

## PREFEMORAL OOPHORECTOMY IN RED EARED TERRAPINS (*TRACHEMYS SCRIPTA ELEGANS*)

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### Summary

In captive female chelonians, such as the red eared terrapins (*Trachemys scripta elegans*) reproductive disorders are common pathological findings. These include oophoritis, salpingitis, dystocia, retained or ectopic eggs and follicular stasis.

The purpose of this article is to describe a prefemoral oophorectomy technique for use in *Trachemys scripta elegans*.

Six female turtles (*Trachemis scripta elegans*), weighting an average of 0.97 ( $\pm$  0.25 SD) were presented for oophorectomy in our clinic. After a preoperative examination and the induction of anesthesia, the animals were placed in either right or left lateral recumbency and aseptically prepared for surgery. Acces to the coelomic cavity was obtained with an incision in the prefemoral fossa. After visualisation of the ovary, the vasculature was ligated and the mesovarium was transected and the musculature and skin were sutured.

In 1 turtle a bilateral oophorectomy was achieved through a single incision, in 4 turtles the oophorectomy was performed through both prefemoral fossa and in 1 turtle an unilateral oophorectomy was conducted due to severe hepatic lipidosis and anesthetic risk. All the animals recovered well after surgery. Mean time of the surgical procedure  $\pm$  SD was 86.33  $\pm$  35.12 minutes and mean time to complete recovery  $\pm$  SD was 137.16  $\pm$  44.8 minutes.

The prefemoral oophorectomy technique described here is a safe, practical method for treating disorders of the reproductive tract in the red eared terrapin and can be used elective or curative, as an alternative to plaston osteotomy.

**Key words:** prefemoral oophorectomy, red eared terrapin

Turtles and tortoises can suffer from different reproductive disorders that involve either the ovary in different stages of follicle evolution or the salpinx (4). Some of this pathology could be avoided through oophorectomy.

Although the plaston osteotomy access to the reproductive tract (2, 5) has been successfully used, the prolonged healing time and complications associated with this technique make the use of the prefemoral celiotomy an alternative access to the coelomic cavity (1).

In most chelonian species the prefemoral fossa is relatively small, limiting the exposure and access to the viscera, thus endoscopic assisted techniques have been used for the improvement of manipulation and visualization (3,6). The purpose of this article is to describe a prefemoral oophorectomy technique for use

in *Trachemys scripta elegans* without coelioscopic assistance, with no special equipment necessary for this procedure.

### Materials and methods

Six female turtles (*Trachemis scripta elegans*) were presented for oophorectomy in our clinic. The owner consented to the procedure by signing a written agreement. After a routine preoperative examination, anesthesia was induced, the animals were intubated using different sizes catheters, depending on the size of the patient. For the maintenance of anesthesia, Sevoflurane was used at 3% in oxygen (0,4 l/min) and air (0,6 l/min) and positive-pressure ventilation, at 6 breaths/min was provided, using the SIMV mode of the Drager Cato anesthetic machine. Heart rate was monitored using ECG and when possible ET CO<sub>2</sub>, FiSev were monitored with a capnograph.

The turtles were placed in right lateral recubency on a heated pad, with the left hind limb in extension, to expose the prefemoral region. This region and the surrounding shell were aseptically prepared with Betadine. In the center of the prefemoral fossa, a 2 to 4 cm cranio-caudal skin incision was made and subcutaneous tissue and fat were dissected to expose the transverse and oblique abdominal muscles (Fig.1).



Fig.1. Placement and antisepsia of a red eared terrapin

After exploratory examination of the coelomic cavity, a forceps was used to grasp a less vascularised area of ovarian connective tissue and traction was applied to retract the ovary through the prefemoral incision, until all follicles were visible. The vasculature was then ligated with poliglicolic acid (Surgicryl, 3.0- 5.0, depending on the size of the animal) and the ovary was excised (Fig. 2).

Closure of the coelomic aponeurosis was done with polyglycolic acid in a simple continuous pattern and the skin with 3.0 nylon (Daclon 3.0) in a horizontal mattress. The skin suture wires were extracted one month after surgery (Fig.3).

Postoperatively, antibacterial therapy was instituted for 5 days, using Enrofloxacin at a dose of 5 mg/kg and analgesia using Meloxicam at a 0.2 mg/kg dose.

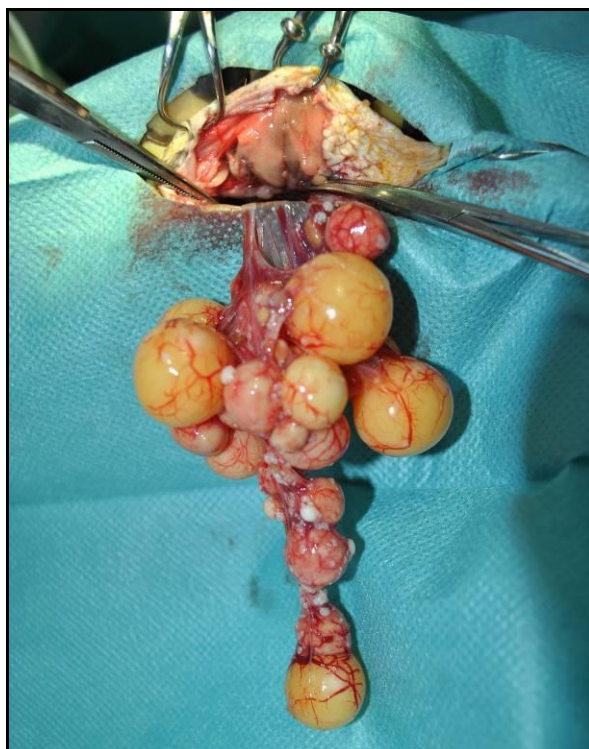


Fig. 2. Clamping of the ovary with follicles in different stages of evolution



Fig. 3. Aspects of the healing process one month after surgery

### Results and discussions

Elective prefemoral oophorectomy was performed on 6 female *Trachemys scripta elegans* turtles, privately owned, weighting a mean  $\pm$  SD  $0.96 \pm 0.25$  kg, but the exact age of the turtles remained unknown.

Results of preoperative hematologic testing were within reference limits. One turtle showed intraoperative macroscopic signs of hepatic lipidosis.

In one case a bilateral ovariectomy was performed through one incision, in the left prefemoral space, this was the largest of all, weighting 1.388 kg. Mean SD surgery time was  $86.33 \pm 35.12$  minutes, mean  $\pm$  SD anesthesia time was  $137.16 \pm 44.8$  minutes and mean  $\pm$  SD recovery time after the skin suture was  $38 \pm 34.05$  minutes, based on the fact that these surgeries were conducted also for student teaching purposes.

All turtles recovered well after surgery and none developed any complications. They were kept outside water for the first 24 h after surgery and started eating the day they were put back in their tanks.

The prefemoral approach to the reproductive tract of chelonians, with no endoscopic assistance was previously described in a loggerhead sea turtle (*Carretta carreta*) by Nutter FB et al. in 2000 (6), but the procedure was considered possible due to the large prefemoral space. We consider this surgical approach possible even in smaller animals, under 1 kg.

Although the coelioscopic assisted procedure (3) offers more visibility and shortens the surgical time, oophorectomy through the prefemoral fossa can be performed with basic surgical equipment, shortening the healing time to 4 weeks,

compared to the 1 to 2 years healing time reported for turtles going through the same procedure with plaston osteotomy approach (5).

### **Conclusions**

The prefemoral oophorectomy procedure described in this article is a safe with minimal risk of postoperative complications technique, that does not need any special equipment and that decreases the healing time to a minimum, compared to the plaston osteotomy approach and can be used successfully for reproductive disorders of the red eared terrapin (*Trachemys scripta elegans*).

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## **CHRONIC KIDNEY DISEASE IN A GERMAN SHEPERD – A CASE STUDY**

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### **Summary**

Chronic kidney disease (CKD) is the most commonly recognized form of kidney disease in dogs and cats. It is defined as any structural and/or functional abnormality of one or both kidneys that has been continuously present for three months or longer (David J. Polzin, 2011). The purpose of this case study was to assess and monitor a 11 year old German shepherd diagnosed with chronic kidney disease over a period of an year based on clinical signs such as vomiting, inapetance, polyuria and polydipsia in conjunction with routine serum biochemistry tests on blood collected by venipuncture in standard tubes and hematological tests on tubes with anticoagulant. Additional examination included urinalysis and ultrasonography. Chronic kidney disease monitoring must consist with regular assessment of blood urea nitrogen, creatinine and total protein serum levels in conjunction with characteristic parameters investigation of the etiopathogenetic factor.

**Key words:** chronic kidney disease, biochemistry, hematology

“Chronic” in the context of CKD means an irreversible and usually progressive loss of kidney function and/or structure. Adaptive compensatory enhancements in kidney function have already reached their nadir, which typically reflects duration of approximately 3 months or longer (4).

Kidneys of dogs and cats with CKD are typically characterized by a permanent reduction in the number of functioning nephrons. Although renal structure and function do not consistently parallel one another, primary kidney diseases usually display evidence of both structural and functional derangements (1).

Once the glomerular filtration rate falls enough to cause the blood urea nitrogen and plasma creatinine concentrations to increase, the diagnosis is straightforward (6).

### **Materials and methods**

A 10 year old German Sheperd was admitted inside the clinic of Bucharest Veterinary Medicine Faculty, in 2010, with hypothermia (37.1 °C), anorexia, vomiting and modified general status.

Blood was collected, by venipuncture, from the cephalic vein in tubes with EDTA for biochemical serum analysis and on tubes without anticoagulant for the complete blood count. Sample examination was carried out immediately after

collection. In some cases, blood samples were collected after treatments with fluids, antiemetic and H<sub>2</sub> receptor antagonist medication. Biochemical and complete blood count analysis was performed inside the Bucharest Veterinary Medicine Laboratory, using an IDEXX VetTest Chemistry Analyzer and respectively an IDEXX VetAutoread Machine.

Initial results shown increased levels in creatinine (9.2 mg/dl), blood urea nitrogen (87 mg/dl), alkaline phosphatase (439 U/L) and total protein (11.6 g/dl) and phosphorous (7.8 mg/dl).

Complete blood count initial results showed decreased levels of hematocrit (33.3%) and hemoglobin (10.7) and increased levels of white blood cells, eosinophils and platelets with value over the device measuring limit.

Thin blood smear microscopic examination, with May Grunwald Giemsa coloring, revealed massive infestation with *Babesia canis*.

Clinical examination of the patient consisted in inspection, lateral and bilateral abdominal palpation of the kidney area and internal temperature appreciation through rectal thermometry as described in specialty literature (7).

Based on clinical signs and patient's history of polyuria and polydipsia in conjunction with results from the biochemical serum analysis and complete blood count, specific babesiosis treatment was instated with fluid therapy with hydroelectrolitic solutions, diuretics, antiemetic, liver protectors, H<sub>2</sub> receptor blocker and specific antidote administration.

Additional investigation methods carried out were ultrasonographic examination and urine specific gravity measurement using a handheld refractometer.

Patient monitoring consisted in regular evaluation, over a year period, of serum parameters such as creatinine, blood urea nitrogen, total protein, phosphorus and alkaline phosphatase.

### **Results and discussions**

Creatinine and blood urea nitrogen serum levels presented inconstant alterations over a year period, with 18 blood sample assessments for each parameter (fig. 1 and 2).

Alkaline phosphatase serum values decreased and stabilized in normal levels, whereas total protein and phosphorus serum levels registered inconstant modifications (table 1).

Initial urine specific gravity findings shown hyperstenuria with a 1.030 value.

Ultrasonography examination revealed right kidney diameter size of 9.7 cm / 3.8 cm and left kidney 9.5cm / 3.6 cm with inflammatory aspect.

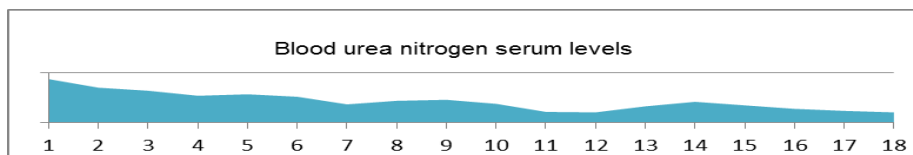


Fig. 1. Blood urea nitrogen serum level monitoring, in a German Sheperd with chronic kidney disease, over a year period (18 assessments)

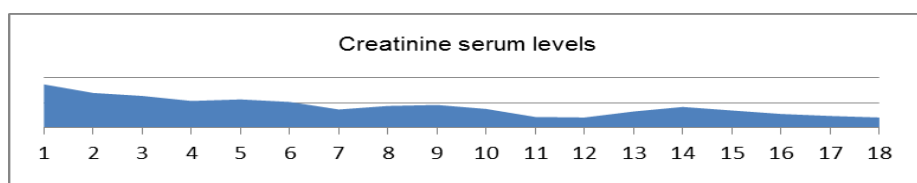


Fig. 2. Creatinine serum level monitoring, in a German Sheperd with chronic kidney disease, over a year period (18 assessments)

Table 1

**Biochemical profile of a 10 year old German Sheperd with chronic kidney disease over a year period (mean  $\pm$  standard deviation)**

Parameter	Value (mean $\pm$ standard deviation)	Range
Creatinine	3.58 $\pm$ 1.8	0.5-1.8 mg/dl
Blood Urea Nitrogen	43.05 $\pm$ 17.84	7 – 27 mg/dl
Alkaline phosphatase	233.12 $\pm$ 96.08	23 -212 mg/dl
Total Protein	7.67 $\pm$ 2.16	5.2 – 8.2 g/L
Phosphorous	6.48 $\pm$ 0.88	2.55 – 6.8 mg/dL

### Conclusions

Treatment of primary cause of chronic kidney disease can reduce the intensity of renal damage.

