinary medicine are widely used for prophylactic purposes, leading to the emergence of multi-drug resistant bacteria, extended spectrum betalactamase gram negative germs becoming a widely spread concern in human and veterinary medicine across the world

Urinary tract infections are a common pathology in cats, gram negative bacilli pathogens being the most frequently implicated, especially *E. coli*. Clinicians are encountering more often than ever, cases of UTI that are resistant at antibiotics from the first line of defense (Pitout et al, 2005, Falca et al., 2011).

MATERIAL AND METHODS

A three years old Domestic Shorthair male cat was presented at the Internal Medicine Clinic of the Faculty of Veterinary Medicine Iasi with urethral obstruction.

The second Domestic Shorthair male cat, two years old was presented in much worse condition, being hypothermic with a rectal temperature of 35º C.

Routine haematology exam using ABC Hematology Analyzer was made. Serum biochemistry using Cormay Accent chemistry analyzer for blood urea nitrogen, creatinine, phosphorus, potassium, aspartat aminotranspherase (AST) and alanin aminotranspherase (AST) was also made. Abdominal ultrasound examination used the Aquila Pro Vet machine (Esoate Piemedical) with probes of 7,5 and 10 MHz.

Urinary sediment were examined cytologically, using May Grunwald Giemsa stain.

The direct bacteriological examination revealed the presence of Gram negative bacteria, followed by cultivation of the urinary sediment on selective media Levine and Mac Conkey. The last step was the cultivation on Oxoid Briliance ESBL medium, used on the screening of ESBL producing microorganism and the phenotypic confirmation using the combined disc method, which revealed a multidrug resistance of the *E. coli* strain. Furthermore stool samples were analyzed from the inhabiting cat and the owners of the first patient

RESULTS AND DISCUSSIONS

The first patient was alert, mildly hypothermic, with normal heart and lung auscultation. The first course of action was to partially empty the urinary bladder via cystocentesis, followed by sedation with medetomidine in a doses of 0.08 mg/kg. An I.V line was initiated to stabilize the patient. Furthermore the obstruction was relieved by inserting a catheter through the urethra into the bladder.

For the second patient, the owners reported a recurrent cystitis with multiple urethral obstructions in the past three months, which was treated repeatedly with Enrofloxacin, without a urinary culture and antibiogram. In the course o the treatment, the patient received a three days treatment with a first generation cephalosporin, of unknown doses. The cat begun to exhibit signs of dysuria after the urinary diet was changed for another label.

Abdominal ultrasound of the first patient revealed signs of renal congestion and an extremely thickened bladder wall (Fig. 1).

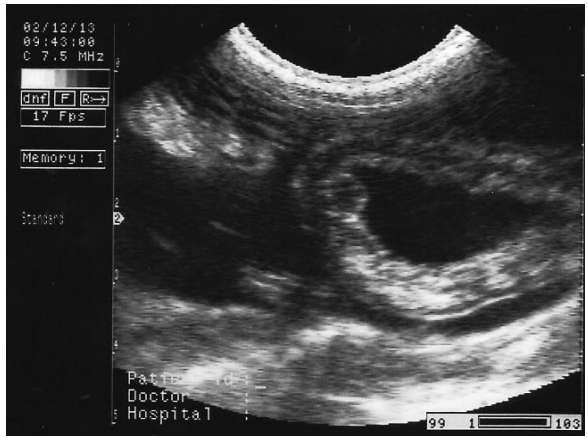

Fig. 1 Thickened bladder wall (cystitis) in a three years old, Domestic Shorthair male cat

Blood biochemistry excluded uremic intoxication or renal failure, but indicated elevated transaminase (AST and ALT). The CBC revealed hemoconcentration and neutrophilia, signs of dehydration and infection.

Considering the fact that the patient had recently ended an antibiotic treatment, the urinary coulter and the antibiogram were irrelevant, not being able to identify the germ causing the urinary tract infection (UTI). The cytological examination of the urine sample

revealed the presence of bacteria, epithelial cells and leukocytes, amorphous debris, struvites (Fig. 2), spermatozoa and erythrocytes.

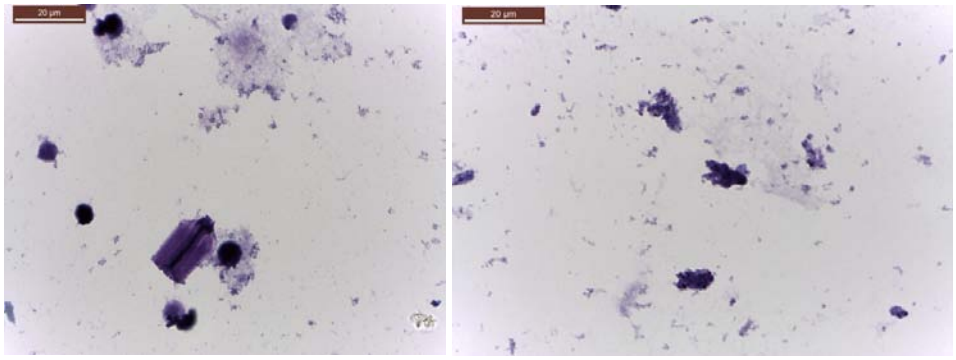

Fig. 2. Urine sediment -presence of struvites crystals, epithelial cells and amorphous debris, cocoid germs and leukocytes. MGG stain

The large number of cocoid germs and leukocytes indicated a bacterial cystitis. Considering the fact that UTI relapse may be the consequence of a multidrug-resistant pathogenic bacterial strain, due to abuse of antibiotics, we considered that the best course of action would be to start a treatment based on supporting the immune system, nonsteroidal anti-inflammatory drug (meloxicam) and high doses of glycosaminoglycans, alongside with closely monitoring of the patient`s general status and renal function, until the urine sample can provide after microbiological exams, the answer for the specific antibiotic treatment.

For the second case, a two years old male cat, abdominal ultrasounds showed a thickened urinary bladder, nephritis and further into the evolution of the diseases the presence of liquid in the abdominal cavity (exsudative peritonitis), suggesting a feline infectious peritonitis (Fig 3).

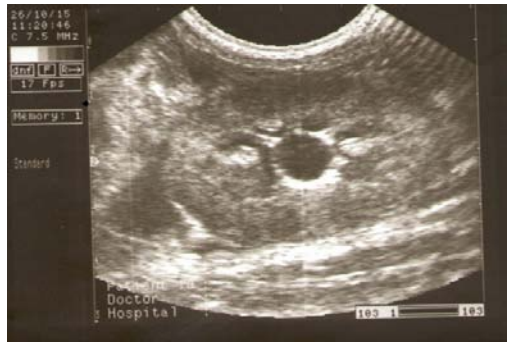

Fig. 4 Chronic nephritis and exsudative peritonitis,
male cat, Domestic Shorthair, 2 years old

Blood biochemistry revealed a stage IV acute renal failure: elevated creatinine (5,4 mg/dL), blood urea nitrogen (220 mg/dL) and phosphoremia (7,6 mg/dL). Treatment was initiated, a urinary catheter was inserted, enrofloxacin was administered giving the fact that according to the owner’s statement the cat has never received antibiotics of any kind, and the specific medication for renal failure, such as phosphorus binders, renal diet and I.V. fluid administration.

After urine sample collection cytological examination revealed the presence cellular casts and neutrophilia, which are signs of infection with renal involvement also (Fig 4).

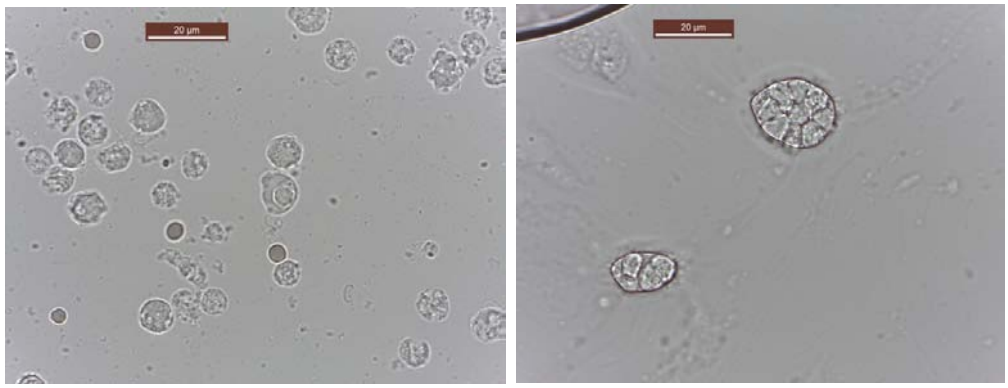

Fig. 5 Neutrophils, epithelial cells and cellular casts in urine sample, male cat, Domestic Shorthair, 2 years old

In the seventh day of antibiotics, the patient had a fever spike, with a body temperature of 40.1 º C. The best course of action was considered to add another high spectrum antibiotic to the treatment: amoxicilin/clavulanic acid. The physical state improved, but in another couple of dayss the symptoms reappeared.

After two weeks from the first admission of the first patient, a urine sample was collected again through cystocentesis, and was sent for microbiological exam. To confirm the presence of multiple antibiotic resistant strains, the sample was on special environment Oxoid Brillince ESBL used for screening ESBL strains. Phenotypic confirmation of ESBL strains was achieved by combined discs technique.

After following the protocol of identification, examining the morphological, biochemical and serological features, the strain detected was an *E. coli*, the enteroivazive type (EIEC). The passing of bacterial colonies on Oxoid Brillince ESBL medium and the formation of characteristic colonies, strengthened the suspicion of a ESBL positive strain (Fig 5).

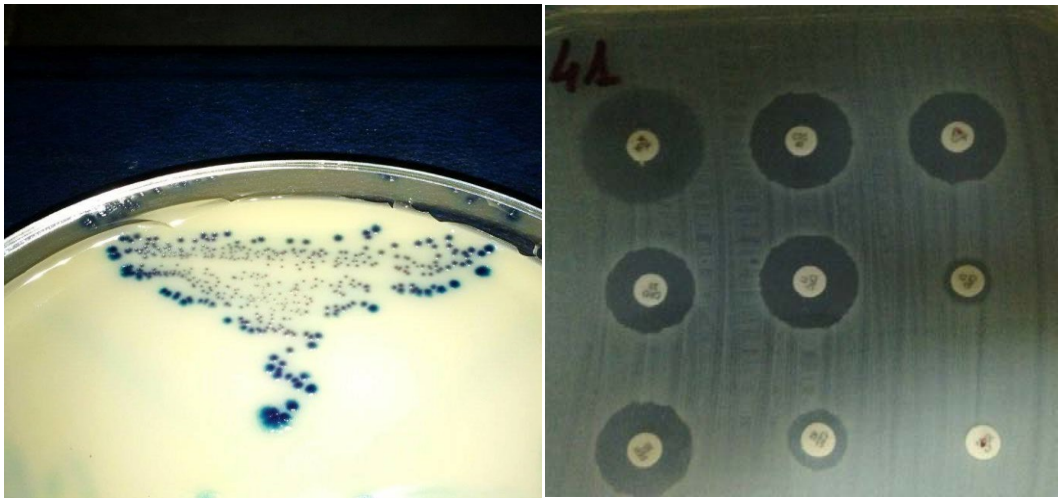

Fig. 5 *E. coli* colonies on Oxoid Brillince ESBL medium and the aspect of the antibiogram

After testing the phenotype resistance, using the combined Discs technique, we found multiple resistance against the following panel of antibiotics: ceftazidime, ceftazidime / clavulanic acid, cefpodoxime, cefpodoxime / clavulanic acid and cefotaxime, cefotaxime / clavulanic acid (CLSI standard 2014).