Patients with chronic kidney disease must be evaluated at least every month by clinical and complementary methods such as blood biochemistry and complete blood count evaluation as well as ultrasonography and urinalysis in conjunction with parameters characteristic for the ethiopathogenetic factor.



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## **ELECTROCARDIOGRAPHIC PARAMETERS IN NEW - BORN CALVES (172 CASES)**

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### **Summary**

172 new - born calves with ages between 1 hour-3-4 weeks have been investigated. The calves were clinically healthy. The examination was realized in standing position and the ECG parameters were: a milivolte amplitude of 10 mm, a rate of ECG tract rolling of 25 mm/sec, unipolar and bipolar legs derivations. The length of ECG component parts (sec): P wave =  $0.05 \pm 0.013$ ; P-R interval =  $0.12 \pm 0.027$ ; QRS complex =  $0.047 \pm 0.012$ ; QT interval =  $0.250 \pm 0.051$ ; T wave =  $0.079 \pm 0.016$ ; R-R interval =  $0.525 \pm 0.015$ ; P-T interval =  $0.376 \pm 0.074$ ; T-P segment =  $0.147 \pm 0.1$  and cardiac frequency electrocardiographically calculated based on R-R interval was  $123.15 \pm 33.6$ . The amplitude of ECG waves in new born calves in D<sub>2</sub> derivation is: P wave =  $0.154 \pm 0.072$  mV; ventricular complex =  $0.910 \pm 0.485$  mV; T wave =  $0.410 \pm 0.244$  mV. The values of electric axes are: P wave =  $50.02 \pm 29.69^\circ$ ; ventricular complex =  $-95.77 \pm 38.67^\circ$  and T wave =  $77.42 \pm 30.28^\circ$ .

Our conclusions were: estimation of ECG values in new born calves is very useful, considering the diverse pathology in this specie at this specific age: cardiomyopathies consecutive to a selenium deficiency, congenital heart malformations and others.

**Key words:** calves, new-born, electrocardiogram, standard leads.

Electrocardiography is a very important method for the diagnosis of cardiovascular diseases (6). The study of electrocardiographic parameters in this specie is useful as cardiovascular diseases (heart congenital defects, cardiomyopathy produced by tocopherol deficiency – selenic cardiomyopathy, pericarditis s.a.) also influence the calves (2).

### **Materials and methods**

172 new born calves have been evaluated. They were healthy individuals, aged several hours to 14 days. Animals have been examined awake and in standing position.

The examination has been done with an electrocardiograph Nihon-Kohden – Cardiofax. The working parameters were: amplitude 1Mv = 10mm (5 and 20mm) and the speed of the rolling paper 25-50mm/sec.

Bipolar and unipolar limbs leads were used.

The next statistical values were estimated:

- arithmetic mean;
- standard deviation;
- variance;
- error of the mean;
- variability ratio (%).

### Results and discussions

There are a few bibliographical data on characteristic electrocardiographic values in calves. These available data show only the length of ECG components. There are only a few data available on values of ECG wave amplitude. This fact is due to the lack of a standard system of derivation, but also to a cardiac and non-cardiac pathology (electrolyte disturbances, lack of acid base balance), which can influence the amplitude of ECG waves (1, 4).

All these factors can modify the amplitude of ECG waves, as there is a large variability of statistical values (variance and variability index)

The estimation of ECG parameters length has been done mainly in D2 lead, because in this lead the amplitude of the ECG wave is the highest and therefore can be easily examined.

The estimation of ECG waves amplitude has been realized in all 6 standard leads (3, 5).

As a first remark, calves electrocardiogram is included in B electrocardiographic group or the equine type: ventricular complex is in an opposite plan to P and T wave (figure 1)(3, 5, 7).

Values of ECG waves amplitude in new born calves are included in tables 1-3.

Values of ECG components length are included in table 4 and values of electric axis of ECG waves in table 5.

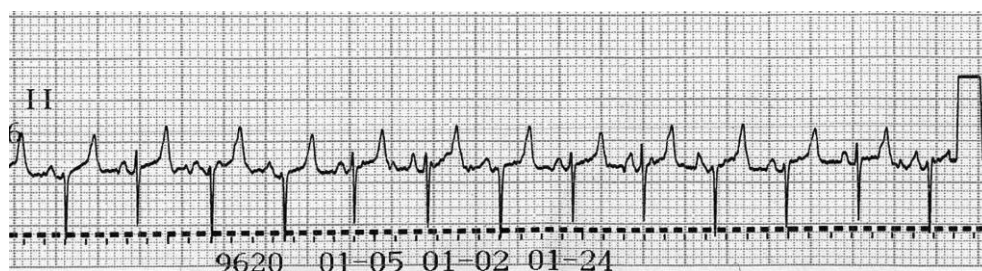


Fig. 1. Electrocardiogram aspect in new born calves

Table 1

**P wave amplitude values in new born calves**

P wave amplitude mV					
	n.	Arithmetic mean	Standard deviation	Variance	Variability ratio
D1	122	0.146	0.079	0.006	54.10
D2	148	0.154	0.072	0.005	46.75
D3	142	0.089	0.070	0.004	78.65
aVR	132	0.139	0.069	0.004	49.64
aVL	124	0.084	0.068	0.004	80.95
aVF	140	0.094	0.090	0.008	95.74

Table 2

**Ventricular complex amplitude values in new born calves**

Amplitude of ventricular complex mV					
	n.	Arithmetic mean	Standard deviation	Variance	Variability ratio
D1	140	0.626	0.384	0.147	61.34
D2	157	0.910	0.485	0.901	53.29
D3	154	0.874	0.484	0.234	55.37
aVR	147	0.612	0.416	0.173	67.97
aVL	142	0.618	0.342	0.117	55.33
aVF	151	0.832	0.451	0.203	54.20

Table 3

**T wave amplitude**

T wave amplitude mV					
	n.	Arithmetic mean	Standard deviation	Variance	Variability ratio
D1	126	0.225	0.196	0.038	87.11
D2	155	0.410	0.244	0.059	59.51
D3	147	0.327	0.227	0.051	69.41
aVR	141	0.270	0.184	0.034	68.14
aVL	133	0.204	0.157	0.024	76.96
aVF	148	0.339	0.204	0.041	60.17

Table 4

<b>ECG components length (I.)</b>					
ECG components length (sec.)					
	P	P-R	QRS	Q-T	T
n.	165	166	170	169	169
Arithmetic mean	0.05	0.12	0.047	0.25	0.079
Standard deviation	0.013	0.027	0.012	0.051	0.016
Variance	0.0001	0.0007	0.0001	0.002	0.0002
Variability ratio	26	22.5	25.53	20.4	20.25

<b>ECG c4omponents length (II.)</b>				
	R-R	P-T	TP	F.C.
n.	167	164	164	167
Arithmetic mean	0.525	0.376	0.147	123.15
Standard deviation	0.150	0.074	0.100	33.607
Variance	0.022	0.005	0.010	1129.5
Variability ratio	28.57	19.68	68.02	27.28

Table 5

<b>Value of electric axis (<math>^{\circ}</math>)</b>					
Value of electric axis of ECG wave( $^{\circ}$ )					
	n.	Arithmetic mean	Standard deviation	Variance	Variability ratio
P wave	145	50.02	29.69	882.04	59.35
QRS	143	- 95.77	38.67	1485.47	40.37
T wave	148	77.42	30.28	917.41	39.11

**Remarks on statistical data****A. Amplitude of ECG wave**

P wave is the representation of atrial electric activity and P wave amplitude is in most of the situations low. P waves had a monophasic aspect, on the same side with T wave.

Ventricular complex is on the opposite side to P and T waves (B electrocardiographic group) and the highest amplitude is seen in D2 lead.

As mentioned before, there are a lot of physiological and pathological factors (without any clinical signs) that modify the amplitude of ECG waves. As an

example of physiological factors there is the stress induced increase of ventricular contraction strength that can increase the amplitude of ventricular complex.

There is also evidence in this specie and at this age that a head deviation towards the left or the right side can cause significant changes of ECG waves amplitude in different leads. These are physiological changes as they are produced by compression of carotidian sinus.

All these factors are increasing the statistic index regarding variability and variability ratio

#### ***B. The length of ECG components***

The estimation of ECG components has a particular importance as the significance is as it follows:

P wave = atrial depolarisation

P-T = atrial systole

QRS = ventricular depolarization

Q-T = ventricular systole

R-R = length of a cardiac cycle, used to estimate cardiac frequency

TP = general diastole

P-T = general electric activity (atrial and ventricular systole)

Statistical indexes are homogenous, excepting TP segment, RR interval and cardiac frequency. The indexes above are influenced by different physiological and/or pathological factors (without clinical signs in these situations).

#### ***C. Cardiac electric axis***

Cardiac electric axis is positive for P and T wave and negative for ventricular complex. There is an increased variability of these indexes following the variability of ECG wave amplitude.

### **Conclusions**

Electrocardiogram is one of the main methods to evaluate the heart in animal species. It is used to study arrhythmia, changes of cardiac cavities volume and indirect information of acid base imbalance or electrolytic imbalance.

New born calves electrocardiogram is included in B electrocardiographic group, with a ventricular complex on an opposite side to P and T wave.

We used the next parameters: the millivolt amplitude =10 mm and the speed of rolling paper 25 or 50mm/sec. We used legs leads (derivations)

The length of ECG component parts (sec): P wave =  $0.05 \pm 0.0001$ ; P-R interval =  $0.12 \pm 0.0007$ ; QRS complex =  $0.047 \pm 0.0001$ ; QT interval =  $0.250 \pm 0.002$ ; T wave =  $0.079 \pm 0.0002$ ; R-R interval =  $0.525 \pm 0.022$ ; P-T interval =  $0.376 \pm 0.005$ ; T-P segment =  $0.147 \pm 0.01$  and cardiac frequency electrocardiographically calculated based on R-R interval was  $123.15 \pm 1129.49$ .

The amplitude of ECG waves in new born calves in D<sub>2</sub> derivation is: P wave =  $0.154 \pm 0.005$  mV; ventricular complex =  $0.910 \pm 0.235$  mV; T wave =  $0.410 \pm 0.059$  mV.

The values of electric axes are: P wave = 50.02°; ventricular complex = - 95.77° and T wave = 77.42°.

Our conclusions were: estimation of ECG values in new born calves is very useful, considering the diverse pathology in this specie at this specific age: cardiomyopathies consecutive to a selenium deficiency, congenital heart malformations and others.

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## **PATHWAYS THAT REGULATE BULL SPERM CAPACITATION AND THE ACROSOME REACTION**

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### **Summary**

Following ejaculation, bull spermatozoa cannot immediately fertilize an egg. In order to become fully functional and competent cells, they will require a finite period of residence in the female reproductive tract. During this period of time, sperm cells suffer an amount of molecular, biochemical and physiological changes collectively referred to as capacitation. In the process of capacitation, which is now regarded as the reversible, prior to the fertilization activation process of sperm, bull spermatozoa will gain the ability to: develop hyper activated motility with vigorous nonlinear flagellar motion, bind to the zona pellucida, undergo the acrosome reaction and proceed eventually to fusion with the oolemma and egg fertilization.

Recent advances suggest that capacitation and induction of the acrosome reaction are regulated by multiple interactions between  $\text{Ca}^{2+}$ , sperm plasma membrane enzymes and lipids.

The purpose of this review is to take into consideration some recent contributions towards our understanding of bull sperm capacitation process, to sum up important questions in this field area and to discuss further possible avenues of research.

**Keywords:** acrosome reaction, bull (*Bos Taurus*), sperm capacitation

After leaving the testis, bull spermatozoa are morphologically differentiated but have acquired neither progressive motility nor the ability to fertilize a metaphase II-arrested egg. During epididymal transit, sperm acquire the ability to move progressively; however, they are still fertilization incompetent. Fertilization capacity is gained after residence in the female tract for a finite period of time. The physiological changes that confer on the sperm the ability to fertilize are collectively called "capacitation" (32).

Sperm capacitation is a gradual, essential event prerequisite for fertilization that takes place in vivo during the sequential exposure of spermatozoa to the different compartments of the female genital tract that occurs during sperm transport (25). A complex physiological process that involves biochemical, biophysical and metabolic modifications of all sperm domains, capacitation results in altered plasma membrane architecture and permeability, which ultimately modulates flagellar activity and renders the sperm apical head plasma membrane fusogenic (15). The process has been thoroughly studied since firstly identified as a vital pre-fertilization event mostly in vitro, and our knowledge of the biochemical



modifications therein is impressive (16). Capacitation was first described and defined independently by Chang (8, 9) and Austin (4, 5) and its definition has been modified and narrowed over the years. The two investigators independently reported functional changes on spermatozoa recovered from the female genital tract after mating or preincubating the cauda epididymal spermatozoa with oviductal secretions (35).

It is well known that fertilization represents the endpoint of a capacitated sperm. Nevertheless, the ability of the sperm to undergo a regulated acrosome reaction can be taken as an earlier endpoint of this extratesticular maturational event (32).

Although capacitation, a unique event which happens only in the male gamete, is required before the hyperactivated sperm can bind to and fertilize the egg, the molecular mechanism(s) of the multifaceted process remains elusive. Similarly, the mechanism(s) that enables the zona-bound spermatozoon to undergo the acrosome reaction is even less clear (2). The purpose of this review is to bring together and to discuss diverse data from various investigators in the attempt to understand the pathways that regulate sperm capacitation and the acrosome reaction. Among others, we will discuss about some recent advances that suggest that capacitation and induction of the acrosome reaction are regulated by multiple interactions between  $\text{Ca}^{2+}$  - binding proteins, sperm plasma membrane and lipids.

#### ***In vitro/in vivo sperm capacitation process – how does it work?***

Beside in vivo scenario, capacitation can occur also in vitro spontaneously in a defined medium without the addition of biological fluids, which suggests that this process is intrinsically modulated by the spermatozoa itself such that these cells are preprogrammed to undergo capacitation when they are incubated in the appropriate medium (24). Although numerous hypotheses have been developed, the precise nature of this process is still not very well understood.

Changes associated with sperm capacitation include an increase in respiration and subsequent changes in the motility pattern, called as hyperactivation which is characterized by pronounced flagellar movements and a marked lateral excursion of sperm head in a non-linear trajectory, and in a number of species, removal of cholesterol from the sperm plasma membrane (21), destabilization of sperm membranes (13), an increase in intracellular pH and calcium levels (22), activation of second messenger systems (11, 23, 29), and/or removal of zinc (3). The most important change in sperm after capacitation is its ability to undergo acrosome reaction in response to zona pellucida 3, progesterone and calcium ionophores (12, 24).

Capacitation is characterized by a series of biochemical and biophysical alterations to the cell including changes in intracellular pH, remodeling of the cell surface architecture, changes in motility patterns and initiation of complex signal transduction pathways. These events have been correlated with a dramatic global up-regulation of tyrosine phosphorylation across a number of key proteins. The ensuing activation of these target proteins has, in turn, been causally linked to the

initiation of hyperactivated motility, ability to recognize and adhere to the zona pellucida, and the ability to undergo acrosomal exocytosis (20). Capacitation in sperm does not occur synchronously and is a transient and irreversible process (24, 26).

Ionic environment and ionic fluxes through membrane are highly important in the spermatozoal maturation, capacitation and in initiating the process of gamete interaction (24). Capacitation is dependent on extracellular  $\text{Ca}^{2+}$  and is associated with elevated intracellular  $\text{Ca}^{2+}$  (14, 19). Alterations in sperm plasma membranes, such as loss of cholesterol, initiate the signal transduction pathway that promotes capacitation (19, 33, 34). Albumin is the major protein in the female reproductive tract who is considered to initiate capacitation by facilitating the efflux of cholesterol from sperm plasma membrane. The loss of cholesterol has been suggested to affect the sperm plasma bilayer and make it more fusogenic (2). These membrane alterations are believed to increase permeability to ions such as  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$ , which enter the cytoplasm and stimulate adenylyl cyclase (10, 17, 18) to promote cAMP production, leading to the stimulation of PKA and, ultimately, protein tyrosine phosphorylation (19) and, thus, resulting in hyperactive spermatozoa.

Yanagimachi (35) was the first to use the term hyperactivation to describe vigorous, whiplash-like beating pattern of the capacitating/capacitated sperm flagellum. Although hyperactivated sperm motility is associated with the process of capacitation, the two changes are regulated by independent mechanisms. Abou-haila stated in his review that the possibility of preventing in vitro capacitation with or without blocking the sperm motility exists and it is a result consistent with the suggestion for the occurrence of multiple signaling pathways that regulate sperm capacitation (2).

Most studies have concluded that  $\text{Ca}^{2+}$ ,  $\text{HCO}_3^-$ , and cAMP are the key secondary signaling molecules that regulate sperm capacitation. When bovine spermatozoa are incubated under in vitro conditions that favor capacitation, intrasperm  $\text{Ca}^{2+}$  increases by 6-fold. This increase will stimulate  $\text{Ca}^{2+}$ -dependent ATPase and adenylyl cyclase, two key enzymes involved in signaling cascade. Omission of extracellular  $\text{Ca}^{2+}$  from the medium blocks capacitation but the tyrosine phosphorylation continues (33, 34). These data suggest that  $\text{Ca}^{2+}$  has a role in other events of capacitation. Calcium exerts its effect on sperm capacitation through  $\text{Ca}^{2+}$ -binding proteins that undergo conformational changes upon interaction with the divalent cation. This interaction plays a significant role in several cell signaling pathways by modulating biological activities of multiple enzymes, proteins and ion pumps (6) including  $\text{Ca}^{2+}$ /CaM-dependent kinases (1).

Accumulated evidence suggests that the transmembrane movement of  $\text{HCO}_3^-$  anions into the sperm may be responsible for the reported increase in intrasperm pH during capacitation. Bicarbonate anions are also reported to stimulate adenylyl cyclase, the sperm PM enzyme responsible for raising the levels of cAMP by increased synthesis. The primary target of cAMP is thought to be PKA which is stimulated in capacitating/capacitated spermatozoa (2).

**The sperm acrosome – function and induction of acrosome reaction**

The sperm acrosome plays an important part following species-specific sperm-egg zona binding. The sperm acrosome is a Golgi-derived secretory organelle that resembles the cellular lysosome in many ways; however, the acrosome is considered analogous to a secretory granule. In response to physiological or pharmacological stimuli, the outer acrosome membrane and the overlying plasma membrane undergo fusion and vesiculation leading to the exposure of the acrosomal contents to the extracellular environment. This exocytotic process is called acrosome reaction and its completion is an absolute prerequisite for fertilisation. Burgess and Kelly (7) reported three important features of the acrosome. These features are: (1) the secretory contents are stored over an extended period of time and are present in a concentrated form; (2) the acrosomal contents form a dense structure and are stored for several weeks during sperm formation and development in the testis and subsequent maturation in the epididymis; and (3) the organelle undergoes secretion (exocytosis) as a result of an external stimulus.

The acrosome reaction is initiated by the signal represented by the recognition and binding of capacitated spermatozoa to zona pellucida in a receptor–ligand manner (2). Morphologically, the exocytosis of acrosomal contents or induction of the acrosome reaction occurs in several steps. First, there is a fusion of the sperm plasma membrane and the underlying outer acrosome membrane at multiple sites. The fusion likely occurs gradually in different acrosomal regions, and may not be an all-or-nothing phenomenon. Second, the fusion of membranes causes formation of hybrid vesicles and the release of the acrosomal contents in a time-dependent manner. The process is slow and may be regulated by changes in the sperm membranes and the cytoskeletal elements such as the F-actin bundles present between the sperm plasma membrane and outer acrosome membrane which may help the spermatozoon to gradually penetrate the zona pellucida. Finally, there is a disappearance of acrosomal contents and vesicles that are held together by acrosomal matrix. The powerful action of hydrolytic enzymes (proteinases, glycohydrolases, etc.), released at the surface of the zona pellucida, makes it possible for the hyperactivated spermatozoon to penetrate the egg and fertilize it (2, 28).

Ca<sup>2+</sup> plays a central role in the assembly of fusion machinery (capacitation) and subsequent fusion of the sperm plasma membrane and outer acrosome membrane during acrosomal exocytosis. Watson and his associates reported that calcium was initially associated with the outer acrosome membrane. As the acrosomal exocytosis progressed, Ca<sup>2+</sup> redistributes between the outer acrosome membrane and plasma membrane anterior to the equatorial segment where the membranes fuse during the acrosome reaction. This distribution suggests a direct role for Ca<sup>2+</sup> in the membrane fusion leading to the acrosomal exocytosis (2).

### Conclusions

The aim of this review is to summarise all that it is known at the present moment concerning bull sperm capacitation process and the acrosome reaction. Despite of the fact that the biological phenomenon of sperm capacitation has been known for more than half a century, the molecular basis of this process is still not well understood. Questions that were addressed in the past such as: how is cholesterol movement from the sperm plasma membrane regulated and how does this movement initiate the intracellular signaling, or what is the mechanism by which the cAMP/PKA pathway is stimulated and how does this stimulation lead to cross talk and up-regulation of protein tyrosine phosphorylation or, finally, what is the nature of the substances that are phosphorylated on tyrosine residues and how does the phosphorylation of these substrates impact on the major endpoints of capacitation (e.g. hyperactivation of motility, competence to undergo a regulated acrosome reaction and fertilization) have now some answers. We have highlighted some recent studies which have attempted to explain the main pathways during sperm capacitation and the acrosome exocytosis. Some of these processes appear to be quite unique and therefore request continued investigation and research.

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## **THE CLINICAL AND IMAGISTIC EXAMINATION IN PROSTATIC DISEASES IN DOGS**

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### **Summary**

During the clinical activity, different prostatic diseases can be met, such as benign prostatic hiperplasia, infectious prostatitis or prostatic neoplasms.

To diagnose these diseases, multiple methods can be used, such as the clinical examination, in which the rectal touch examination is an important step, imagistic examination – ultrasound guided fine needle aspiration (FNA), aside from radiography and echography, urethral catheterization and taking samples of prostatic fluid by repeated irrigation and the citological and bacteriological examination of the samples.

Our casuistry is represented by 12 dogs that were brought in with clinical signs of dysuria; we must mention that the clinical symptomatology can be quite diversified, ranging from constipation due to intense hypertrophia of the prostate, to changes in the dog's posture due to pain radiating towards the whole abdomen.

For the dogs suspected with a prostatic disease, a rectal touch examination was performed, along with a radiographic examination of the abdomen in the right and left lateral view, an echographic examination and an ultrasound-guided fine needle biopsy followed by the microscopical examination of the samples obtained.

The applied examination methods lead to diagnosing either a prostatic abscess, or a benign or malign prostatic hiperplasia.

**Key words:** prostate, ultrasound-guided FNA, dysuria, abdominal radiography, ultrasound

To establish a certain diagnosis for any prostatic diseases and to differentiate between benign and malignant morphological changes, a complete clinical examination is required. The examination protocol includes a phisical examination, a rectal touch prostatic evaluation and imagistical procedures, such as an abdominal radiography, with or without contrast substance (1, 6), ultrasound examination of the pelvic region and, when required, an ultrasound-guided fine needle aspiration followed by a microscopical examination of the aspirate. All imagistic methods are important, giving new and improved information on the state of the abdominal and pelvic organs (4).

As the clinical symptoms can be quite diverse, the dogs can be brought in for digestive or urinary disfunctionalities that have as a starting point the hypertrophy of the prostatic gland (4, 5).

Very often, the dogs were brought in for a radiological examination of the dorso-lumbar spine, in lateral recumbency, after being redirected from the clinical examination that showed changes in the spine and hindlimbs position. The modifications included signs of strong lumbar pain, cifosis, the refusal of stationary positions and even colic-like symptoms.

### **Materials and methods**

Twelve dogs were included in this study, of different breeds and ages, that were showing symptoms varying from dysuria to chronic constipation. Three of the examined dogs were aged between 1 and 4 years, while 9 of them were of ages varying between 5 to 14 years old.

For each dog, a complete clinical examination was made, before being redirected to one or more paraclinical examinations – abdominal radiography, ultrasound, ultrasound-guided fine needle aspiration, biochemical examination of the blood and microscopical examination for the urine and the aspirate obtained after the FNA procedure. Each patient had a clinical examination chart prepared.

The radiological examination was performed on the alert dogs, in lateral and ventral decubitus, to attain the latero-lateral and dorso-ventral recumbencies. As a technical material, for the X-ray examination, a Roentgen Eltex 400 and a mobile Roentgen Intermedical Basic 4006 devices were used. For the ultrasound examinations, two ultrasonographic devices were used – an Esaote Aquila and a Logiq F8, supplied by General Electric for research. For a contrast urethrography, a non-ionic solution was used, based on ioversol (OptiRay 300).

The ultrasound examination of the abdomen was performed on the alert animals, in dorsal decubitus, exposing the abdomen and pelvic areas, in bidimensional mode. For the microscopical examination of the urinary sediment, a Motic B1 Series microscope was used.

The radiographic images were taken with the alert animal, in latero-lateral and ventro-dorsal recumbency. Two images were often needed to evaluate the size and direction of hypertrophy and to see the degree of topographical changes brought by the modification of the size of the prostatic lobes.

Normally, the prostate is located immediately caudal to the urinary bladder, completely surrounding the urethra, and does not extend cranially to the pelvic brim, in young, sexually mature dogs. Criteria for determining the normal size of the prostate have been published, but are not usually used due to their difficult methodology.

The most useful method used can be used by drawing a line, in the lateral radiographic view of the abdomen, parallel or perpendicular to a line drawn from the sacral promontory to the cranial edge of the pubic bone, creating the pubic – promontory axis distance. The normal canine prostate size should be less than 70% of that distance (2).



### Results and discussions

Out of the twelve examined dogs, 9 of them were of ages over five years, while three of them were 1 to 5 years old.

The twelve patients were grouped, according to their diagnosis as in the following chart:

Table 1

The list of patients, listed by diagnosis

No.	Breed	Age	Diagnosis
1.	German Shepherd	9 years	Prostatic abscesses
2.	Rottweiler	5 years	Prostatic abscesses
3.	German Shepherd Mixed breed	12 years	Prostatic abscess + urolithiasis
4.	Mioritic Shepherd	13 years	Prostatic abscess
5.	Amstaff mixed breed	13 years	Prostatic abscess + urolithiasis
6.	Mixed breed	13 years	Prostatomegaly – adenocarcinoma
7.	Caniche	8 years	Malignant prostatic hypertrophy – adenocarcinoma
8.	Pekingese	10 years	Prostatomegaly – adenocarcinoma
9.	Pekingese	14 years	Prostatic adenocarcinoma – metastatic
10.	Amstaff mixed breed	13 years	Benign prostatic hypertrophy
11.	German Shepherd	5 years	Benign prostatic hypertrophy
12.	German Shepherd	3 years	Benign prostatic hypertrophy

*Benign hyperplasia* of the prostate was diagnosed in 3 dogs with ages of 3 to 13 years, that were not castrated and were never used for reproductive purposes.