The owner of the first patient had also a healthy cat at home, with no history of antibiotic therapy, living in the same space and microclimate as the already mentioned patient. Assuming that the cat could be a carrier of a pathogenic strain with multiple antibiotic resistances, the testing of a feces sample from the healthy cat was recommended. After testing the feces sample from the inhabiting cat, we isolated a strain of *E.coli* EIEC with the same spectrum of resistance which has strengthened our suspicion of an phenomenon of in-cross resistance and the elaboration of extended spectrum beta-lactamases enzymes, hypothesis supported by some authors (Hordijk J. et al., 2013) knowing the fact that the administration of fluoroquinolones antibiotics (ciprofloxacin, enrofloxacin) may result in the emergence of drug-resistance and hence the emergence extended spectrum beta-lactamase enzymes (Axel, 2012).

The latest antibiogram for the first patient, revealed a limited number of antibiotics which could be used to treat the recurrent cystitis, among which Meropenem, Imipenem, Piperacilina and Tazobactam, Tobramicin and Nitrofurantoin. The recommended treatement was Nitrofurantoin, 4 mg/kg, for 10 days, after which the clinical sings improved.

The second patient had a more serious multi-drug resistance, being only moderate sensitivity to Chloramphenicol and Lincomycin/Spectinomycin. Beta-lactam antibiotics (penicillins, cephalosporins) as first choice antibiotics in both human and veterinary use, is a major factor in the emergence of ESBL enzymes (Cristina et al., 2012).

Production of extended spectrum beta-lactamases enzymes by Gram negative bacteria poses a threat to public health worldwide. ESBL enzymes are encoded by genes that are located on mobile genetic elements (Mărculescu A., 2007). Therefore, between people and pets, or within the same species can occur horizontal transfer of bacteria carrying resistance genes ESBL (Carattoli et. al, 2008).

CONCLUSIONS

Urinary infections with Extended-Spectrum betaLactamase-positive E. coli was detected in two male cats, one of them having suggestive signs of feline infectious peritonitis coinfection. Confirmation of resistance genes common to both strains will be achieved by PCR and sequencing.

REFERENCES

1. Carattoli A., 2008, *Animal reservoirs for extended spectrum beta-lactamase producers*.,Clin. Microbiol. Infect. 14:117–123.
2. Clinical and Laboratory Standards Institute (CLSI)., 2014, *Performance Standards for Antimicrobial Susceptibility Testing,* M100-S25.
3. Cristina R., Doma O. A., Moneang C. L., 2012, *Use of antibiotics and about quinolones in veterinary therapy*, *Veterinary Drug,* Vol. 6 (2), 4-67.
4. Dalhoff A., 2012, Global Fluoroquinolone Resistance Epidemiology and Implictions for Clinical Use, Interdisciplinary Perspectives on Infectious Diseases, 2012, 1-37

5. Falca C., Solcan G., Mot T., Morar D., Papuc I., Vulpe V., Pop C., Vlagioiu C., Giurgiu G., Mircean M., Braslasu C.M., 2011, *Medicina interna a animalelor*, vol. 2, Ed. Eurostampa, Timișoara
6. Hordijk J., Schoormans A., Kwakernaak M., Duim B., Broens E.,Dierikx C., Mevius D., Wagenaar J-2013, *High prevalence of fecal carriage of extended-spectrum β-lactamase/ AmpC-producing Enterobacteriaceae in cats and dogs*, Frontiers in microbiology, vol. 4, 242/1-5
7. Mărculescu A., Cerenea M., Nueleanu V., Oros N.A., Chereji R., 2007- *Rezistența microbiană față de antibiotice*, Rev. Medicamentul Veterinar/Veterinary Drug Vol.1, No.1, 44-51.
8. P. Damborg , E. M. Broens, B. B. Chomel, S. Guentherx, F. Pasmansk, J. A. Wagenaar, J. S. Weese, L. H. Wielerx, U. Windahl, D. Vanrompay and L. Guardabassi, 2015, *Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspectives for Targeted Research and Policy Actions,* Journal of Compare Pathology,1-14.
9. Pitout J. D., Nordmann P., Laupland K. D., Poirel L., 2005, *Emergence of enterobacteriacae producing extended spectrum β-lactamase (ESBLs) in the community*, Journal of Antimicrobial Chemotherapy, 56, 52-59.

RUPTURE OF PREGNANT UTERUS IN A GOLDEN HEAD LION TAMARIN FEMALE-CASE STUDY

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Abstract:*A 12 years old golden head lion Tamarin female, 126 days pregnant, was brought to consult, with bloody vulvar discharge. This supposed to give birth with at least one day prior to the presentation to our clinic, but showed no prodromal sign, even tough the general state of the animal was considerably modified. The foetus viability was checked by ultrasound examination. After establishing foetal death, due to the risk of aggravation of mother's general condition, performing urgent hysterectomy was decided. During the surgical intervention, uterine rupture with the presence of the foetus with his placenta outside the uterus, in the abdominal cavity was noticed. Thus, ovariohysterectomy was performed after extracting the dead fetus, in order to save the mother's life.*

Keywords*: lion tamarin, uterine rupture, ovariohysterectomy, diagnosis, treatment*

INTRODUCTION

The Lion tamarin is one of the 35 species of small monkeys *Callithrichidae* family, which includes Marmosets and Tamarins. Having a rainforest habitat in the S-E of Brazil, it is a species with a population of about 3,200 specimens in the wild and about 500 of them in captivity. Their habitat has been destroyed due to forest exploitation and agriculture, which gradually led to the isolation of this species and can even lead to its disappearance (Goldizen, 1988).

There are four species of lion tamarin, all of which are endangered: golden lion tamarin (*Leontopithecusrosalia*), golden head lion tamarins (*Leontopithecuschrysomelas*), the black lion tamarin (*Leontopithecuschrysopygus*) and black-headed lion tamarin (*Leonthopithecuscaissara*) (MaLinda, 2013).

The body length of the lion tamarin ranges from 200-336 mm, plus a long tail, weight is about 800g, they are omnivorous animals, diurnal, with seasonal breeding. In the wild, the peak of reproductive activity takes place from late March to mid-June (end of rainy season) and most births take place in the period from September to February (rainy season) (Kingston, 1969). In captivity lion tamarins do not exhibit reproductive seasonality, yet calving are not distributed throughout the entire year. The breeding season lasts from March to September and no calving takes place during winter (Kay et. al., 1996).

Lion tamarin females reach sexual maturity at the age of 15-20 months, but because of social structures, reproduction doesn't occur until the age of 2½ years old. Sexual cycle lasts 29 days, with relatively erased oestrous signs (MaLinda, 2013). Gestation lasts 125 days, the female cyclic activity resuming one month post-partum, but will not get pregnant until the next breeding season. Usually lion Tamarins give birth once a year. He lives up to 8 years in the wild, but can live much longer in captivity. The oldest lion tamarin died at the age of 31 years in a zoo in San Antonio (Tyler et al. 2010).

This case study presents the rupture of the pregnant uterus and ovariohysterectomy in a non-human golden head lion tamarin monkey.

MATERIAL AND METHOD

The patient, a golden head lion tamarin female, aged 12 years, presented at the consultation after reaching gestation period (126 days) with a bloody vulvar discharge since the previous evening. Owners asserted that monkey was at the 7th or 8th gestation, and that each time a few days pre-partum, the abdomen ptosis was commonly noticed and in about 2 days following that, spontaneously birth took place, aspects which were unnoticed this time.

The animal was taken the night before referral to our clinic at a private clinic where radiological and ultrasound examinations were performed, after which a viable foetus was found.

The patient was clinically examined by inspection and palpation, his weight was in the physiological limits of species, 739g, as well as the heart and respiratory rates, the rectal temperature waswithin physiological limits too, 39,2˚C (39,1 ^{o} -40,6 ^{o} C)(Elizabeth, 2015). The general state of the animal was modified, with depression, lack of urination and defecation for 24 hours, no interest to food or water. At the palpation of the ventral aspect of the caudal abdomen, the presence of a hard object was observed, as well as some portions of the foetal body. In order to verify the viability of the foetus, an ultrasound examination was conducted, with a 7,5MHz convex transducer. The gestational sack and the foetus were identified in the mid-inferior abdomen, but no foetus heart beat was found, thus the intrauterine foetal death was established.

RESULTS AND DISCUSSION

Foetal dystocia due to large size compared to the mothers are often found in non-human primate monkeys, so it is particularly important to inform owners about this aspect (Hill, 1969; Norton et al., 2005; Schlabritz-Loutsevitch et al., 2008).

Since most parturitions in this species occur during the night, special attention should be given to females showing prodromal signs during the day, with an increased chance of developing late night dystocia signs (Ford et al., 1998). In this particular case, due to the fact that the patient has experimented each time (7 or 8 times) aeutocicdelivery, the owners did not pay attention to prodromal signs, ignoring the animal's anxiety, weakness and depression.

The dystocia diagnosis is determined by a thorough physical examination, including pelvic canal inspection, supplemented by medical imaging exams. Radiographic examination may provide important data on the size of the litter, the foetal volume compared to maternal pelvic size, data about the positioning of the foetuses, pelvic shape abnormalities due to previous trauma, metabolic bone diseases etc.(Davidson, 2009).

Ultrasound provides data on foetal viability like foetal heart rate frequency and intensity, the presence of some anomalies like hydrocephalus,anasarca, diabetic pregnancy(Plunkett, 2000).Most cases of dystocia in Marmosets and Tamarins require caesarean section treatment (Traas, 2009).

After performing ultrasound and establishing the foetal intrauterine death, because of the animal's visibly modified general condition (depression, anorexia, weakness) and the risk

of dead foetus alteration with repercussions on maternal condition,it was decided toperformhysterotomy.

The animal was placed in the dorsal decubitus position and received pre-operative Atropin 0,04mg/kg s.c., Cefazolin 25mg/kg s.c. and Ketamin 10mg/kg i.m (Fig.1)., anesthesia was maintained on inhaled isoflurane between 1-2%, using inhalation mask for small animals (Fig.2).

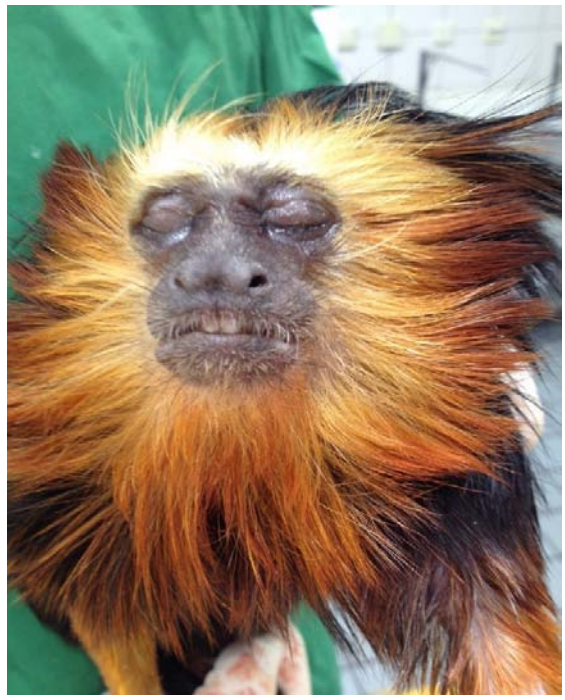


Fig.1. The golden head lion tamarin female-aspect during the preparation for surgery

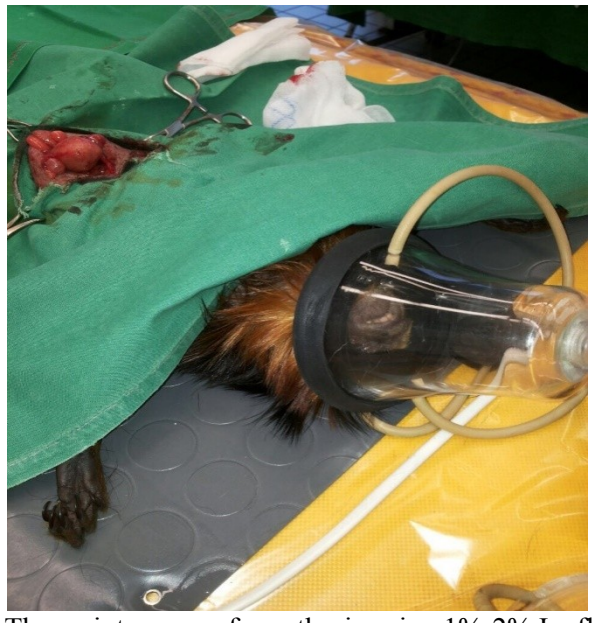
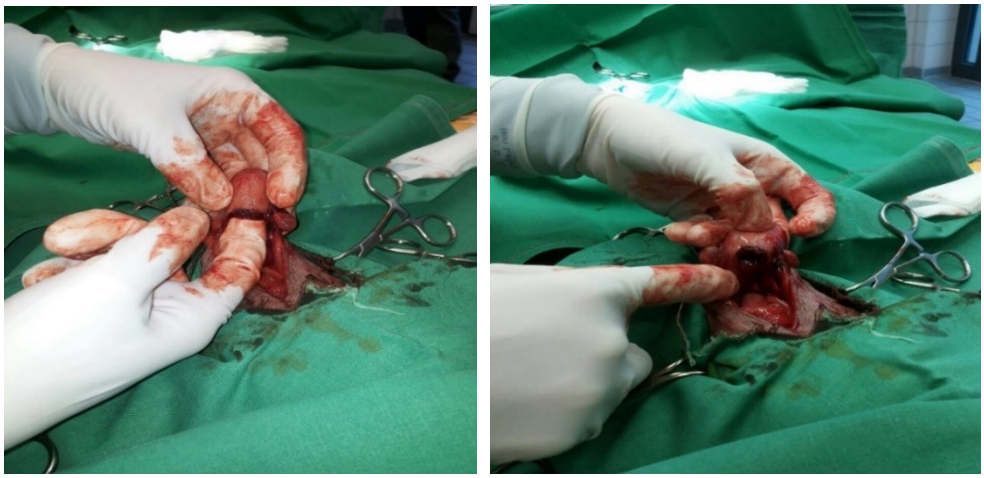


Fig.2. The maintenance of anesthesia using 1%-2% Izoflurane mask-aspect during the surgery

Laparotomy on the lineaalba was performed, the presence of the conceptus was signaled, all together with his placenta, outside the uterus, in the abdominal cavity. The uterus presented an approximately 2cm long rupture, dorsal to the vagina. Perforating wound edges were devoid of vitality, rupture occurred a few hours before surgery, most likely because of the efforts to expel the foetus. No external trauma signs were noticed. Another possible cause of the uterine rupture could be the excessive foetal volume, knowing that the lion Tamarin usually has twin pregnancy, triplets or even quadruplets (Elizabeth, 2015),and when there is only one fetus, dystocia risk greatly increases.

The fetus was extracted, along with the placenta (Fig.4) and ovariohysterectomy operation was performed because the uterus was already very contracted and was showing a significant rip (Fig. 3 (a,b)).



(a) *(b)*

Fig.3. (a,b). The uterine rupture-aspect during the surgery

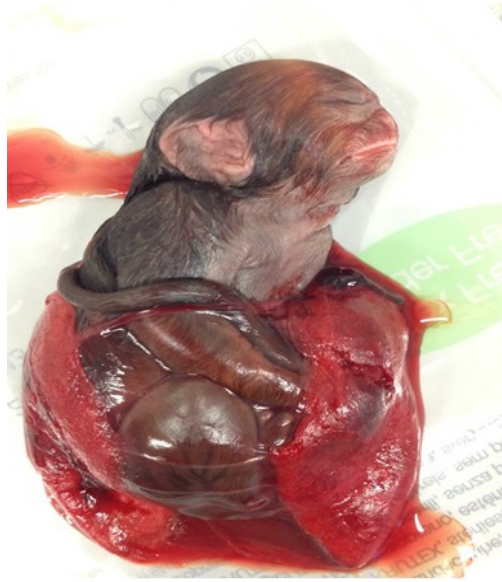


Fig. 4. The lion tamarin dead foetus, male, edematous, along with the bidiscoidal, hemochorial placenta

The abdominal suture was then performed, in a continuous thread with Monosyn 1,5 metric, followed by subcutaneous suture with the same type of thread and ending with intracutaneous skin suture with Monosyn 1,5 metric.

Postoperative treatment consisted in electrolyte rebalancing, antibiotherapy with Vetrimoxin LA and analgesia with Metacam 1.5 mg / ml, also local to the operation wound, we used the aluminum spray.

CONCLUSIONS

Ovariohysterectomy on inguinal way was the optimal treatment in this case, considering that the uterine structure was already seriously affected, as well as the fact that the female was already at her 7^{th} -8^{th} pregnancy. The foetus presented cadaver alterations, being edematous. Most likely the uterine rupture occurred during efforts to expel during labor, at least 12 hours before the presentation to our clinic due to excessivefoetal volume dystocia.

REFERENCES

1. Davidson, A. P., 2009- Dystocia management. In J. D. Bonagura& D. C. Twedt (Eds.), Current Veterinary Therapy XIV (pp. 992e998). St Louis, MO: Saunders/Elsev
2. Elizabeth R. Magden , Keith G. Mansfield, Joe H. Simmons, Christian R. Abee, 2015-Nonhuman Primates in: Laboratory Animal Medicine, Third Edition.
3. Ford, R. B., &Mazzaferro, E. M., 2005- Section 1 Emergency Care. In R. B. Ford & E. M. Mazzaferro (Eds.), Kirk and Bistner's Handbook of Veterinary Procedures and Emergency Treatment (8th ed). (pp. 113e121). St Louis, MO: Saunders/Elsevier.
4. Goldizen A.W., 1988- Tamarin and marmoset mating systems: Unusual flexibility. Trends in Ecology and Environment, 3: 36-40.
5. Hill, W. C. O., 1969- Obstetric mishaps in marmosets. Acta Zool. Pathol. Antverp., 48, 149e154.
6. Kay R.F., Meldrum D.J., 1996- A new small platyrhine from the Miocene of Columbia and the phyletic position of Callitrichidae.
7. Kingston W.R., 1969- - Marmosets and tamarins. Laboratory Animal Handbook, 4:23-250.
8. MaLinda D. Henry, Sarah J, Hankerson,Jennifer M. Siani, Jeffrey A. French, James M. Dietz, 2013- High rates of pregnancy loss by subordinates leads to high reproductive skew in wild golden lion tamarins (*Leontopithecusrosalia*), Hormones and Behavior, Volume 63, Issue 5, May 2013, Pages 675-683.
9. Norton, T. M., Basinger, R. R., Waschak, M., Keller, B., Stedman, N., Quist, C., et al., 2005- Prosimian morbidity, mortality, and medical management on St. Catherines Island: 1990e2005. In: Proc. AAZV, AAWV, AZA/NAG Joint Conf., 148e152.
10. Plunkett, S. J., 2000- Emergency Procedures for the Small Animal Veterinarian (2nd ed.). Edinburgh: W.B. Saunders.
11. Schlabritz-Loutsevitch, N. E., Moore, C. M., Lopez-Alvarenga, J. C., Dunn, B. G., Dudley, D., & Hubbard, G. B., 2008- The baboon model 388 Nonhuman Primates in Biomedical Research (Papiohamadryas) of fetal loss: maternal weight, age, reproductive history, and pregnancy outcome. J. Med. Primatol., 37, 337e345.
12. Traas, A. M., 2009- Surgical management of dystocia. In: Proc. Int. Vet. Emerg. Crit. Care Symp.
13. Tyler Long C., Luong H.R., Mckeon P.G., Megan A., 2010- Uterine Leiomyoma in a Guyanese Squirrel Monkey (Saimiri sciureus sciureus)- J Am Assoc Lab Anim Sci, v 49 (2), 2010 Mar.

EFFECTS OF CONGENITAL HYDROCEPHALUS IN DOGS ON BRAINSTEM AUDITORY EVOKED RESPONSES - BAERs

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Abstract:*Congenital hydrocephalus (CH) is a neurological disorder characterized by the abnormal accumulation of cerebrospinal fluid within the cranium. It is not a specific disease, but rather a multifactorial disorder with a variety of pathophysiological mechanisms. The diagnosis is based on the clinical features and imagistic examination of the brain.BAERs is the electrophysiological test trying to assess the impact of hydrocephalus on activity of the brainstem.*

The aim of this study is to describe the waves' morphology, amplitudes, latencies and intervals, and to evaluate the clinical usefulness of the BAERs in dogs with CH.

The BAERs were recorded in 10 normal control subjects and 13 dogs with congenital hydrocephalus. The electrophysiological evaluation was carried out with the Neuropack S, MEB 9400K electrodiagnostic system (Nihon Kohden) in the auditory brainstem response program (ABR) with surface electrodes. Examination was made under sedation with medetomidine hydrochloride 30µg/kg, inj. i.m.

BAERs examination revealed an increase in the amplitude values of III and V waves, and a prolongation of the I-V interval in all patients, even though they presented different degrees of hydrocephalus. P values obtained were = 0.023 for wave III amplitude, respectively 0.018 for V and 0.032 for I-V interval value. The differences between CH and healthy dogs were rare and did not reach statistical significance criteria (P > 0.05) relating to I, II, III and V waves latencies or I-III, III-V intervals. Statistically significant differences between sex and laterality of ear stimulated were no found in both groups.

In conclusion, BAERs gives valuable information about the severity of brainstem changes induced by hydrocephalus. BAERs recorded with surface electrodes characterized by increase of amplitudes without changes in latencies of the waves may show an impairment of strength between the nuclei of the auditory pathway.

Keywords: *brainstem auditory evoked responses, congenital hydrocephalus, dog, electrophysiology*

INTRODUCTION

In dogs, hydrocephalus is a common congenital or acquired neurological disorder characterized by the abnormal accumulation of cerebrospinal fluid within the cranium. It is not a specific disease, but rather a multifactorial disorder with a variety of pathophysiological mechanisms (Thomas 2010; Hecht and Adams 2010;Shihab, 2011).

CH is more frequentin toy-breed dogs such as the Chihuahua, Toy poodle, Boston terrier, Maltese, English bulldog, Pug, Pomeranian, Yorkshire terrier, and Pekingese (Vullo et al. 1997; Thomas 1999; Esteve-Ratsch et al. 2001; Ohlerth and Scharf, 2007; Woo et al. 2010).The causes of CH are different and include genetic factors, intrauterine or prenatal infection,developmental anomalies or bleeding in the brain (Thomas 1999). Thomas et al (1999, 2010) has revealed that CH,also occurs secondary to a diverse range of nervous system abnormalities such as meningomyelocele, Chiari malformation, Dandy-Walker syndrome or cerebral hypoplasia.

Hydrocephalic dogs show altered mental states ranging from depression to hyperexcitability, disturbed consciousness, incoordination, circling, seizures, as well as symptoms such as dilated and fixed pupils, visual and auditory impairment, blindness,

ventroor ventrolateral strabismus and abnormal shape of the skull. Some of them also exhibit no overt clinical signs at all (Vullo et al. 1997; Adamiaket al. 2012)

Hydrocephalus is diagnosed through clinical neurological evaluation and by using cranial imaging techniques such as ultrasonography, computer tomography, magnetic resonance imaging or pressure-monitoring techniques.BAERs is the electrophysiological test trying to assess the impact of hydrocephalus on activity of the brainstem. The BAERs test is a noninvasive investigation and can be reliably repeated without limits. It allows monitoring and the evolution of hydrocephalus in time, and it is not cost-prohibitive.