A 3 year – old German Shepherd was brought in at the Radiology Laboratory of the Iasi Faculty of Veterinary Medicine with clinical signs of chronic constipation and with varying degrees of lameness on the hindlimbs. After the clinical examination, a lateral X-ray image of the lombo-sacral spine was required, a lesion of that section of the spine being suspected.

On the radiographic image, a round mass of increased radioopacity was observed pressing dorsally on the terminal segment of the colon. Also, the urinary bladder was distended, moving cranially the intestinal mass, and there was also a slight increase in opacity in the L7-S1 area. Also, in this area, the presence of an osteophyte was observed.

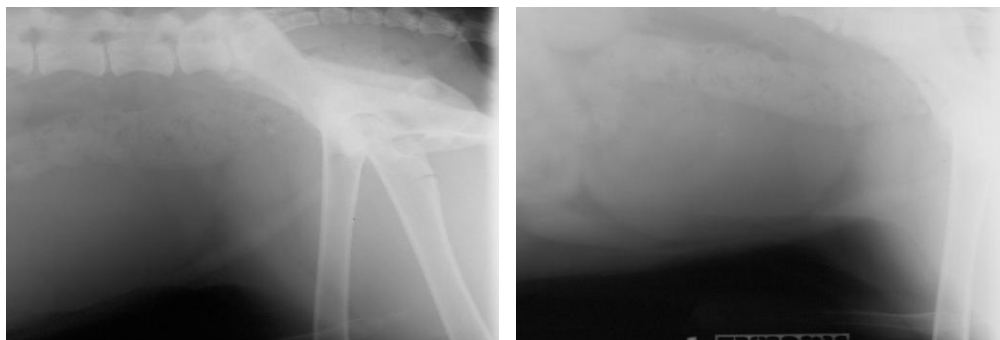


Fig. 1. Latero-lateral recumbency of the abdomen

After the radiographic examination, the patient was redirected to an abdominal ultrasonography that confirmed the enlargement of the prostate, but it also showed that the structure of the prostatic parenchima was normal. Also, the urinary bladder was found to be distended, with a hyperechoic structure of the wall. The liver parenchima appeared to be normal.

The prostatic size decreased after neutering.

*Malignant hyperplasia of the prostate* was diagnosed in 4 dogs.

One of these dogs was a Pekingese 14 year-old dog that was brought in the Emergency Care Unit in the Iasi FMV with a history of anuria and perianal tumours. There was a urethral catheterism performed prior to presentation at a private practice and the clinician suspected the presence of a urolith in the prostatic area of the urethra.

To ascertain the presence of said urolith, the dog was redirected to the Roentgen Laboratory for an abdominal radiography. The image was taken with the alert animal in latero-lateral recumbency and showed that, while there were no radioopaque uroliths, there was a significant enlargement of the prostate that seemed to press on the urethral lumen. The urinary bladder was distended and with an increased volume, displacing, cranially, the intestinal mass.

Both the kidneys and the prostate showed an irregular pattern in radioopacity, therefore, the dog was then sent to an abdominal ultrasonography.

The ultrasonographic images showed that the prostate was enlarged and the parenchima had a non-homogenous echoic structure. An ultrasound-guided fine needle aspirate was performed and the tissue sample was sent to the laboratory for citological interpretation. Unfortunately, the result showed that the tissue changes in the prostate were of a malign character, most probable a metastasis of the tumoral structures located perianally.

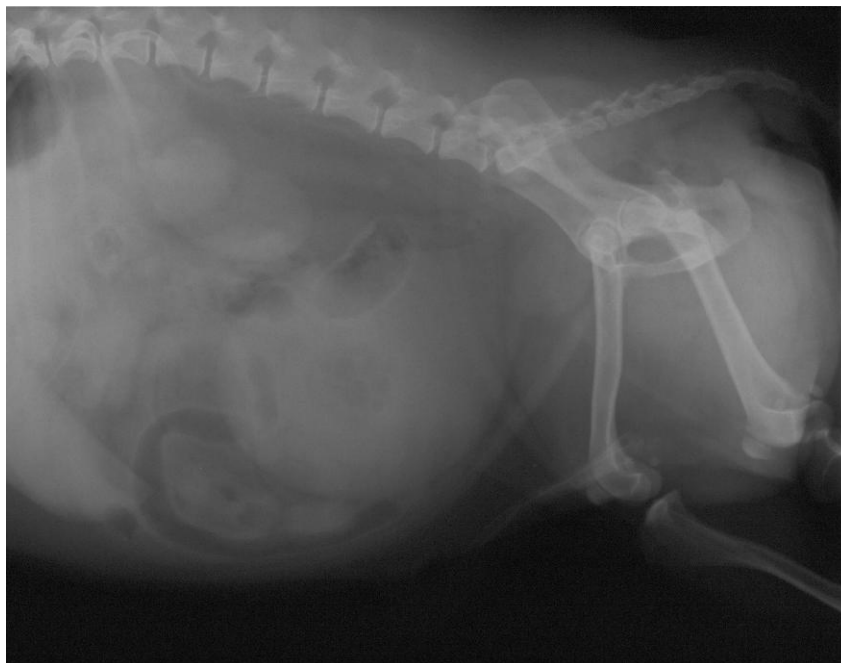


Fig. 2. Abdominal LL recumbency – distended urinary bladder and prostatic radioopacity

*Infectious prostatitis* – translated by the presence of abscesses in the prostatic parenchima and the enlargement of the prostate, was diagnosed in 5 dogs, out of all the age groups.

A Caniche breed, male dog, aged 4 years, was brought in with a suspicion of urolithiasis, translated through signs of stranguria, painful urination, traces of blood in the urine and changes in the lumbo-sacral and hindlimbs positions. Also, there could be observed modifications of the general state of the dog, such as a pronounced apathy, anorexia, fever and strong abdominal pain. The fever was initially believed to be the sign of an infectious process that appeared following the lesioning of the vesical and urethral mucosae by the passing of small-sized uroliths. However, after radiological and ultrasound inspection, no uroliths were detected.

However, on the radiographical image, an area of increased opacity was detected caudally of the urinary bladder. The prostatic silhouette axis was close to 85% of the pubic – promontory axis distance. However, despite the urolithiasis suspicion, no uroliths were detected on the radiographic image. An urethral catheterisation was performed, to ascertain the permeability of the urethral duct, and a ioversol – based contrast solution was injected to confirm the reduced size of

the intra – prostatic urethral lumen. The contrast substance underlined the urethral wall, but the radioopaque line was discontinued in the prostatic area.

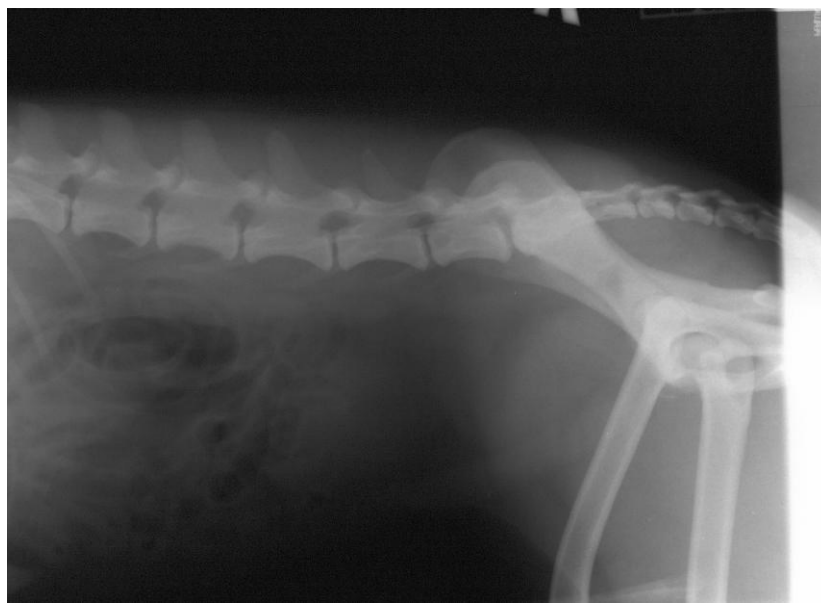


Fig. 3. Latero-lateral recumbency of the abdomen with radioopacity in the prostatic area – without contrast substance

After the radiographic examination, the dog was redirected to an ultrasonographic abdominal examination to assess the prostatic parenchima and to locate the eventual presence of the urinary microlithiasis. No urinary stones or sand were detected, however, three cavitary lesions were detected in the prostatic mass.



Fig. 4. Urethral catheterisation



Fig. 5. Contrast X-ray image

At the same time, urine and blood samples were taken and sent for biochemical and microscopical examinations. The biochemical blood profile showed the presence of an infectious process, while the microscopical urinary sediment analysis revealed the presence of blood, white blood cells and bacillary microorganisms in increased numbers.



Fig. 6. Prostatic abscesses as seen in the ultrasonographic examination of the prostate

The urine was prelevated using a mostly sterile method and was sent for an uroculture and the results for a bacterian infection were positive.

### Conclusions

Sometimes, the prostatic lobes, by increasing in size, can press dorsally on the colon, changing its topography and modifying the size of the lumen, creating difficulties in the transit of the faeces, creating the basis of a chronic constipation. Other times, before reaching the constipation stage, the dog can show signs of dysuria, translated by a decrease in the size of the intra-prostatic urethral lumen by compression.

Quite often, the general state of the animal can be changed, being expressed through a lack of appetite, apathy, and even prostration when the pathology has a long evolution and the dog becomes organically exhausted.

Both the clinical and imagistic examinations are required and give equally important information regarding the different pathologies that the patients are

presented with. The clinical examination can act as a filter, gathering the information and being able to redirect the dogs to the required paraclinical examinations. In all the cases, we coupled the radiographic examination with an ultrasonographic image of the abdominal organs to ascertain whether the changes that appeared in the size influenced, in any way the structure of the internal parenchima of the prostate. Quite often, the radiographic image managed to narrow and single out the cause of the changes in the general state of the patient, while the ultrasonographic image of the abdomen managed to lead to a more exact diagnosis. The ultrasound-guided fine needle aspiration managed to give information in regards to the ethiological cause of the prostatic modifications and, when used in the case of a prostatic abscess, it can provide the biological material for a bacterial culture and antibiogram, giving the clinician the exact ethiological cause and sensitivity degree of the pathogen towards certain antibiotics.

The imagistic examinations that we performed, both the ultrasound and the abdominal radiography, bring important information on different sides of the same pathology. While the radiographic image brings forth information regarding the general size and the eventual changes in the abdominal topography, the ultrasound examination of the prostate can bring information regarding the inner structure of the organ, being able to show any change in the echoic structure and the existence of any cysts or abscesses. Both paraclinical examinations are required to achieve a certain diagnosis, in tandem with other paraclinical examinations.

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## **CONTRIBUTIONS IN COMPUTED TOMOGRAPHY DIAGNOSTIC OF SPINAL NEOPLASMS IN CAT**

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### **Summary**

Extradural tumors are the most common category of neoplasm affecting the spinal cord. Of feline extradural tumors, osteosarcoma is the most common primary vertebral neoplasm. Both MRI and CT evaluations are more sensitive than radiographic evaluation in detecting vertebral lesions, and in particular bone lysis which is not apparent radiographically and can be demonstrated readily using CT.

The study was developed on an 8 years old feline male patient, European breed that was initially presented with symptoms like dysuria, urinary retention and spinal/back pain in cranio-lumbar region.

Radiographic evaluation showed narrowing of L2 – L3 intervertebral space and also a density change in vertebral body characterized by increased radiopacity. These findings were confirmed after CT evaluations that showed osteolysis in the 3<sup>rd</sup> cranio-dorsal portion of L3 body, sclerosis of entire vertebrae's body and inhomogeneous aspect of medullar parenchyma in the osteolysis area.

Histopathologic evaluation showed the presence of an intramedullary malignant neoplastic proliferation of bone tissue constituted of fusiform cells with visible ovalar or round vesicular nuclei and atypical mitosis. Bone cortical perforation and microfocal granulocytic inflammatory infiltrate were also present.

CT evaluation can lead to identification of osteolytic and sclerotic lesions associated with early vertebral extradural tumors because of the possibility of section evaluation and also high contrast obtained for the bone tissue.

**Key words:** computed tomography, extradural tumor, cat

Tumors affecting the spinal cord may be classified as extradural, intradural-extramedullary, and intramedullary (11).

Extradural neoplasms comprise approximately 50% of all spinal neoplasms, while intradural-extramedullary tumors and intramedullary tumors constitute 30% and 15%, respectively (11).

Extradural tumors are the most common category of neoplasm affecting the spinal cord. Sarcomas, including osteosarcoma, chondrosarcoma, fibrosarcoma and hemangiosarcoma are the most common primary vertebral neoplasm in dogs and cats (10).

Of feline extradural tumors, osteosarcoma is the most common primary vertebral neoplasm (7). Of the intradural-extramedullary tumors, meningiomas have been infrequently reported in the spinal cord of cats.

Intramedullary tumors of cats are extremely rare and usually have features consistent with primitive neuroectodermal tumors or they have glial origin (7).

The most common applications for spine CT include suspected intervertebral disk disease, spinal stenosis, or spinal masses (6).