Until now, BAERs traces in congenital hydrocephalus were described only in children. The aim of this study is to describe the waves' morphology, amplitudes, latencies and intervals, and to evaluate the clinical usefulness of the BAERs in dogs with CH. To our knowledge, this is the first report of BAERs recording in dogs, using surface electrodes.

MATERIAL AND METHODS

The BAERs were recorded in 10 normal control subjects and 13 dogs with congenital hydrocephalus. The study was conducted at the Faculty of Veterinary Medicine, Department of Clinical Sciences, Internal Medicine (Neurology), and was performed in accordance with the guidelines and upon approval of the Animal Care Committee of the University of Agricultural Sciences and Veterinary Medicine, Iasi, Romania.

Clinical examination were followed by transfontanelar ultrasound exam using an Aquilla Pro Vet machine with probes of 7,5MHz The electrophysiological evaluation was carried out with the Neuropack S, MEB 9400K electrodiagnostic system (Nihon Kohden) in the auditory brainstem response program (ABR). Examination was made under sedation with medetomidine hydrochloride (Domitor, Pfizer, Finland) in dose of 0.03 mg kg, inj. i.m. The dogs were positioned in sternal recumbency on a padded table in a sound-attenuated room.

The waves were recorded with circular surface electrodes. The active electrode was placed at the vertex, the negative electrodes just rostral to the opening of the ear canal and the grounding retrooccipitally, on the median line. The area on which the electrodes were placed was trimmed, degreased with Skin Pure NIHON KOHDEN and covered with special adhesive paste (EEG Paste Elefix® NIHON KOHDEN). Impedance of electrode was kept below 5 Ω. Alternating click stimuli of 0.1 ms were issued by an audio headset type device inserted into the auditory channel. These through earphones were used to provide a better seal in the external auditory decreasing external artefacts and avoiding ear canal collapse. Monaural and binaural stimulation were performed at the intensity of the stimulus of 90 decibels sound pressure level (dB SPL). Contralateral ear was masked with pure white noise 40 dB below that of BAERs stimulus. The sound stimulus was 1000 times repeated and averaged (Venkervan Haagen et al. 1989), using a High-cut filter of 100 Hz and a Low-cut filter of 3000 Hz (Kawasaki and Inada, 1994; Arnold, 2007). A minimal of 2 series with 1000 stimuli each were averaged for response reproducibility, in order to identify any differences. The average of those two examinations was considered the final value. Artifactual data were automatically rejected; the tests were repeated when rejected waveforms represented more than 5% of the average. The statistical interpretation of the results was made with the software Statistical Package for the Social Sciences for Windows (SPSS) 20. Wilcoxon Signed Ranks Test for 2 paired samples was used to determine the presence/absence of

statistical differences between the twoears (right and left) or between an ear (right or left) and binaural stimulation in the two groups. The significance threshold was $P < 0.05$.

RESULTS AND DISCUSSIONS

Congenital hydrocephalus was present in 13 dogs (8 males and 5 females), 9 of them belonging to purebreeds (Bichon, Siberian Husky, Chihuahua, Akita Innu, French Bulldog, Poodle, Yorkshire Terrier, King Charles Cavalier, Pug) recognized as having an increased risk in the development of this pathology (de Lahunta 2009; Thomas 2010) and four dogs were crossbreed. Dogs' ages ranged from 2 months to 6 years, 12 dogs being less than 7 months,and only one had 6 years. Regarding the age of appearance of clinical signs, it is known that HC is most often diagnosed in the first months of life (Podell2002; Dewey 2008; Lorenz et al. 2011),but some dogs can develop specific neurological signs of this disease in adulthood. Shihab et al. (2011) in a study that involved treatment of CH by ventriculo-peritoneal shunt reported that hydrocephalic dog can reach the age of 9 years (111 months). An epizootic study on 564 dogs showed that over 30% of patients diagnosed with HC were older than 2 years (Selby et al. 1979).

Neurological deficits were seen in 11 of 13 dogs. Thus, macrocephaly (fig. 1) was encountered in five dogs, also ascertaining thefronto-parietal fontanelle persistence. This can be explained by the appearance of hydrocephalus before ossification of skull (Platt 2004; Thomas 2010; MacKillop 2011). Ultrasound exam revealed different degree of dilation of the lateral ventricles and of the third ventricle, cerebral parenchyma being represented by a thin layer in the cerebral falx (Fig. 2). Cerebrospinal liquid in some cases was rich in hyperechoic suspended particles. Brain parenchyma might be viewed as a hyperechoic band in the cerebral falx. In 8 from 13 dogs was observed a ventrolateral strabismus, called "sun sunset", caused by the deformation of the head and orbits (fig. 3).

Figure 1. Chihuahua dog, female, 2.5 month-old
Congenital hydrocephalus. Macrocephaly

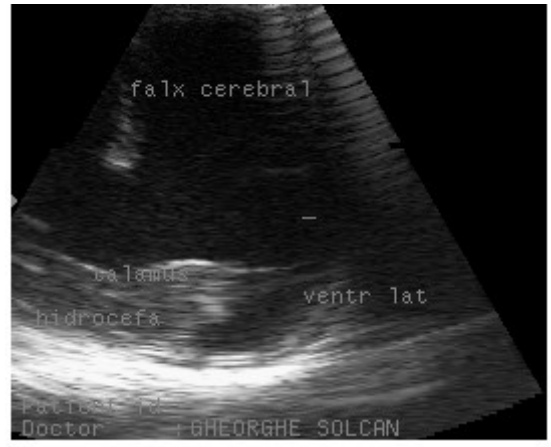

Figure 2. Pug, male, 3 month-old. Transcranial ultrasound through the open fontanels. Major dilation of the lateral ventricles and of the third ventricle, cerebral parenchyma represented by a thin layer in the cerebral falx. CSF rich in hyperechoic suspended particles. Severe congenital hydrocephalus.

Figure 3. Yorkshire dog, two month-old, female. Ventrolateral strabismus left eye.

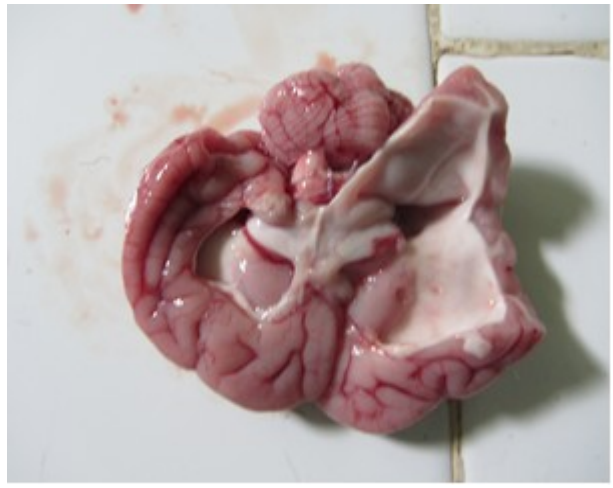

Figure 4. Four month-old crossbreed dog. Severely dilated cerebral ventricles, brain parenchyma reduced to 2-3 mm in thickness.

Behavior and consciousness changes were observed in 10 dogs and epileptic seizures were presented in 8 patients. 7 dogs showed signs of brainstem, too. Thus, the manege was present in 7 patients (3 being on the right and 4 on the left side), torticollis on the right side in 2 dogs and bilateral nystagmus in 5 patients.

5 patients in the study were euthanized. Necropsy examination revealed dilated cerebral ventricleswith reduced brain parenchymato 2-3 mm in thickness (fig. 4), without evidence of the presence of obstacles in the CSF circulation.

In a study on hydrocephalicYorkshire Terrierdogs conducted by Woo (2010), ventrolateral strabismus occurred only in severe hydrocephalus, when ventricular volumewas higher by10% of brain volume. In 5 of patients with strabismus was noticed bilateral blindness with the presence of pupillary reflexes.

BAERs testing was performed on 13 dogs with CH. In all patients, BAERs examination revealed an increase in the amplitude values of III and V waves, and a prolongation of the I-V interval in all patients, even though they presented different degrees of hydrocephalus (Table 1). P values obtained were = 0.023 for wave III amplitude (fig. 5), respectively 0.018 for V (fig. 6) and 0.032 for I-V interval value (fig. 7).

Table 1.

Mean values of BAERsamplitudes and intervals in hydrocephalic and healthydogs

AMPLITUDES (μV)	***I***	***II***	***III***	***V***
Healthy dogs	2.14±0.35	2.20±0.56	2.44±0.51	2.46±0.9
Hydrocephalus dogs	2.12±0.27	2.17±0.46	4.56±0.45	5.89±0.7

INTERVALS (ms)	*I-III*	*III-V*	*I-V*
Healthy dogs	2.002±0.05	1.80±0.08	2.9±0.5
Hydrocephalus dogs	2.10±0.34	1.87±0.06	4.14±0.3

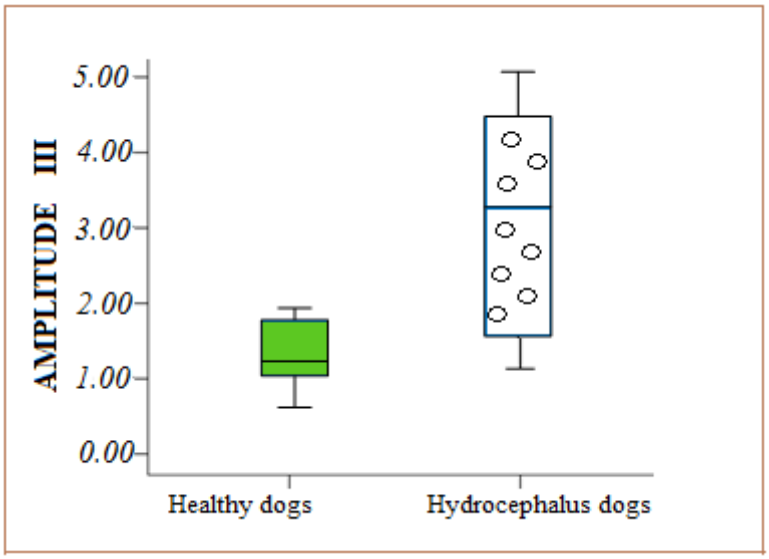

Figure 5. Values of the amplitudes of wave III obtained in healthy vs hydrocephalic dogs for an intensity of sound of 90 dBSPL.

Increasing of the amplitudes of the waves III and V reflects increased activity of the neurons from olivary complex located in the upper and lower colliculus, activity probably due to injuries descending auditory pathways in the brain. Although there have been less studied in the veterinary medicine, Mulders et al. (2000) have demonstrated in rats that descending auditory pathways from the auditory cortex to olivary complex they areextended to the nuclei olivocochlear terminating at the cochlea, and assume that the same thing happens to the dog, thus explaining BAERs anomalies recorded.

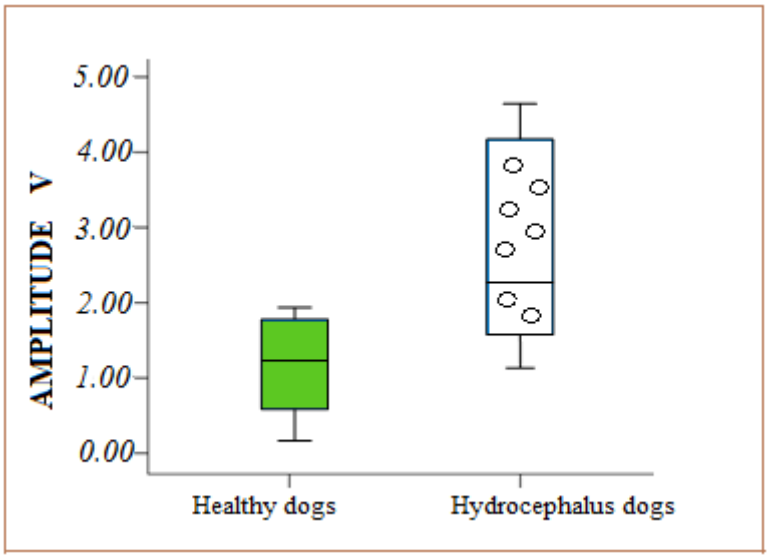

Figure 6. Values of the amplitudes of wave V obtained in healthy vs hydrocephalic dogs for an intensity of sound of 90 dBSPL.

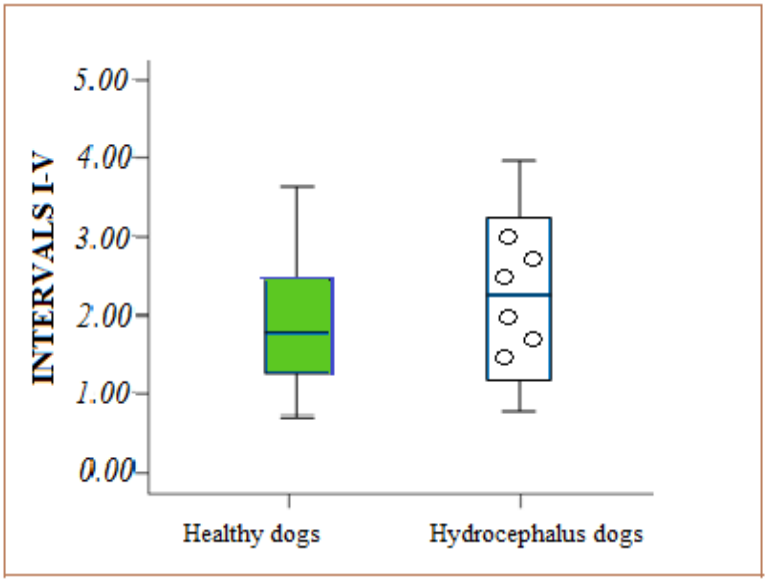

Figure 7. BAERs testing performed on the healthy and hydrocephalicdogs.Values of the intervals I-V obtained for an intensity of sound of 90 dBSPL.

Though in all patients with hydrocephalus auditory pathways are affected, the mechanism by which the brainstem is involved remains open to speculations.A direct cause of brain stem functionsdisturbanceappear to be represented by increased intracranial pressure. Nagao et al., (1979)experimentally, showed interruption of neuronal activity in the rostral brainstem by increasing the intracranial pressure in cats, reporting an increase of the amplitude of III and V waves, and a prolongation of the I-V intervals. Increasing of the intracranial pressure induce cerebral ischemic lesions on the brainstem by altering blood flow to the penetrating vessels of the basilar or posterior cerebral artery.

The differences between CH and healthy dogs were rare and did not reach statistical significance criterion ($P > 0.05$) relating to I, II, III and V waves latencies or I-III, III-V intervals. Statistically significant differences between sex and laterality of ear stimulated were no found in both groups.

CONCLUSIONS

In dogs with congenital hydrocephalus, the BAERs test showed values for the amplitudes of waves III and V, and interval I-V higher than reference ones, regardless of the degree of hydrocephalus. These resultscan be attributed to the inhibition of neurons from the auditory cortex or that of descending auditory pathways.

Always, the BAERs results should be interpreted in conjunction with other exams: neurological, blood, cerebrospinal fluid; ultrasound, radiographic, magnetic resonance or computer tomography.

ACKNOWLEDGMENT - *this paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES:

1. Adamiak Z, Jaskolska M, Pomianowski A, 2012.Lowfield Magnetic Resonance Imaging of canine hydrocephalus. Pakistan Veterinary Journal 32, 128–130.
2. Arnold S, 2007. The auditory brainstem response. In: R. Roeser, H. Hosford-Dunn, & M. Valente's (Eds.) Auditory: Diagnosis, treatment strategies, and practice management (2nd ed.). New York: Thieme-Medical Publishers.
3. De Lahunta A, 2009. Veterinary neuroanatomy and clinical neurology, Third edition, W.B. Saunders, Philadelphia.
4. Dewey W.C, 2008. A practical guide to canine and feline neurology, Second edition, Wiley-Blackwell, Iowa, U.S.A.
5. Esteve-Ratsch B, Kneissl S, Gabler C, 2001.Comperativeevaluation of the ventricles in the Yorkshire Terrier andthe German Shepherd dog using low-field MRI. VeterinaryRadiology and Ultrasound 42, 410–413.
6. Hecht S, Adams WH, 2010. MRI of brain disease in veterinarypatients. Part 1: Basic principles and congenitalbrain disorders. Veterinary Clinic Small Animal 40, 21–38.
7. Kawasaki Y, Inada S, 1994. PAEks of brainstem auditory evoked potentials in dogs. Vet. Res. Commun.;18:383–396.
8. Lorenz DM, Coates JR., Kent M, 2011. Handbook of veterinary neurology, Fifth edition,.Ed.Sounders, Philadelphia.
9. MacKillop E, 2011. Magnetic resonance imaging of intracranial malformations in dogs and cats Veterinary Radiology & Ultrasound, Vol. 52 (1), S42–S51.
10. Mulders WH, Robertson D, 2000. Evidence for direct cortical innervation of medial olivocochlearneurones in rats. Hear Res, 144:65–72.
11. Nagao S, Roccaforte P, Moody R, 1979. Acute intracranialhypertension and auditory brainstem responses. Part 1. J.Neurosur 8, 51: 669-676.
12. Ohlerth S, Scharf G, 2007. Computed tomography in smallanimals – Basic principles and state of art applications.Veterinary Journal 173, 254–271.
13. Platt R.S, Olby JN, 2004. Canine and feline neurology, 3rd , British Small Animal Veterinary Assoc.
14. Podell M, 2002. Canine and feline hydrocephalus. Standards of care. EmergCrit Care Med;4:1–8.
15. Selby LA, Hayes HM, Becker SV, 1979. Epizootiologic features of canine hydrocephalus. Am J Vet Res;40:411–413.
16. Shihab N, Davies E, Kenny PJ, Loderstedt S, Volk HA, 2011. Treatment of hydrocephalus with ventriculoperitonealshunting in twelve dogs. Veterinary Surgery 40,477–484.
17. Thomas WB, 1999.Nonneoplastic disorders of the brain.Clinical Techniques in Small Animal Practice 14, 125–147.
18. Thomas WB,2010. Hydrocephalus in dogs and cats. VeterinaryClinic Small Animal 40, 143–159.
19. Vullo T, Manzo R, Gomez DG, Deck MDF, Cahill PT,1997.Diagnosis of cerebral ventriculomegaly in normal adultBeagles using quantitative MRI. Veterinary Radiologyand Ultrasound 38, 277–281.
20. Venker-van Haagen AJ, Siemelink RJG, Smoorenburg GF, 1989. Auditory brainstem responses in the normal beagle, Quarterly, 11:3,129-137.
21. Woo DC, Choi CB, Nam JW, Ryu KN, Jahng GH, Lee SH,Lee DW, Kim HY, Kim HY, Ahn KJ, Choe BY,2010. Quantitative analysis of fydrocephalic ventricular alterationsin Yorkshire terriers using magnetic resonanceimaging. VeterinarniMedicina 55, 125–132.

OBSERVATIONS REGARDING INCIDENCE, CLINICAL AND THERAPEUTIC ASPECTS OF DIGITAL DERMATITIS IN HOLSTEIN COWS

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Abstract:*Digital dermatitis is a worldwide disease causing lameness in cattle, especially when housed. The lesions can be very painful, and affected dairy cows may avoid moving and may stop eating, which lowers milk production, decreases the condition of the body, and can lead to reproductive problems and increased likelihood of culling if it is let untreated from the first signs of disease. The study was performed from January 2013 to June 2015 on 160 dairy Holstein cows (free stall housing) at "Action Felix" dairy farm in Oradea, Bihor county. For detecting and treating different podal diseases clinical exam is performed twice a year (spring and autumn) on all the cows and also when lameness occurs on some cows.The cows were restrained in lateral decubitus using a chute, all hoofs were examined and also the corrective trimming was performed. After clinical examination was performed and on which digital dermatitis occurred two bandage types were performed one with oxytetracycline powder 20% and lincomycin powder 10%.The incidence of digital dermatitis was 40% in the spring decreasing until 20% in autumn of the total number of cows, appearing in different stages of evolution. After treatment was applied the evolution was positive in both procedure, healing occurring in 1 to 4 weeks depending on the severity of the initial lesion.*

Keywords: *cow, digital dermatitis, lameness*

INTRODUCTION

Digital dermatitis (DD) was first described in Italy by Cheli and Mortellaro (1974).

Digital dermatitis is a worldwide disease causing lameness in cattle, especially when housed. Different species of bacteria have been cultured from digital dermatitis lesions, including Fusobacterium spp., Bacteroides spp., Campylobacter spp. and Peptococcus spp. (Ohya et al.), however spirochaetes are the bacteria most commonly associated with digital dermatitis (Walker et al.)

The lesions can be very painful, and affected dairy cows may avoid moving and may stop eating, which lowers milk production, decreases the condition of the body, and can lead to reproductive problems and increased likelihood of culling if it is let untreated from the first signs of disease.

Our objective was to observe the incidence, clinical stages of the disease and try two types of treatment.

MATERIAL AND METHOD

The study was performed from January 2013 to June 2015 on 160 dairy Holstein cows (free stall housing) at "Action Felix" dairy farm in Oradea, Bihor county. Twice a year(spring and autumn) a clinical examination is performed on all the cows, also in this periods corrective hoof trimming is performed and on the cattle with lameness curative trimming is performed.

All the cow were restrained in lateral decubitus using a chute, all hoofs were examined and the ones that presented digital dermatitis were cleaned using sterile gauze, hydrogen peroxide 3 % and treated using topical treatment with oxytetracycline 20% powder (Fig.1.) and lincomycin 10% powder and a wound dressing was applied (Fig.2.).

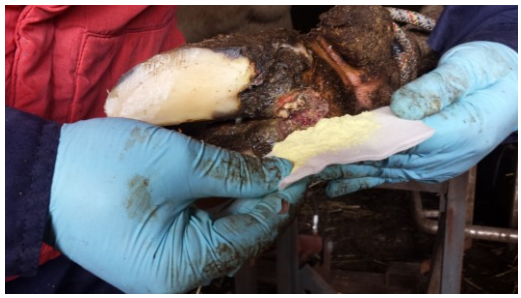

Fig.1. Oxytetracycline powder

Fig.2 Bandage

The wound dressing was changed every two days until healing was achieved.

Digital dermatitis was classified in four stages depending on the degree of the lesion: S0 no lesion; S1 (Fig.3.) lesion smaller than 2 centimeters, incipient lesion, local erythema, lameness stage 1 (Shearer et al.); S2 (Fig.4.) lameness present stage 2-3 (Shearer et al.), lesion bigger then 2 centimeters easily bleeding; S3 (Fig.5.) lameness stage 4-5 (Shearer et al.), hair growing from the lesion and on the lesion surface a septic discharge is present.