Both MR imaging and CT are more sensitive than radiography in detecting vertebral lesions, and in particular bone lysis which is not apparent radiographically and can be demonstrated readily using CT. Vertebral canal tumors can be difficult to localize to the intradural, extradural, or intramedullary compartments on noncontrast CT images. Injection of intravenous contrast medium and, more important, CT myelography are more precise for determining the location of masses in the vertebral canal with respect to the dura. MRI is also good for localizing these kind of lesions; however, with MR imaging it is more difficult to localize vertebral canal masses with respect to the dura because of lower spatial resolution (10).

### **Materials and methods**

The study was developed on an 8 years old feline male patient, European breed that was initially presented with symptoms like dysuria, urinary retention and spinal/back pain in cranio-lumbar region.

The imagistic evaluation took place in the Laboratory of Imagistic Diagnostic from the Surgery Department of the Faculty of Veterinary Medicine from Timisoara.

Radiographic evaluation was made with Multix Swing (Siemens) radiographic installation. There were obtained images from two standard incidences. The imaging processing was made utilizing computed radiography system Direct View Vista CR (Carestream) and AQS Vet Standalone application (Artz + Praxis GmbH).

CT evaluation was made initially by simple scanning (conventional CT) and followed by 2 ml/kg in bolus intravenous administration of iodine based medium contrast (Iopamiro 370 – Bracco, Italy). The scanning was made at 2 minutes intervals after intravenous administration (after evaluation technique described by Schwarz and Saunders 2012).

During CT scans, the animal was placed in dorsal recumbency and under general anesthesia (dissociative type). There were used Xylazine 2% (Narcoxy – MSD Animal Health) and ketamine 10% (Ketaminol - Intervet).

After the CT scanning of the patient with Somaton definition AS (Siemens), the images of the scans were reconstructed utilizing standard multiplanar reconstructive techniques (MPR). The slice thickness was 1 mm and, for a better



view of the present lesions, a 3D reconstruction was made through VRT application using the workstation's image analysis software.

### **Results and discussions**

Clinical evaluation of the patient showed symptoms like disuria, urinary retention and spinal pain in cranio lumbar region.

Radiographic evaluation made in two standard incidences showed narrowing of L2 – L3 intervertebral space and also a density change in vertebral body characterized by increased radiopacity (vertebral sclerosis) (Fig. 1).

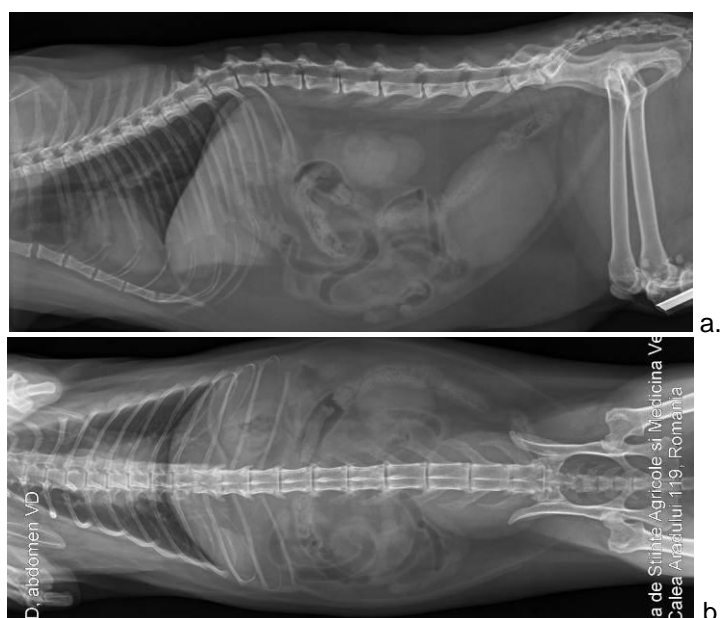


Fig. 1. Conventional radiographic images a. lateral incidence; b. ventro-dorsal incidence

The patient received nonsteroidal anti-inflammatory drugs. Also, the gallbladder was emptied by manual presson.

After three days from the first evaluation, the general status of the patient deteriorated. This lead to motor and sensitive deficit on both hind limbs.

Neurologic examination showed no proprioceptive activity on both hind limbs and no superficial sensitivity of lumbar spine's cranial portion in caudal direction.

Pain sensitivity was present but considerable diminished in both hind limbs. Patellar, tibial cranial and plantar reflexes were diminished. Muscle tone evaluation showed spastic contraction of hind limbs.

Pre – contrast CT images (fig. 2) showed osteolysis in the 3<sup>rd</sup> cranio-dorsal portion of L3 body, sclerosis of entire vertebrae's body and inhomogeneous aspect of medullar parenchyma in the osteolysis area.

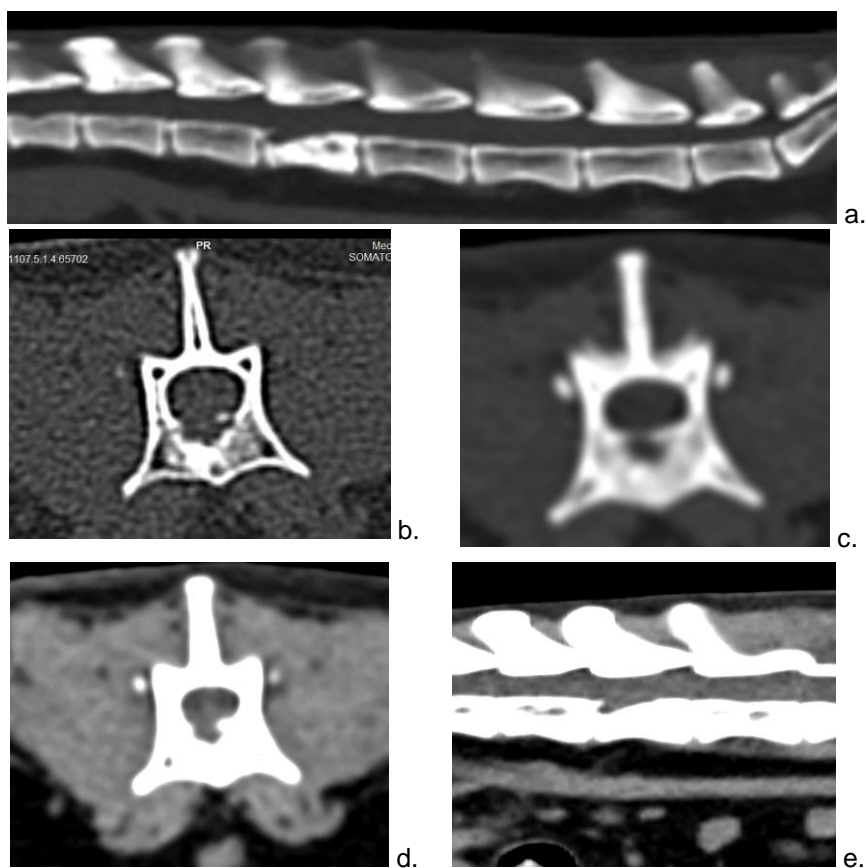


Fig. 2. Pre-contrast CT image (MPR a. and e. sagittally plane; b – d. transverse plane)

Other authors (1, 8) consider that the most frequent changes associated with spinal neoplasia are sclerotic reaction in compact bone and vertebrae's body osteolysis.

CT in particular is more sensitive for identifying bone loss, cortical bone detail than conventional radiography (10, 11).

Pre – contrast CT images (fig. 3) showed contrast enhancement in the area of osteolysis and a mass impression in medullar canal with dorsal displacement and medullar compression.

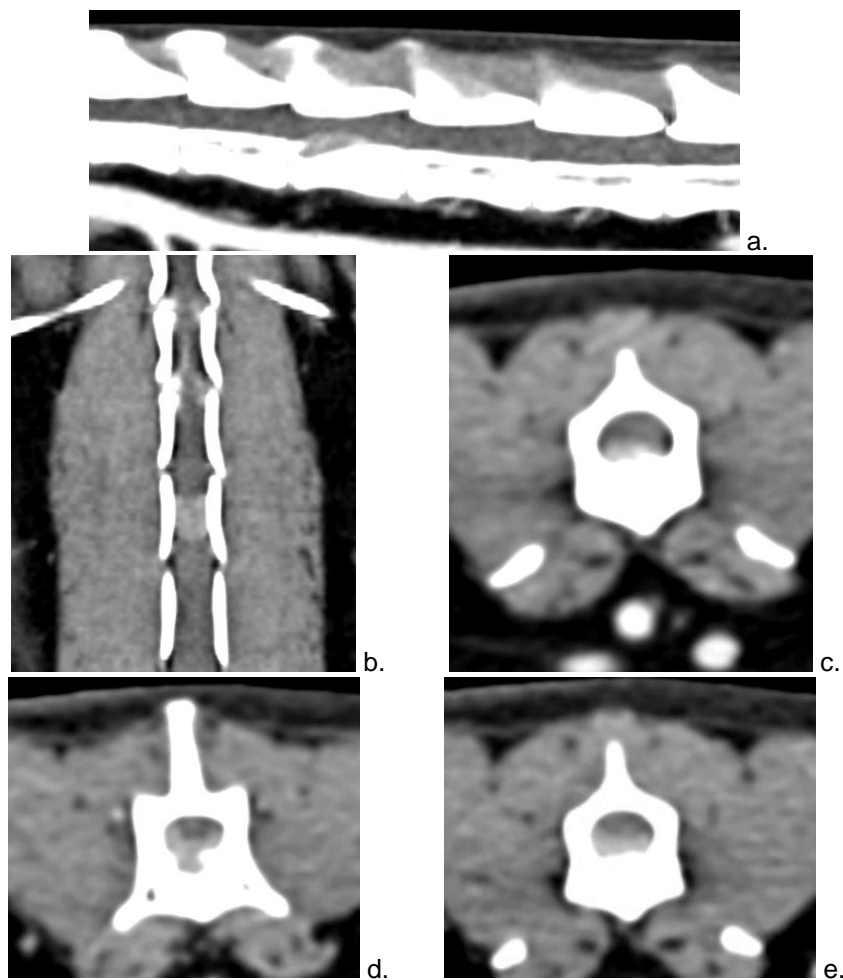


Fig. 3. Post-contrast CT image (MPR a. sagittally plane; b. dorsally plane, c – e. transverse plane)

Pre- and post – contrast changes showed on CT images is suggesting the presence of an extradural spinal tumor with origin in vertebrae's body.

The changes observed in this study are specific to extradural spinal tumor. The same changes are mentioned by Schwarz and Saunders 2012 (9).

The surgical exploration made with the purpose of biopsy analysis, showed ventral extradural medullar compression through a mass of soft tissue in L3 area with irregular surface and infiltrative aspect in the vertebrae's body.

Histopathologic evaluation showed the presence of an intramedullary malignant neoplastic proliferation of bone tissue constituted of fusiform cells with visible ovalar or round vesicular nuclei and atypical mitosis. Bone cortical perforation and microfocal granulocytic inflammatory infiltrate were also present. Malignant sarcomatous proliferation can correspond to an high grade conventional intramedullary osteosarcoma.

Knowing the severity of the neurologic symptoms an poor prognosis of osteosarcoma with axial localization, the patient was euthanasied.

Feline bone tumours are rare (0.5% of all feline tumours). Most are malignant (67–90%), with malignant osteosarcoma the most common primary bone tumour (70–80%), then fibrosarcoma and chondrosarcoma (2).

Generally, dogs or cats with an extradural metastatic neoplasm or vertebral neoplasm have a poor prognosis, and palliative therapy only is attempted (4). Removal of an affected vertebrae (Vertebrectomy), particularly in the cranial lumbar region, may be attempted in selected cases (3).

Axial osteosarcoma carries a poorer prognosis due to increased difficulty with local resection and control (5).

Bitetto et al 1987 (2) showed in a study that the cats with axial tumors that were not euthanatized at the time of diagnosis (6 of 7), the median survival time was 5.5 months.

### **Conclusions**

CT evaluation can lead to identification of osteolytic and sclerotic lesions associated with early vertebral extradural tumors because of the possibility of section evaluation of the images and also high contrast obtained for the bone tissue.

Post – contrast CT scans allow the identification of the lesions. This lesions present neoformation vessels and density changes due to contrast substance accumulation at this site.

### **Acknowledgements**

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## **A RETROSPECTIVE STUDY OF THE EFFICACY OF FEMORAL HEAD AND NECK OSTECTOMY IN DOGS AND CATS (2008-2013)**

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### **Summary**

Medical records of 41 dogs and 8 cats underwent unilateral femoral head and neck ostectomy (FHO) from 2008 to 2013 at the Surgery Clinic of the Faculty of Veterinary Medicine Timisoara, were reviewed. We report the signalment, bodyweight, breed, history, etiology, clinical features and outcome after surgery. In this study we included all dogs were diagnosed with unilateral avascular necrosis of the femoral head, and all cats with femoral head and neck fracture. The problems that was identify in early postoperative time include persistent lameness, discomfort after vigorous exercise, stiffness in cold weather and difficulty jumping and climbing stairs. When total hip replacement procedure is not feasible femoral head and neck excision is a satisfactory outcome to small dogs and cats, however, slightly intermittent lameness may remain.

**Key words:** cat, dog, femoral head, ostectomy

Femoral head and neck excision arthroplasty in dog was first described in 1961 by Spreull and Ormrod (17, 23). The techniques and surgical approaches used as well as the results of the procedure vary (9, 10, 13, 15, 16, 21, 22). In many studies (1, 2, 4, 5, 11, 18) the findings have been based solely on the outcome of owner questionnaires. According to several retrospective studies, the functional results of femoral head ostectomy were better in cats and small dogs, than they were in large dogs (8). The Off and Matis – 2010 suggests that an owner's perception of outcome after this procedure in their animal was much different from the more objective evaluations (16).

Recent studies question the usefulness of techniques of the femoral head and neck ostectomy versus total hip replacement (THR) procedures (3, 20). Additionally, hip replacement technology has made huge advancements in the last 20 years with success rates reported of >80% (12, 14). *Miniature, micro* or *nano* THR turned out practical and effective surgery in medium-size dogs and small breed dogs and cats with coxofemoral disease (6, 7, 14, 24).

The aim of this study was to investigate the efficacy of FHO using the data records of the Surgery Clinic of Faculty of Veterinary Medicine Timisoara. Our objective was to report signalment, bodyweight, breed, history, etiology, clinical

features and outcome after application of the unilateral femoral head and neck ostectomy (FHO).

### Materials and methods

From 2008 to 2013, femoral head ostectomy was carried out in 41 dogs with and 8 cats. In this study were included 41 dogs with unilateral avascular necrosis of the femoral head (Legg-Calvé-Perthes disease) and 8 cats with femoral head and neck fracture. The diagnosis was based on history, clinical and radiographic examination.

A craniolateral approach to the hip was used in all cases. After reflection of the joint capsule and transection of the round ligament, the limb was rotated outwards 90°. Osteotomy of the femoral neck was achieved using an oscillating saw, with or without removal of the lesser trochanter.

After surgery, all owners were trained to apply at home, for the next 4 weeks, the program of postoperative rehabilitation - Home Therapy Program (25).

The obvious were extracted the following information: signalment, bodyweight, breed, history, etiology, clinical features and outcome after surgery.

The patients were clinical evaluated at one week and of one month postoperatively. The radiographs were evaluated preoperatively, immediate postoperatively and two months postoperatively (results not included in this study). Based on the owners' observations versus medical staff evaluation the postoperative functional outcome was recorded. Findings recorded included subjective assessments of lameness, and specific complications. Outcomes of medical staff evaluation were graded as excellent, good, fair, or poor, according to the degree (score) of lameness – table 1.

Table 1

**Lameness degree assessing scale**

Degree	Description	Outcome
0	Normal attitude in station and in walking – without lameness	excellent
1	In walking difficulties, especially at rapid carriage – fine lameness	good
2	In walking difficulties, intermittent lameness in rapid walking	good
3	Evident lameness at every step, pain	fair
4	The leg pull out of support in station and in walking, intense pain	poor

All statistical analyses were performed with commercial statistical software.

### Results and discussions

Forty-nine coxofemoral articulations in 41 dogs and 8 cats were treated by FHO. Median age was 9.6 months (6 months to 2 years) for dogs and 2.6 years (8 months to 6 years) for cats. Twenty-six dogs (20 male [0 castrated] and 22 female [1 spayed]) and 8 cats (3 male [2 castrated] and 5 female [2 spayed]) were registered. Median body weight was 6.5 kg (4-18kg) for dogs and 2.9 kg (1.7 – 4.3 kg) for cats.

The distribution on breeds for the dog shown: cross breed - 26.8% (11/41), Poodle – 19.5% (8/41), Bichon – 17.1% (7/41), Pekingese – 9.8% (4/41), 4 Yorkshire terrier -9.8% (4/41), West highland terrier – 7.3% (3/41), Shih Tzu – 7.3% (3/41), Mops – 2.3% (1/41). Seven cats have been breed European shorthair – 87.5% and one cat – Birman 22.5%.

In our study, indication in dogs was avascular necrosis of the femoral head caused by Legg-Calvé-Perthes disease; most patients suffering from this disease weighed <15 kg (16, 19).

Based on the owners observations, the locomotion on the operated leg at a slow gait was normal in 40 patients (81.6%) and it remained normal at a fast gait in 31 (63.2%). Lameness was seen in 11 patients (22.4%) after strenuous exercise and in 5 patients (10.2%) during cold weather. Of the 49 owners, 91.8% (45) have declared themselves satisfied with the outcome of the surgery. This data recorded were similar with another study (2, 4, 5, 10, 11, 16, 18). The information obtained from pet owners subject to FHO has allowed us to identify, in early postoperative time the following problems: persistent lameness, discomfort after vigorous exercise, stiffness in cold weather and difficulty jumping and climbing stairs. Another authors (1, 4, 16, 18) reported similar results.

Outcomes of medical staff evaluation were presented in table 2.

Table 2

**Functional outcome in according with lameness  
degree assessing scale**

Species	Outcome			
	Excellent no/%	Good no/%	Fair no/%	Poor no/%
<b>Dogs n=41</b>	-	12 / 29.3%	18 / 43.9%	11 / 26.8%
<b>Cats n=8</b>	1 / 12.5%	6 / 75%	1 / 12.5%	0
<b>%</b>	<b>2.1</b>	<b>36.7</b>	<b>38.8</b>	<b>22.4</b>

The results were excellent in 2.1% of cases, good in 36.7%, fair in 38.8%, and poor in 22.4%, resulting 61.2 percent of patients that have had less than excellent results which is a high failure rate. Similar conclusions were formulated and other authors (12, 16). Our evaluation indicated that cats had the fewest postoperative problems. However, the present study revealed discrepancies



between the results of objective clinical evaluations data and subjective observations by owners with regard to FHO. The current assumption that small dogs compensate better after FHO than large dogs must be revised.

Off and Matis - 2010 (16) shows that the pain reduction afforded by FHO occurs at the cost of some loss of limb function even in small dogs, in which lameness may be difficult to detect with the naked eye because of their rapid motion.

Therefore, FHO should be restricted to exceptional circumstances, where joint preservation is not possible or when infection or other contra-indications preclude joint replacement, even in small dogs (12, 16). This may be due to the owner's financial constraints for expensive cost of the THR, unavailability of a surgeon or appropriate implant, or skeletal immaturity of the patient (12).

The FHO is chosen as the surgical treatment option for a variety of conditions: hip dysplasia, trauma, avascular necrosis of the femoral head (Legg-Calvé-Perthes disease) or degenerative joint disease (arthritis). Our observations obtained from relationships with the owners of dogs and cats, cause us to believe, that in our country there are still financial constraints that limit access to therapeutic options more expensive as THR. In these circumstances, although the results of this work show a low rate of success after the FHO, this technique is still a therapeutic option for cats and small young dogs with Legg-Calvé-Perthes disease.

### **Conclusions**

The evident discrepancies exist between the results of objective clinical evaluations (medical staff evaluation) data and subjective observations by owners with regard to outcomes after FHO.

Although FHO offers a low rate of success, it remains a salvage procedure practiced in medical conditions and socio-economic well-defined.

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## **DIAPHRAGMATIC HERNIA IN DOGS AND CATS: A REPORT OF 43 CASES (2001-2013)**

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### **Summary**

Medical records of 26 dogs and 17 cats undergoing surgical repair of diaphragmatic hernia from 2001 to 2013 at the Surgery Clinic of the Faculty of Veterinary Medicine Timisoara, were reviewed. We report the signalment, bodyweight, breed, history, etiology, clinical features and outcome after surgery. Thoracic simple radiographs revealed evidence of diaphragmatic hernia in only 76% of the animals, and additional imaging tests were often needed to confirm the diagnosis. Thirty-nine hernias were repaired through a midline celiotomy and 4 required a partial median sternotomy combined with midline celiotomy. All hernias were sutured primarily without the use of tissue flaps or mesh implants. In the postoperative period 86% of the animals developed pneumothorax. Postoperative survival rate was 88.4%.

**Key words:** cat, diaphragmatic hernia, dog

True diaphragmatic or pleuroperitoneal hernias are defined as subtotal diaphragmatic defects in which the serosa on the thoracic surface of the diaphragm remains intact, thus preventing direct communication between the pleural and peritoneal cavities (4, 12, 13, 19). True diaphragmatic hernias are rare (10, 19).

The false diaphragmatic hernia is a protrusion of abdominal viscera through an opening in the diaphragm and is caused mainly by trauma such as an automobile accident - traumatic diaphragmatic hernia, and rarely by congenital defects - congenital pleuroperitoneal hernia and congenital peritoneopericardial hernia (2, 6, 9, 20). Both congenital hernias have been diagnosed in cats and dogs (1, 19). Up to 85% of diaphragmatic hernias in small animals are traumatic in origin, 5 to 10% are congenital, and the rest are of unknown etiology (2, 21).

Postoperative survival rates ranging from 52% to 94% after surgical correction of a diaphragmatic hernia have been reported in the literature (2, 5, 8, 10, 18, 21). Surgical repair within the first 24 hours of trauma has the highest mortality rate (33%) (16). In another study perioperative survival rate of 89.7% was observed in dogs and cats that received surgical intervention within 24 hours of admission (8).

Based on these findings, the aim of this study was defined to describe the situation at the Clinic of Surgery, Faculty of Veterinary Medicine Timisoara on our casuistry with diaphragmatic hernia in dogs and cats.

### **Materials and methods**

The information used in this study was collected from the medical evidences of the Surgery Clinics of the Faculty of the Veterinary Medicine Timisoara from the past fourteen years (2000 - 2013).

The data base consists exclusively of clinical accounts of the canine and cats cases diagnosed with diaphragmatic hernia. We have determined the type of diaphragmatic hernia and causes, the utility of diagnostic methods used the types of diaphragmatic tears, the organs herniated, the share of diaphragmatic hernia from all the cases of the Surgery Clinics of the Faculty of the Veterinary Medicine Timisoara and from all patients with traumatic pathology, the clinical features types and outcome after surgery. Dogs and cats with diaphragmatic hernia were also classified as dyspneic or not dyspneic at the time of evaluation on the basis of high respiratory rate.

The diagnosis for diaphragmatic hernia was set following corroboration of data from medical history with those obtained after clinical and radiographic simple examination or contrast radiographic studies, and confirmed by exploratory celiotomy.

Surgery for all hernias consisted of the primarily suture of the diaphragmatic breach without the use of flaps of tissue or mesh implants. Surgical herniorrhaphy was performed via a ventral midline celiotomy approach to the diaphragm in all cases. All patients were ventilated during general inhalatory anesthesia for surgical repair by use manual ventilation or a mechanical ventilator.

All statistical analyses were performed with commercial statistical software.

### **Results and discussions**

Forty-three cases (26 dogs and 17 cats) were identified with diaphragmatic hernia. Median age was 4.4 years (0.3 to 12.5 years) for dogs and 2.5 years (0.1 to 12 years) for cats. Twenty-six dogs (15 male [5 castrated] and 11 female [8 spayed]) and 17 cats (8 male [5 castrated] and 9 female [7 spayed]) were registered. Any particular predilection for sex, or breed was not observed. The distribution of the sex and age of the animals in this series was found to be similar with other studies (8, 17).

All dogs registered hernias were categorized as traumatic diaphragmatic hernias. Causes of traumatic diaphragmatic hernia identified by anamnesis, clinical and radiological examination and / or confirmed by exploratory celiotomy included in dogs motor vehicle accidents (14/26 [53.8%]), blunt trauma (7/26 [27%]), and unknown trauma (5/26 [19.2%]). Eight (30.7%) dogs sustained other orthopedic

injuries - fractured ribs, fractured pelvis, and fractured femur and / or soft tissue traumatic injuries - pleural effusion, hepatic congestion, urinary bladder rupture, and muscular hematomas.

Hernia identified by anamnesis, clinical and radiological examination and / or confirmed by exploratory celiotomy included in cats four congenital hernias (23.5%) of which three peritoneopericardial hernia (17.6%), one pleuroperitoneal hernia (5.8%), and thirteen traumatic diaphragmatic hernias (76.5%). Causes of traumatic diaphragmatic hernia in cats included dog attack (5/13 [38.4%]), falling (4/13 [30.8%]), motor vehicle accidents (3/13 [23.1%]), and unknown trauma (1/13 [7.7%]). Five of 13 (38.4%) cats sustained dog attack orthopedic injuries including fractured long bones, fractured ribs, fractured pelvis, coxofemoral luxation or vertebral column fracture and four of 13 (30.8%) sustained other soft tissue traumatic injuries – pulmonary contusion, hepatic congestion, liver lobe torsion, spleen rupture, intestinal incarceration. Two of these cats required euthanasia.

The rate of diaphragmatic hernia diagnosis was 0.38% from all animals examined (11,235) in our clinic and of 2.1% reported at animals with trauma history.

Severe injuries of the thoracic cavity and organs have also been reported in more than 39% of dogs and cats with musculoskeletal trauma (9). Pulmonary contusions, pleural effusion, hemothorax, pneumothorax, and rib fractures are the most common of these injuries. Between 2% and 5% of animals with fractures have a diaphragmatic hernia (10). High incidences of pneumothorax, pneumomediastinum and intrathoracic and intrapulmonary hemorrhages have previously been reported in diaphragmatic hernias (5, 8, 10, 21) in according with present study that revealed higher incidence rate of pneumothorax (86%).

Nine of 26 (34.6%) dogs at the preliminary clinical examination had dyspnea and nineteen of 26 (73%) presented enteric signs – emesis, hematemesis, diarrhea or constipation. Twelve of 17 (70.6%) cats at the preliminary clinical examination had dyspnea and nine of 17 (53%) presented cardiovascular abnormalities. Regardless of the etiology of the diaphragmatic defect, clinical signs related to diaphragmatic hernia are largely associated with dyspnea, effusion or dilation of the gastrointestinal tract (2, 8, 9, 15).

Thoracic simple radiographs revealed evidence of diaphragmatic hernia in only 76% of the animals (33/43), and in ten cases (24%) additional imaging tests - barium sulfate or a water soluble iodinated positive contrast medium administered by orogastric intubation - were often needed to confirm the diagnosis.