Fig.3. S1 digital dermatitis

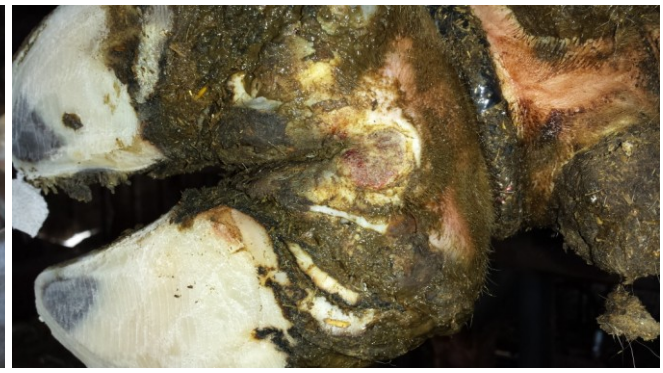

Fig.4. S2 digital dermatitis

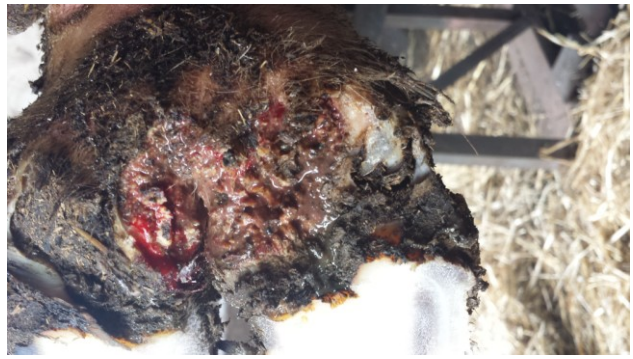

Fig.5. S3 digital dermatitis

RESULTS AND DISCUSSIONS

From the total number of cows on the 2 year study we encountered an incidence of digital dermatitis between 20% and 40% depending on the season (Fig.6), the higher incidence was encountered in the spring of 2013.

Fig.6. Incidence of digital dermatitis

Season/Year	Incidence %	Incidence number of cows
Spring/2013	40,62	65
Autumn/2013	29,37	47
Spring/2014	30,62	49
Autumn/2014	21,25	34
Spring/2015	27.5	44

The increasing incidence in the spring period was attributed to the weather conditions, cold and high humidity, also the farm has an outdoor paddock that the cows can use freely, but in the winter the sole is humid. Other studies also show a higher incidence in winter period (Wells et al., 1999; Laven, 2006.)

We encountered the presence of digital dermatitis in some cases on both hind legs on the same animal.

Regarding the stage of digital dermatitis from the total number of affected cows (239 heads) on the study period this was: S1 – 57 cows (23,84%); S2 – 146 cows (61,08%); S3 – 36 cows (15,06%).

Topical treatment with oxytetracycline 20% powder was performed on 189 cows with different stages and the rest of 50 cows were treated with lincomycin 10% powder also with different stages of the disease, the time needed to heal was between 1 to 4 weeks from the treatment debut (Fig.7.).

Fig.7. Type of treatment and time needed to heal

Stage of DD and treatment	Number of cows treated	Time until healed 7 to 10 days	Time until healed 11 to 20 days	Time until healed 21 to 31 days
S1 oxytetracycline 20%	43	32	11	-
S2 oxytetracycline 20%	122	-	109	13
S3 oxytetracycline 20%	24	-	5	19
S1 lincomycin 10%	14	9	5	-
S2 lincomycin 10%	24	1	21	2
S3 lincomycin 10%	12	-	7	5

CONCLUSIONS

Digital dermatitis has a high rate of incidence in dairy farms causing lameness which causes economical loses for the farmer if it is let untreated.

In our study the incidence was between 20% to 40% decreasing each year due to a better management of the disease.

Both treatments had good results, healing occurring between 7 to 31 days from the beginning of the treatment.

A better education of the farmer and employees in detecting and preventing the disease is recommended.

Hoofs should be kept clean and dry, by maintaining a clean environment, this significantly reducing the incidence and prevalence of digital dermatitis.

Hoof trimming is an action that must be included in the farm program.

REFERENCES

1. Cheli, R., Mortellaro, C.M., 1974. La dermatite digitale del bovine. In: Proceedings 8th International Meeting on Diseases of Cattle, Milan, Italy, pp. 208–213.
2. Laven R.A., Logue D.N., 2006. Treatment strategies for digital dermatitis for the UK. The Veterinary Journal 171, 79–88.
3. Ohya, T., Yamaguchi, H., Nii, Y., Ito, H., 1999. Isolation of Campylobacter sputorum from lesions of papillomatous digital dermatitis in dairy cattle. Veterinary Record 145, 316–318.
4. Shearer J.K., Van Amstel S., Gonzalez A., 2005. Manual of Foot Care in Cattle. W.D Hoards and Sons Company, Fort Atkinson, USA, 16–21 .
5. Walker, R.L., Read, D.H., Loretz, K.J., Nordhausen, R.W., 1995. Spirochaetes isolated from dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Veterinary Microbiology 47, 350–352.
6. Wells S.J., Garber L.P., Wagner B.A., 1999. Papillomatous digital dermatitis and associated risk factors in US dairy herds. Preventive Veterinary Medicine 38, 11–24.

THE INCIDENCE OF *CAMPYLOBACTER* ON BIRD CAECA

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Abstract

In L.S.V.S.A. Iaşi, between 01.03. - 31.12.2014. isolation and bacteriological analysis for Campylobacter spp., 170 caeca samples of poultry were made from six slaughtering units located in three depatrments:Iasi, Bacau and Vaslui, as follows: AG - 40 samples, AT - 41 samples, 10 samples YF-MK-40 samples, RT-41 and VB samples - 28 samples. The strains isolated and confirmed by classical biochemical tests was subsequently confirmed by PCR tests, phenotypic identification and differentiation between Campylobacter coli and Campylobacter jejuni. The incidence obtained was as follows: 39.41% (67 strains) belong to Campylobacter coli, 52.94% (90 strains) of Campylobacter jejuni, and 7.65% strain PCR test became negative.

Keywords: *Campylobacter*; poultry; slaughtering; differentiation PCR; resistance;

INTRODUCTION

Infections and food-poissoning caused by *Campylobacter spp.,* have been identified in numerous countries (Ireland, Netherlands, USA, Russia, etc.). In Romania, this genus has been studied, particularly in the period 1985-1995, by Rusu V. *et al*., Peacock L. *et al*., Niţulescu C., *et al*. manly in study of the incidence in the etiology of animal diseases, and in many cases of disease in humans (2). Lately, it is considered certain involvement of *C. jejuni* in producing Guillain-Barré syndrome in humans (1, 2). They are often involved in human and animal pathology, strains that show a particular high resistance to some antimicrobials, particularly antibiotics (3).

Campylobacter spp., are microaerophilic bacteria, that prefers a concentration of 10% for carbon dioxide and 85% for nitrogen. The absence of one out of the two specified concentrations, inhibits the growth of the bacteria. This species of microorganisms are able to form colonies on solid selective media at a incubation temperature of 41,5˚C, but not at 25°C.

Termotolerant *Campylobacter spp*. has the following characteristics: Gram-negative bacteria that grows at a temperature of 41.5˚C; morphological are slightly curved bacilli; mobility characteristic, positive oxidase, glucose, lactose, and sucrose negative, and does not produce gas hipurat hydrolyzed as described in SR ISO 10 272.

Campylobacter spp. presents mobility and specific biochemical characteristics. Biochemical tests for species differentiation are: sensitivity to nalidixic and cephalothin, detection of indoxyl acetate and sodium hipurat hydrolysis reaction (SR ISO 10 272). It is resistant to cephalexin and in general oxidase and catalase positive (exp. *C. upsaliensis*), often produces hipurat hydrolysis , not urea, (*C. lari*) and does not produce gas.

MATERIALS AND METHODOS

A.N.S.V.S.A. program regarding the monitoring of *Campylobacter coli/jejuni,* from caeca bird, established for L.S.V.S.A. Iaşi, the period of this study respectively, it was carried out between 01.03.2014 and 31.12.2014.

Isolation and bacteriological tests were performed on 170 samples of poultry caeca, from six slaughtering units, considered to be representative from Iași, Bacău and Vaslui county. There have been shipped, received, analyzed - 170 caeca samples, from the following encoded units:

AG - 40 samples AT - 41 samples YF -10 evidence, MK - 40 samples, RT - 41 samples and VB - 28.

Sampling from each designated slaughterhouse was made monthly, one sample/week (4 samples for each month). One sample was constituted by 10 poultry caeca for one lot/day of slaughter.

Working method was established according to standard ISO/TS 10272/2006 (without enrichment stage), supplemented by requirements of the OIE Manual. We used the following culture media: mCCDA, Karmali Agar - obligatory/constant; Preston, Skyrim, CAT, etc. - alternatively; Muller Hinton blood agar, broth/Columbia blood agar, Brucella broth, etc.; Broth thioglycolate - average retention/bird. Incubation at 41,5^{0}C was made in an electronic thermostat – LEEC for concentration control of the O_2(5%), CO_2 (10%), N_2 (80-85%).

Fig. 1. Electronic thermostat - LEEC

For species identification the following tests were required: morphological characterization, micro-aerophyile development and mobility, aerobic development at 25°C and 41,5°C (thermophile strains), hydrolysis of sodium hipurat and indoxyl acetate, sensibility/resistence at nalidixic acid, resistance at cephalotine, positive reaction for oxidase and catalase. Strains confirmation ware made by PCR method at NRL for *Campylobacteriosis in Animals*.

RESULTS AND DISCUSSION

After incubation we observed the development of bacterial colonies with morphological characters specific for *Campylobacter spp.* as follows.

- On mCCD agar fine or medium opac - gray colonies, sometimes with metallic luster, and the tendency to spread (fig. 2, 3).
- On Karmali agar it produces fine, gray, flat and wet, sometimes looking like dew drops colonies, with a tendency to spread (fig. 4).
- On Columbia blood agar fine, gray, non hemolytic or alpha hemolytic colonies (fig. 5).
- Other forms of presumptive *Campylobacter spp.* colonies are: gray opaque, gray with different shades, etc.

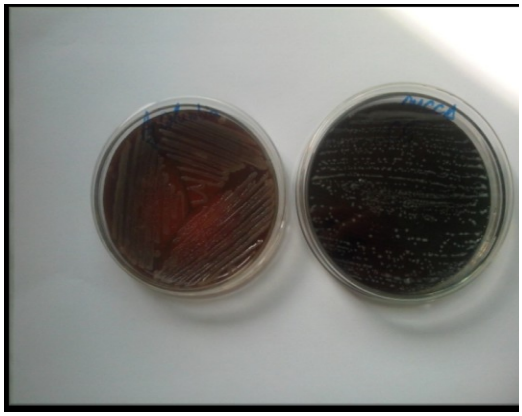

Fig. 2. Prezumtive colonies of *Campylobacter spp.* mCCDA agar- fine or medium opaque - gray colonies, with metallic luster and the tendency to spreading

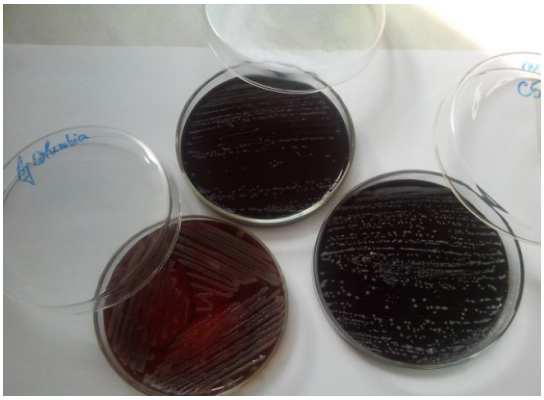

Fig. 3. Fine grey-white colonies in mCCD agar. Abundant colonies of *Campylobacter spp., in Columbia* blood agar