Thirty-nine hernias were repaired through a midline celiotomy (90.7%) and four cases (9.3%) required a partial median sternotomy combined with midline celiotomy (three cases of the cats with liver lobe torsion, spleen rupture, intestinal incarceration and one dogs with massive pleural adhesences. From thirty-nine patients with traumatic diaphragmatic rupture 29 cases (74.4%) received surgical intervention within 24 hours of trauma, and 10 (25.6%) received surgical intervention after 2 days of trauma (2-96 days).

Three types of diaphragmatic tears were identified in thirty-nine traumatic diaphragmatic hernia: circumferential – 35.9% (14/39), radial – 41.1% (16/39), and combination circumferential-radial - 23% (9/39). The tears location, left to right distribution, were identified in left-sided in 44% of cats (6/13), right-sided in 38% of cats (5/13), ventral in 15% of cats (2/13). In the dogs, tears was location in left-sided in 38.5% (10/26), right-sided in 42.3%, (11/26), ventral in 15.4% (4/26), and multiple in 3.8% (1/26). In previous studies (3, 7, 21), the left sided hernia was believed to be more common, and in another study (11) the right sided location was indicated to be common. However, this predilection was not observed in this study. In essence, left to right distribution is probably uniform in dogs and cats, with sporadic cases having bilateral or multiple tears (5, 7, 8, 9, 16, 21).

The liver, small intestine, and pancreas tend to herniate through right-sided tears, whereas the stomach, spleen, and small intestine more commonly herniate through left-sided tears. Which organs occupy a hernia depend on various factors, including the site of the diaphragmatic tear, proximity of the organs, and range of movement of supporting ligaments or mesentery (10).

Five of 43 (11.6%) cases (two dogs and three cats) died or were euthanatized postoperatively. Post-mortem examination confirmed that one dog died of multiple organ failure, one cat died as a result of acute pulmonary edema and two cats with vertebral column fracture required euthanasia. Postoperative survival rate was 88.4%. Any particular predilection for patients with traumatic diaphragmatic rupture that received surgical intervention within 24 hours of trauma, and animals that received surgical intervention after 2 days of trauma was not observed.

Postoperative survival rates reported in the literature (2, 5, 8, 9, 10, 14, 18, 21) ranging from 52% to 94% after surgical correction of a diaphragmatic hernia.

### **Conclusions**

Blunt force trauma likely to cause diaphragmatic hernia in animals includes motor vehicle trauma, falling from heights, and animals attacks.

Diaphragmatic hernias his strong associated with severe injuries of the musculoskeletal system thoracic and abdominal cavity and organs

Thoracic simple radiographs revealed evidence of diaphragmatic hernia but, additional imaging tests were often needed to confirm the diagnosis.

If patient is stabilized appropriately prior to surgery, the survival rate is equally good for animals operated on early time or on long time after diaphragmatic hernia was produced.

The predilection location of the diaphragmatic tears, left to right distribution, was not observed in this study.

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## EQUINE KELOID - CLINICAL AND THERAPEUTICAL ASPECTS

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### Summary

In the present study were performed observation about the equine keloid concerning his clinical appearance and therapeutic. In this study were took in observation 18 horses, pure and mixed Romanian horse breed. The keloid have predilection for hind lambs. The size of keloid in variable and different from the size of a chestnut to the size of a hand ball. The keloid structure is based on collagen fibers, confirmed by the histopathology tests were it can be seen the anarchic distribution and big diameter of the collagen fibers. The keloid therapy is difficult because the attempts with corticosteroids bandage, triamcinolone acetonid and silicone gel produced uncertain results.

After surgical treatment by ablation of the keloid, good results were obtained; the skinless area was covered with skin and protected by a bandage. In cases of large keloid scars the results are poor because the diseases reoccur. In our casuistry we saw recur disease and complications in 4 cases. The complications were characterized by chronic lymphangitis, chronic edema and skin sclerosis.

**Key words:** equine, keloid, clinical, therapy

Equine keloid is benign proliferation with tumoral appearance of dermal collagen following traumatic injuries.

The equine keloid is one of the most serious pathology of the wound healing tissue, especially in horse (3). Between the complete healing of the wound and the beginning of the keloid tissue may be a variable time line. Equine keloid appears mostly after accidental wounds that are untreated or improperly managed.

Equine keloid is often localized at the limbs extremities (pastern, fetlock, hock), where there is a poor muscular layer and a rich conjunctive tissue. Humans and horses are the only mammals known to naturally develop excessive granulation during wound healing; however, similarities and differences between fibroblast populations and associated collagen have not been reported(5,6,9).

The keloid has a tumor-like shape and its structure is mostly type III and type II collagen, which has a fast growth both in horizontal and vertical pattern.

The goal of this study is to assets and to evaluate the limit between normal healing and Keloid, the evolution and the therapy of this healing process which is a common complications of cicatrisation in horses.

### Material and methods

Clinical investigation and observations were performed during 10 years, on horses addmitted at the Surgery Clinic of the Veterinary Faculty in Cluj-Napoca for diagnostics and therapy.

For this study were used 18 horses of different ages that were exploited for extensive work and also for sport. For each case the next aspects were recorded: size, localization, expansion, exterior aspect, tegument health status, presence and/or absence of necrotic tissue, presence of fistulas, regional changes due to the tumor compression, and patient's general health status.

Regarding the therapeutic procedure it must be mentioned that all horses were submitted to preoperative examination before the surgical intervention.

Ablation of the keloids were performed made through the elliptical incision of the peritumoral tissues with the scalpel at the base of tumor, and also keeping the marginal wound epithelium intact(4,7). The excision and the removal of the tumor was performed systematical, progressive, and controlling the haemorrhage by a good hemostasis. The hemostasis was made differently in each case by forcipressure, mediated suture, electro-cauterization, thermo-cauterization, and hemostatic buffers (7).

In cases of small size keloids, the skin around the removed tumors was isolated by sharp and blunt dissection and by mobilizing it the wound was closed by interrupted suture pattern, on this way the wound surface that resulted after the keloid ablation was covered entirely by skin. Where the tumors were large in size the ablation process was performed by removing a little part each time, this was made to protect the surrounding anatomical structures (tendons, bones, nerves, sinovial sheath). After ablation topical therapy was applied for hemostatic and healing purpose (7).

### Results and discussions

In our study, regarding the localization of the keloid, this was found in 85% of cases at the extremities of the limbs (metatarsal region, metacarpal region, pastern), mostly at the posterior limbs (Fig.1 and 2).



Fig.1. Fetlock localization



Fig.2. Pastern localization

Beside the previous localizations, the tumors were found sporadically in atypical area as the frontal (Fig.3) and cervical region.



Fig. 3. Frontal region localization

Other criteria that we followed in our research were the size of the tumors and their shape. The size of the tumors varied from the size of a nut to the size of a handball. The base of the tumors was either a pedicle or a large implantation area (Fig. 4).



Fig. 4. Large implantation base

There is a large variability in what concerns the shape of the tumors, meaning that the most frequent shapes were: round, spherical, oval, bilobated

(Fig.5.) and easy elongated. Due to this aspect in many cases the tumors have had a large implantation area.



Fig. 5. Bilobated keloid

At the examination of the tissues surrounding tumors, the next aspects were encountered: tumefaction, size enlargement, induration, painless.

The aspects of the examined keloids were: the small ones had a smooth, glossy, red-pink surface, covered sometimes with dirt; the large keloid had an irregular cauliflower-like shape, with large creases, with a rough thick tegument, with debridements and with necrotic tissue and large deep fistulas. On the surface, some keloids were covered by dark crusts under which were necrotic areas with ulcers and suppurations, all these next to a ichorous odor.

The presents and the growth of the tumors, in time, perturbed the venous blood flow, resulting edemas, limphangitis, which in time had led to dermal sclerosis etc.

In section, the keloid aspect is fibrouse tissue, compact, dens, fatty (Fig. 6), white-pink in color, with dark areas to the surface and white in profound aspect.



Fig. 6. The dens appearance on the section

Histological, the keloid appears as an agglomeration of collagen fibers, unorganized with an amorphous appearance in section (Fig. 7).

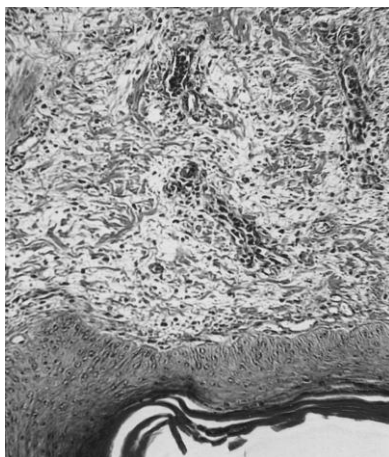


Fig.7. Collagenic microstructure of the keloid

The hematological findings from horses with keloid were inconclusive. The surgical therapy was efficient in all 7 cases where the tumors size was little, the tissue gap being covered with the surrounded skin. In 4 cases, because of the large gap that resulted after the tumor removal, 20-25cm, the surgical intervention was not followed by successfull, even though next to it, topic medication with antibiotic powders, oxytetracilin spray, antibiotic ointments or other substances were applied(1,2,8). It must be mentioned that more the surgical intervention is delayed more the skin structure changes by collagen infiltration, followed by less resistance of the skin. This will negatively modify the wound closure and its healing process.

### **Conclusions**

Equine keloid appears as a wound healing complications in most cases because of unproper, incomplete or incorrect wound therapy.

The most frequent localization is at the posterior limbs, at the metatarsal, pastern and/or fetlock regions.

Equine keloids clinically appears as a tumor-like growth to vary in size, with or without complications, such as necrosis of the tumoral tissue.

The biological structure of the developed tissue composed from thick collagen fibers, with a circular and anarchic pattern.

The most efficient therapy is total ablation of the tumor and the wound closure with a skin flap by interrupted pattern suture.

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## ARTERIAL BLOOD PRESSURE MEASURED BY OSCILLOMETRIC METHOD IN CATS WITH HYPERTHYROIDISM

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### Summary

The study was carried out on seven cats, aged between 8 and 14 years, diagnosed with hyperthyroidism. The serum thyroxine ( $T_4$ ) concentration in all cats included in this study was higher than 5  $\mu\text{g/dl}$ . Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) were measured by oscillometric method. The mean SBP, DBP and MBP values were  $183 \pm 16,5$  mmHg,  $122,8 \pm 10,4$  mmHg,  $145,2 \pm 13,8$  mmHg, respectively. Oscillometric blood pressure measurement is an effective method in detecting hypertension in cats with hyperthyroidism.

**Key words:** hyperthyroidism, blood pressure, cats

Blood pressure may be assessed by direct or indirect methods. In a clinical setting, the indirect techniques (oscillometric or Doppler ultrasonographic methods) are more readily applicable for the routine measurement of blood pressure (3, 4).

Hypertension is a sustained elevation of blood pressure above that which is normal for individual (5). Persistent hypertension can lead to damage a variety of organs such as the eye, kidney, heart and central nervous system (3, 5). Unlike human, the cats suffer predominantly from secondary hypertension. The most common cause of hypertension in cats are the chronic renal disease and the endocrinopathies. Therefore, elevation of blood pressure can be an early diagnostic marker of various diseases (4, 5, 8).

The aim of this study was to assess the prevalence and the type of the arterial hypertension measured by oscillometric method in cats with hyperthyroidism.

### Materials and methods

The study was carried out on seven cats aged between 8 and 14 years, of different breeds, diagnosed with hyperthyroidism to the Veterinary Clinics of the Faculty of Veterinary Medicine Timisoara. In all cats included in this study, the serum thyroxine ( $T_4$ ) concentration, measured through electrochemiluminescence method, was higher than 5  $\mu\text{g/dl}$ .



The systolic blood pressure (SBP), the diastolic blood pressure (DBP), the mean blood pressure (MBP) and the pulse frequency were determined by oscillometric method, using the CARDEL Veterinary Monitor 9401 device. The blood pressure was recorded on the left thoracic member, with the limb of the cat at the level of the heart, using a cuffs width of about 30-40% of the forearm circumference. The measurement of the arterial blood pressure was performed in the owner's presence, before the physical examination, after a 10-15 minutes period of accommodation with the room and measurement devices.

There were performed five consecutive measurements for each cat, at 1-2 minutes interval and the arterial blood pressure value was calculated as the mean of five consecutive measurements.

In this study, the arterial hypertension was define as SBP and/or DBP higher then 150/90 mmHg. These are the limits between normal blood pressure and hypertension presented in *Guidelines for the identification, evaluation and management of systemic hypertension in dogs and cats*, established by Veterinary Blood Pressure Society and ACVIM Hypertension Consensus Group (3).

### Results and discussions

The systolic blood pressure have recorded a mean value of 183,0 mmHg, 95% of the values were between 170,6-195,4 mmHg. The mean value of diastolic blood pressure was 122,8 mmHg and 95% of the values were between 115,0-129,9 mmHg. The mean value of the mean blood pressure was 145,2 mmHg (table 1). The interindividual variations amplitude regarding the blood pressure value was between 30 and 48 mmHg.