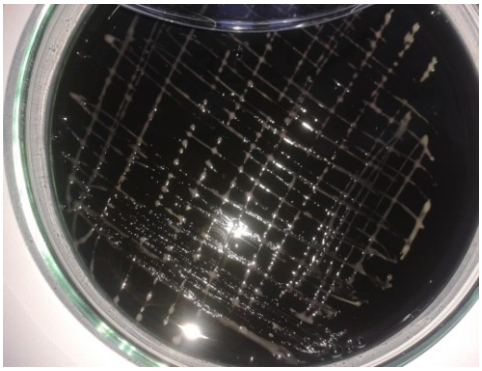

Fig. 4. Fine grey-white colonies of prezumtive *Campylobacter spp.*, in Karmali agar with dewdrops aspect with a tendency to spread

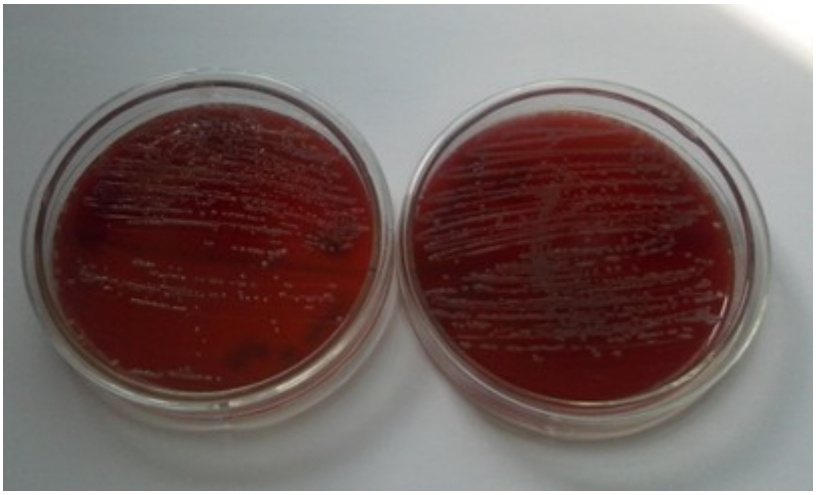

Fig. 5. Fine, gray, non hemolytic or alpha hemolytic of *Campylobacter spp.* colonies on Columbia blood agar

Microscopic examination of possible *Campylobacter spp.* leaded to the identification of specific following morphologic characters: Gram (-), slightly curved bacilli, aspect of flaying seagulls (fig. 5)

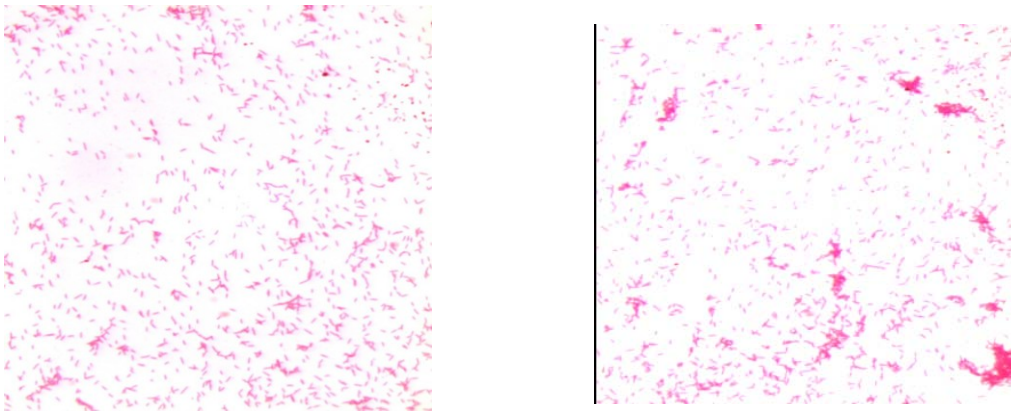

Fig. 5 Morphological characteristics - Gram (-), slightly curved bacilli, aspect of flaying seagulls

The biochemical assays required for species identification, showed a microaerophile development at 41,5^{0}C - with production of characteristic colonies for *C. coli/jejuni* (fig. 6); sodium hipurat hydrolysis give a positive reaction (+) for *C. jejuni* (fig. 7) and a negative reaction(-) for *C. coli*; for both strains, indoxyl acetate hydrolysis, oxidase and catalase give positive reaction (+) (fig. 7).

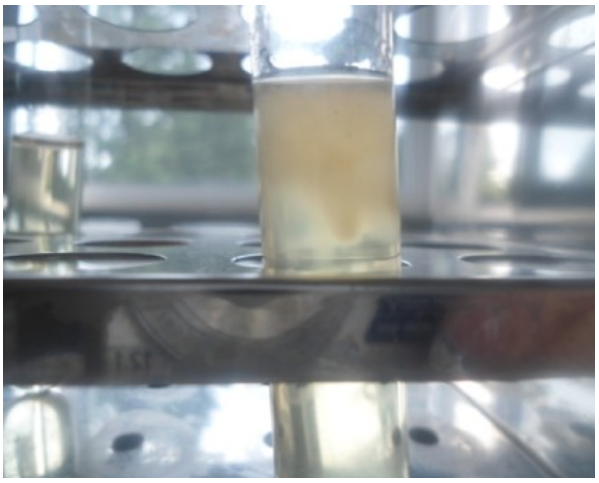

***Fig 6. Campylobacter spp.*, in thioglycolate broth – forming a characteristic superior ring**

Fig. 7 Positive reaction for indoxyl acetate hydrolysis and for oxidase reaction

Out of the 170 examined samples, we isolated 157 strains of *Campylobacter spp.:* 90 (52,94%) strains for, - *C. jejuni, and* 67 (39,41%) strains for *C. coli*. 13 presumptive strains of *Campylobacter spp.,* obtained after incubation in specific medium, could not be confirmed by PCR.

The emergence of resistance to antibiotics some strains of *Campylobacter spp.*, isolated from chicken meat is associated with the use of antimicrobials in feed administrated to increase production and for health protection during growth period.

Results obtained in some studies, regarding the use of antibiotics in raising broilers, allow correlations between the guided use of groups of tetracyclines, cephalosporins, quinolones, macrolides in farm and the incidence of resistant strains of *Campylobacter* spp. isolates from humans with all the difficulties encountered in establishing human therapy for these diseases (4).

Devising strategies regarding the use of antimicrobials in birds (and other animals) to bring to a minimum the appearance of resistant strains capable of producing illness in humans is very difficult, because of *Campylobacter spp.* adaptability. This species we have identified (C. *jejuni* and *C. coli*) to have chromosomally mediated resistentence to erythromycin due to alteration of the ribosome (5). After sequencing of the 23S rRNA genes from resistant *Campylobacter spp.* Trieber and Taylor (1999) identified some mutations, and coreleted to be responsible for resistance fo chlarithromicin (7).

This study capitalizes, isolated and confirmed the presence of *C. coli and C. jejuni* **strains** as part of the National program for Campylobacter spp. control in bird. Studies regarding their resistance to different concentrations (minimum inhibitory concentration -MIC) of antimicrobial substances, used in Europe, in the period 2014-2020 are coordinated by National Reference Laboratory (NRL) and under UE supervision. EURL – AR (E.U. Reference Laboratory – Antimicrobial Resistance) is in charge to provide scientific advice in organization, implementation and evaluation of monitoring schemes for antimicrobial resistance made by LNR's from UE Country.

CONCLUSION

Out of the 170 examined samples 92.35% (157) was positive for *Campylobacter spp.: (*90 for *C. jejuni, and* 67 for *C. coli)* and we raise an alarm regarding animal and human health.

In our concept, antimicrobial specific prevention and treatment measures applied by farmers, are abusive and does not limit *Campylobacter spp.* development, but increase their resistance.

REFERENCES

1. Apostu S., 2006. - "Microbiologia produselor alimentare". Vol. I. Editura „ Risoprint". Cluj-Napoca, pag. 84-90;
2. Bârzoi D., Meica S., Neguț M., 1999 – "Toxiinfecții alimentare". Ed. Diacon Coresi, București, pag. 422-454;
3. Hansson, I., Olsson Engvall, E., Vågsholm, I., Nyman, A. 2010. – „Risk factors associated with the presence of *Campylobacter* positive broiler flocks in Sweden." Prev. Vet. 96: 114 – 121;
4. Pezzotti G., 2003 – "Occurrence and resistance to antibiotics of *Campylobacter Jejuni* and *Campylobacter Coli* in animals and meat in northeastern Italy". *International Journal of Food Microbiology.* Vol. 82, pag 281-287;
5. Programul Național de verificare a *Campylobacter spp*., în cecumurile de pasăre. Nota de serviciu 652-653/2013;
6. Taylor D.E., 1992 - „*Campylobacter jejuni* - current status andfuture trends." Washington: American Society for Microbiology; p. 74-86;
7. Trieber C.A., Taylor D.E., 1999 – „Abstracts and final program of the 10th International Workshop on *Campylobacter*, *Helicobacter* and related organisms." Baltimore:University of Maryland School of Medicine; Abstract CA6. p. 3;
8. Stănescu V. și Apostu S., 2010. "*Igiena, inspecția și siguranța alimentelor de origine animală"*. Vol. I/II Editura „ Risoprint". Cluj-Napoca;

COLECYSTECTOMY WITH BILIODIGESTIVE DERIVATION (FLÖRKEN LATERO-LATERAL CHOLEDOCHO-DUODENO ANASTOMOSIS) IN A SPHYNX CAT WITH COMMUNE BILE DUCT TUMOR *CASE STUDY*

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Abstract:*An 11-year-old 3.1 kg, castrated, female Sphinx cat was referred to the Clinic of the Faculty of Veterinary Medicine Bucharest with acute onset vomiting, loss of appetite, anorexia, faintness, sharp breath, inability to exercise and lethargy. The results from a complete blood (cell) count (CBC) and serum chemical profile, submitted at that time were abnormal. CBC showed anaemia (HCT 19.4% and HGB 6.1g/dL) and high levels of WBC (25.50K/µL) and GRANS (20.40K/µL). The patient had hyperglycaemia (Glu-179mg/dl) and the presence of bilirubin and GGT elevation supported the existence of an extrahepatic biliary obstruction (GGT-18U/L, TBIL-19.1mg/dl). The ALT was 577U/L. The rectal temperature was 36.1ºC, the patient was jaundiced, with mild dehydration (persistent skin fold thickness 2-3 seconds) and mild abdominal pain on abdominal palpation as evidenced by vocalization and splinting of the abdomen.*

Abdominal ultrasound showed a bile duct obstruction due to a 6mm round formation in the commune bile duct, attached to the wall, and abundant sludge in the gallbladder. The established treatment consisted of rehydration and electrolyte balance and parenteral nutrition. The patient was submitted to intravenous fluidotherapy with Sodium Chloride 0.9 %, Aspatofort, Ondansetron, Metoclopramide and Duphalyte, CRI for 3 days.The decision to take the patient to surgery was based primarily on the acute development of clinical signs (vomiting, lethargy, poor appetite) and on the existence of extrahepatic biliary obstruction, despite the risk of high bilirubin levels.We performed a laparotomy focused on the gallbladder and liver to minimize anesthetic time and associated morbidity. We used a complex isoflurane inhalatory protocol for anaesthesia. Colecystectomy with biliodigestive derivation (Flörken latero-lateral choledocho-duodeno anastomosis) was the selected technique, due to the position of the tumor.Post-surgery, the cat was monitored in the hospital for three days. Treatment consisted of fluids, anti-nausea medication (ondansetron, metoclopramide), antibiotics (amoxicillin), and nutritional support via syringe feeding. She was discharged on oral amoxicillin, (12 days) and Denamarin.

A chemistry panel was performed after ten days and it showed the decrease of ALT to 213U/L, GGT was 19U/L, TBIL-1.4mg/dl. At three months after surgery, the complete blood (cell) count (CBC) and serum chemical profile showed normal values (Glu-83mg/dl, GGT-0U/L, TBIL-<0.2mg/dl, ALT-120U/L).

Surgery is the elective treatment in commune bile duct tumor in cats. The condition is an emergency due to the fact that it affects all other organs (heart, lungs, kidneys). Chronic hemolytic anaemia has been linked to bilirubin cholelithiasis.

Key words: *feline, cholecystectomy, bilirubin, choledochoduodenostomy, biliodigestive derivations.*

Total ablation of the gallbladder in cats is an operation that presents a high degree of difficulty and it is performed in severe cases, acute blockage of the cystic duct, as a result of gallbladder neoplasia or other severe inflammatory conditions. An 11-year-old 3.1 kg, castrated, female Sphinx cat was referred to the Clinic of the Faculty of Veterinary Medicine Bucharest for acute onset vomiting, loss of appetite, anorexia, faintness, sharp breath,

inability to exercise and lethargy. The differential diagnoses at this time included hemolytic jaundice, feline infectious peritonitis, and hepatic neoplasia (primary or metastatic).

The results from a complete blood (cell) count (CBC) and serum chemical profile submitted at that time were abnormal. CBC showed anaemia (HCT 19.4% and HGB 6.1g/dL) and high levels of WBC (25.50K/µL) and GRANS (20.40K/µL). The patient had hyperglycaemia (Glu-179mg/dl) and the presence of bilirubin and GGT elevation supportted the existence of an extrahepatic biliary obstruction (GGT-18U/L, TBIL-19.1mg/dl). The ALT was 577U/L. The rectal temperature was 36.1ºC, the patient was jaundiced, with mild dehydration (persistent skin fold thickness 2-3 seconds) and mild abdominal pain on abdominal palpation as evidenced by vocalization and splinting of the abdomen.

Abdominal ultrasound showed a bile duct obstruction due to a 6 mm round formation in the commune bile duct, attached to the wall, and abundant sludge in the gallbladder.

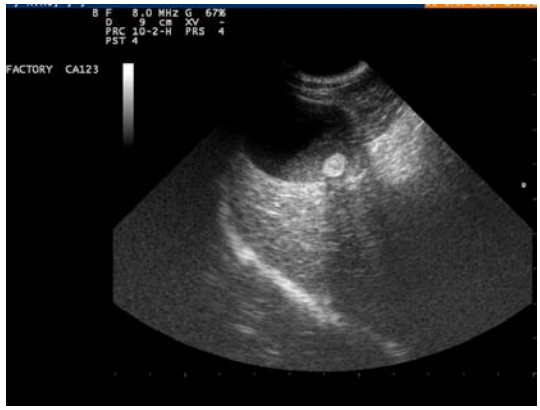

Image 1. Ultrasound aspect of the gallbladder (orig.)

The established treatment consisted of rehydration and electrolyte balance and parenteral nutrition. The patient received intravenous fluido-therapy with Sodium Chloride 0.9 %, Aspatofort, Ondansetron, Metoclopramide and Duphalyte, CRI for 3 days.

The decision to take the patient to surgery was based primarily on the acute development of clinical signs (vomiting, lethargy, poor appetite) and on the existance of an extrahepatic biliary obstruction, despite the risk of high bilirubin level.

We performed a laparotomy focused on the gallbladder and liver to minimize anesthetic time and associated morbidity. Considering our patient anesthetic status (ASA 3 patient) we decided on a balanced anesthesia protocol in order to minimize the risks and to maximize our patient's safety and comfort. The patient was premedicated with Butorphanol 0.2 mg/kg. Induction was performed with propofol (4 mg/kg), while maintenance after intubation, with isoflurane and fentanyl CRI 0.02 µg/kg/min. The patient had a long recovery from anesthesia and was extubated 2 hours after surgery. Considering the duration of the procedure and our patient's breed, the most important complication was hypothermia (lowest value 35.8ºC) despite our efforts to warm it with an electric blanket and warm fluids.

Cholecystectomy with biliodigestive derivation (Flörken latero-lateral choledocho-duodeno anastomosis) was the selected technique, due to the position of the tumor.

The first step was to identify and dissect the gallbladder from the hepatic tissue. We used an electro scalpel in order to also cauterize all the anterior wall of the gallbladder. The common bile duct was enlarged almost 10 times (image 2). Next step was the ligature of the cystic duct. We used 3/0 polydioxanone.

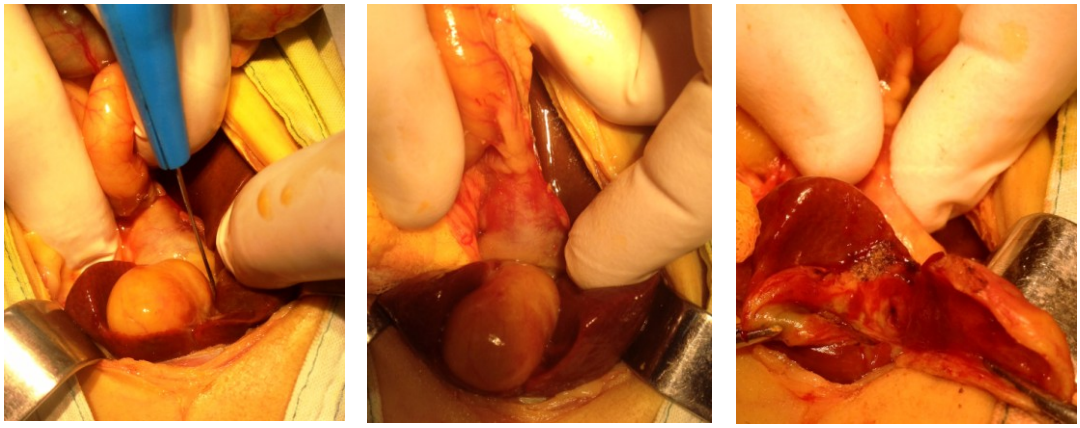

Image 2. Identification and the dissection of the gallbladder from the hepatic tissue (orig.)

Due to the fact that the opening of the common bile duct into the duodenum was placed under doubt and we were unable to catheterize the Oddi sphincter, we decided to open the common bile duct laterally and to open the duodenum on the greater curvature (image 3).

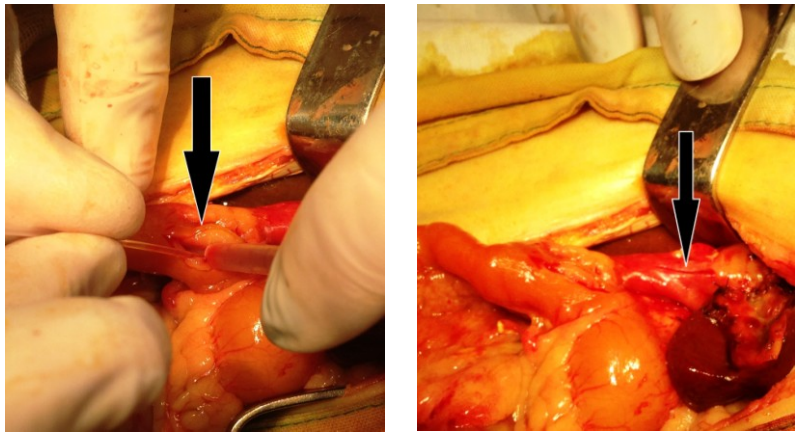

Image 3. Incision of the duodenum on the greater curvature (left) and lateral incision of the common bile duct (right)(orig.)

The final step was to perform the latero-lateral choledocho-duodeno anastomosis using 4/0 polydioxanone. We performed a Schmieden suture to connect the duodenum with the common duct (image 3).

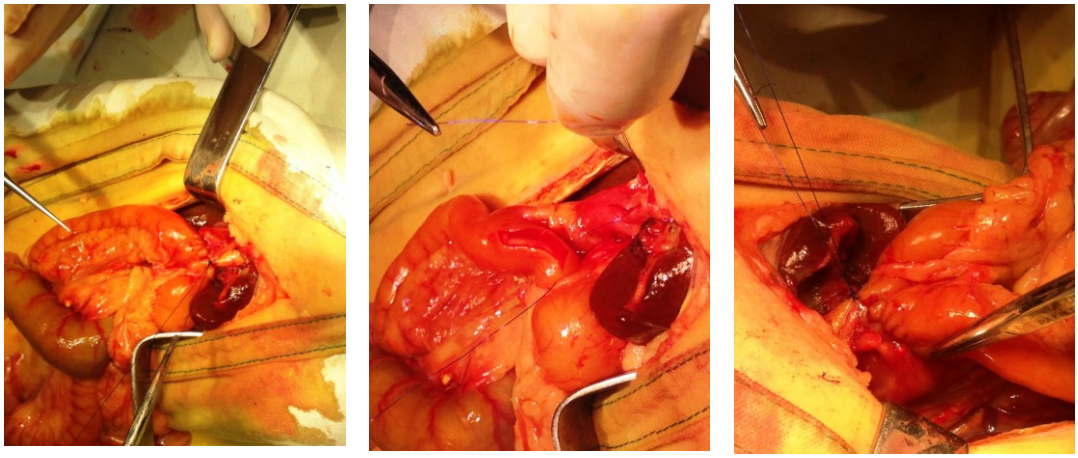

Image 4. Latero-lateral choledocho-duodeno anastomosis using the Schmieden suture (orig.)

The abdominal cavity was closed using a simple Continuous nonlocking suture with 2/0 nylon monofilament. We performed a subcuticular suture to close the skin.

Post-surgery, the patient remained in the hospital under observation for three days. Treatment consisted of fluids, anti-nausea medication (ondansetron, metoclopramide), antibiotics (amoxicillin), and nutritional support via syringe feeding. The patient was discharged on oral amoxicillin, (12 days) and Denamarin. A chemistry panel was performed after ten days and showed the decrease of ALT to 213U/L, and GGT was 19U/L, TBIL-1.4mg/dl. At three months after surgery, the complete blood (cell) count (CBC) and serum chemical profile boasted normal values (Glu-83mg/dl, GGT-0U/L, TBIL-<0.2mg/dl, ALT-120U/L). We performed an ultrasound check 15, 30 and 90 days after surgery and the abdominal cavity and the hepatic tissue had a normal aspect every time.

CONCLUSION

Surgery is the elective treatment in commune bile duct obstruction in cats. This condition is considered an emergency due to the fact that is affects all other organs (heart, lungs and kidneys). Chronic hemolytic anemia has been linked to bilirubin cholelithiasis.

REFERENCES

1. Budras, K. D., McCarthy, P. H., Fricke, W., Renate Richter - Anatomy of the Dog, 2007, Fifth Edition, Schlütersche Verlagsgesellschaft mbH & Co. KG, Hans-Böckler-Allee 7, 30173 Hannover
2. Fossum, Theresa Welch - Small Animal Surgery, Second Edition, 2002, Mosby editure, (ISBN 10: 0323012388 / ISBN 13: 9780323012386)
3. Gallardo-Meza, A. F., González-Sánchez J. M., Villarroel-Cruz R., Piña-Garay M. Á., Medina-Andrade M. Á., De la Barquera A. M., González -Torres L. - Hepatico-duodeno-anastomósis, técnica de elección para el tratamiento de Quiste de Colédoco. Seguimiento a largo plazo. Un estudio interinstitucional, Revista Mexicana de Cirugía Pediátrica, Vol. 16, No. 2, Abril-Junio 2009
4. Morrison, W. B. - Cancer in dogs and cats: medical and surgical management, Second Edition, 2002, ISBN 1-893441-47-4, China
5. North, Susan M., Banks, Tania Ann - Small Animal Oncology: An Introduction, 2009 Elsevier Limited, ISBN: 978-0-7020-2800-7