Table 1  
**Descriptive statistic of the blood pressure values and the pulse frequency on the cats with hyperthyroidism**

Variables	n	Mean	Standard deviation	Standard error mean	Range
<b>SBP (mmHg)</b>	<b>7</b>	<b>183</b>	<b>16.5</b>	<b>6.2</b>	<b>48.0</b>
<b>DBP (mmHg)</b>	<b>7</b>	<b>122.8</b>	<b>10.4</b>	<b>3.9</b>	<b>30.0</b>
<b>MBP (mmHg)</b>	<b>7</b>	<b>145.2</b>	<b>13.8</b>	<b>5.2</b>	<b>45.0</b>
<b>Pulse frequency</b>	<b>7</b>	<b>148.2</b>	<b>10.9</b>	<b>4.1</b>	<b>33.0</b>

In this study, the blood pressure measured by oscillometric method has revealed the presence of hypertension in 85% of the cats (figure 1). In all hypertensive cats the SBP/DBP values were higher than 160/109 mmHg.

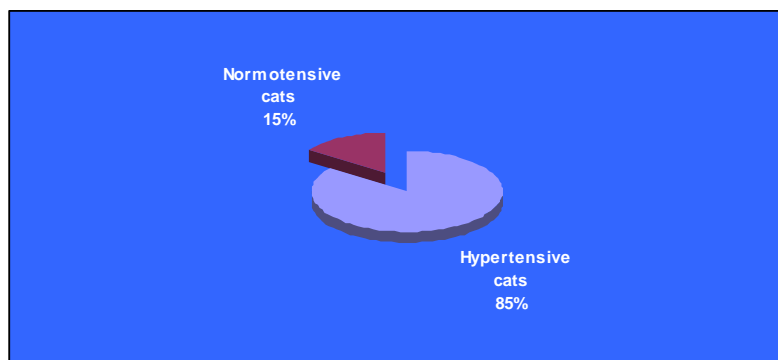


Fig.1.The prevalence of hypertension in the cats with hyperthyroidism

The relationship between hyperthyroidism and arterial hypertension is not yet sufficiently clarified, as there are controversies regarding the arterial hypertension prevalence of the cats with this disease. Thus, Kobayashy et al. (6) have found the arterial hypertension in 87% of cats diagnosed with hyperthyroidism, while, more recent, Syme and Elliot (10) reported a prevalence between 9 and 12%.

The hyperthyroidism leads to the increased of the  $\beta$ -adrenergic receptors number and their sensibility to the catecholamines action. Also, the thyroid hormones promotes the cardiac hypertrophy due to activation of protein synthesis, after triiodothyronine binds to receptors of myocardial cell nuclei. Also, L-thyroxine has a direct inotrope effect and the thyroid hormones amplify the sympatho-adrenergic effects. All this changes leads to the rise of blood pressure as a result of increased cardiac output and vasoconstriction due to sympathoadrenergic disturbance (5).

There was no significant correlation between the blood pressure value and serum thyroxine concentration (table 2).

Table 2

**Correlations between serum  $T_4$  concentration, blood pressure, and pulse frequency on hyperthyroid cats**

Variable	s	$T_4$ ( $\mu\text{g/dl}$ )	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	Pulse frequency
$T_4$	<b>Spearman's correlation</b>	1.000	0.286	0.143	0.394	0.964**
	<b>p</b>	-	0.535	0.760	0.819	< 0.001
	<b>n</b>	7	7	7	7	7

**\*\*Correlation is significant at the 0.01 level (2-tailed)**

**Note:** SBP-systolic blood pressure; DBP-diastolic blood pressure; MBP-mean arterial blood pressure

Although the increase in blood pressure is determined by the thyroid gland hypersecretion, clinical trials revealed no significant correlation between serum thyroxine concentration and the blood pressure level (2).

More, some cats remain hypertensive after restore the euthyroidism, which suggest that some cats could present hypertension non-linked with the hyperthyroidism state (7). In those cases the decline in renal function after the treatment of the hyperthyroid state could explain the occurrence of arterial hypertension. Backer et. al. (1) has found a significant increased of the glomerular filtration rate on the cats with hyperthyroidism compared whit the healthy ones. It was not directly proven if this mechanism is associated with the glomerular hypertension development, but it is possible to be involved in causing renal injury in cats with hyperthyroidism (9).

### **Conclusions**

Oscillometric blood pressure measurement is an effective method in detecting arterial hypertension in cats with hyperthyroidism.

There was no significant correlation between the blood pressure value and serum thyroxine concentration.

In this study, mixed arterial hypertension was found in cats with hyperthyroidism.

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## RESEARCH ON PUERPERAL UTERINE PATHOLOGY

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### Summary

After implementing bacteriological examinations on 132 samples of uterine secretions collected from cows with calving eutocica, 24 hours, 3days, 6 days, 9 days, 15 days and 21 days post-partum were identified following genera: *Streptococcus*, *Enterococcus*, *Micrococcus*, *Staphylococcus*, *E. coli*, *Proteus*, *Pseudomonas*, *Arcanobacterium* and *Fusobacterium*.

Bacteria growth was as follows: 24 hours post-partum was identified in all species described above. Starting on the third day post-partum no bacteria were isolated belonging to the genera *Micrococcus*, *Enterococcus* and *Proteus*, but bacterial associations were present consisting of 2 or 3 genres.

In 36% of the samples collected at 15 days post-partum there were identified associations represented by two bacterial species.

After 9 days post-partum, six cows had puerperal endometritis in pathological secretions identified following joint infections: *Streptococcus*- *Arcanobacterium pyogenes* - *Pseudomonas aeruginosa*; *Staphylococcus* - *Streptococcus*, *Fusobacterium necrophorum* - *E. coli* and *Staphylococcus* – *Streptococcus* – *E. coli*.

**Key words:** puerperal uterus, bacterial flora, uterine infection.

Physiological changes that occur in the cows body during the gestation, at the time of parturition, lactation and during the outburst of the genital reconstruction, to achieve a future conception, demand a lot of their body and significantly reduce the cows resistance to pathogenic or conditionally pathogenic microorganisms, present in their living environment (2, 4, 6).

Therefore, along the entire period of gestation and especially during the puerperium, in order to ensure a normal reproduction, it is necessary to pay special attention to each cow, in order to reduce, as much as possible, the factors that could negatively affect the cow health condition (3, 7, 9).

The uterine infections in cows can be produced by virulent pathogenic bacteria, which usually have a high electivity for the genitals and can cause generalized infections accompanied by abortion (*Brucella abortus*, *Campylobacter fetus*, *Leptospira*, *Listeria* etc.) or by low virulence bacteria or conditionally

pathogenic bacteria, such as: staphylococci, streptococci, Arcanobacterium pyogenes, Fusobacterium necrophorum, Pseudomonas aeruginosa etc., which usually come from the living environment of the animals or reach their genitals through their blood or lymph, and, in cases in which the ovary estrogen-generating function is reduced or abolished, they trigger infection (1, 5, 8).

Usually, in cows, the puerperal infections are localized, for this species has defensive capabilities superior to those of other species females (3) and are not followed by general health disorders. To prevent the puerperal infections in cows, besides ensuring proper zoohygiene and feeding conditions, special attention should be paid to deeply knowing and permanently monitoring the dynamics of the morphological and functional changes of the reproductive system, of the parturients, as well as the clinical manifestations of the infections that may occur during this period, and, if the case is, to determine, by laboratory examinations, the microbial agents responsible for their occurrence (1, 7).

### **Material and methods**

The investigation of the puerperal uterine bacteriology was performed in the period 2012-2013.

**Samples:** 132 uterine secretions samples collected from 22 cows with eutocic calving, every 24 hours, 3 days, 6 days, 9 days, 15 days and 21 days post partum.

Was used culture: broth and nutrient agar; sheep blood agar; broth and VL agar; horse blood VL agar, brilliant green and sodium azide.

Other materials : defibrinated sheep blood; defibrinated horse blood, 0.04 % solution of brilliant green; 0.6 % solution of sodium azide; 16/160 mm tubes; 20/200 mm tubes; sterile plastic Petri dishes with a diameter of 100 mm; reducing mixture; glass lamellas; sterile graduated pipettes; sterile insemination pipettes; sterile pestle mortars, dye solutions for Gram method.

Instrumentation: sterile syringes; vaginal speculum; insemination needle.

Equipment: thermostat set at 37 Celsius degrees; autoclaves; electric oven; fridge; stereomicroscop; optical microscope, anaerostate; temperature-controlled water bath etc.

### **Technique**

#### ***Sampling technique***

The sampling for the bacteriological examination was performed by asepsia techniques, by means of the instruments used for the artificial insemination of the cows, as follows:

- the insemination pipettes used for sampling in the womb, before their introduction into the female genitalia, were protected by the polyethylene bag in which they were preserved, a bag pierced by means of the tip of the pipette, only after crossing the vagina and after the penetration into the cervical canal, in order

to avoid the contamination of the sample with the existing microbial flora of the vagina.

For the sample collection from the uterus, a disposable syringe of 20 ml was adapted at the external tip of the pipette for the necessary samples collection by suction. After customization, by marking the female registration number on the appropriate tube, the samples were transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine of Bucharest, within 3 hours following their collection, where they were processed by complex bacteriological examination.

***The technique for the isolation and identification of the bacteria possibly present in the samples.***

Each sample was examined macroscopically, retaining the data on the sample consistency, appearance, presence of pus or blood, after which it was transferred to a sterile mortar and, if necessary, it was 1/10 thinned with a sterile physiological solution or with nutrient broth. Several smears were examined from the obtained suspension, in order to color them by means of the Gram staining method. Simultaneously, inseminations were performed from each sample with the platinum loop in or on the following culture media: broth and nutrient agar, sheep blood agar; horse blood VL agar, brilliant green and sodium azide.

After the customization, all inseminated media were incubated at 37 Celsius degrees for 24-72 hours and they were daily examined except for the VL agar, horse blood, brilliant green and sodium azide plates, which were incubated for 24-72 hours in anaerobiosis.

After the inseminations, the smears were colored by the Gram staining method and they were examined by light microscopy, by means of the immersion lens, in order to relieve any bacteria present in the collected samples and their main morphobiological features (size, tinctorial affinity, shape, manner of grouping etc.).

Cultures occurring at the surface of the inseminated media were daily examined for 72 hours following their occurrence, with the necked eye and by means of the stereomicroscope, after which, transplants per corresponding media pairs were made of each type of isolated, developed colony in order to obtain pure cultures. After their transplanting, the inseminated culture media were incubated at 37 Celsius degrees, both aerobically and under anaerobic conditions. Smears from the obtained cultures were colored by the Gram staining method in order to check their purity. Pure cultures obtained were subject to identification by means of classical techniques.

The classification of the isolated strains was made by corroborating the data obtained on:

- the examination of the cultivation conditions and of their culture features
- the examination of their morphological features
- the examination of their biochemical properties

### Results and discussions

The results of the bacteriological investigations are synthetically presented in tables 1 and 2.

Table 1

**Bacterial species isolated from vaginal secretions of cows after 1, 3, 6, 9, 12 and 21 days after calving**

No. of processed samples	Isolated bacterial species										Total no. of isolated bacterial strains
	Positive Gram bacteria						Bacteria				
	<i>Streptococcus</i> $\beta$ hemolytic	<i>Staphylococcus</i> $\beta$ hemolytic	<i>Micrococcus</i> spp..	<i>Enterococcus</i> spp..	<i>Arcanobacteriu</i> m pyogenes	<i>Clostridium</i> perfringens	<i>Proteus</i> vulgaris	<i>Escherichia</i> coli	<i>Pseudomonas</i> aeruginosa	<i>Fusobacterium</i> necrophorum	
132	39	27	34	27	6	12	6	41	2	3	197

Table 2

**Bacterial associations present in uterine secretions collected from 22 cows after 1, 3, 6, 9, 12, 15 and 21 days postpartum**

Total no. of processed samples	Bacterial association	Number of positive samples	Remarks
132	<i>Micrococcus</i> spp. <i>Escherichia coli</i>	7	
	<i>Enterococcus</i> spp. <i>Escherichia coli</i>	12	
	<i>Proteus vulgaris</i> <i>Escherichia coli</i>	6	
	<i>Micrococcus</i> spp. <i>Enterococcus</i> spp.	15	
	<i>Staphylococcus</i> <i>Streptococcus</i>	18	
	<i>Clostridium perfringens</i> <i>Micrococcus</i> spp. <i>Escherichia coli</i>	12	
	<i>Streptococcus</i> <i>Staphylococcus</i> <i>Fusobacterium</i> necrophorum <i>Escherichia coli</i>	3	During the period of 6-9 post partum days, the 6 animals were clinically



	<i>Streptococcus</i> <i>Arcanobacterium pyogenes</i> <i>Pseudomonas aeruginosa</i>	2	diagnosed with endometritis
	<i>Staphylococcus</i> <i>Streptococcus</i> <i>Escherichia coli</i>	1	

The analysis of the data in table number 1 shows that: of the 132 uterine secretions samples collected from the 22 cows after 1, 3, 6, 9, 15 and 21 days following the calving, 197 bacterial strains were isolated from 145 that belonged to Gram positive bacteria of the genus *Streptococcus* (39 strains), *Staphylococcus* (27 strains), *Arcanobacterium* (6 strains), *Micrococcus* (34 strains), *Enterococcus* (27 strains) and *Clostridium* (12 strains), and 52 strains belonged to Gram bacteria - of the genus *Escherichia* (41 strains), *Pseudomonas* (2 strains), *Proteus* (6 strains) and *Fusobacterium* (3 strains).

Based on data included in table no. 2, they may say that in none of the samples the presence of bacteria was ascertained, they never isolated a single species, but associations of 2 to 4 bacterial species: in 7 cases, the association *Micrococcus*-*Escherichia coli*; in 12 cases, the association *Enterococcus*-*Escherichia coli*; in 6 cases, the association *Proteus vulgaris* - *Escherichia coli*; in 15 cases, the association *Micrococcus* – *Enterococcus*; in 18 cases, the association *Clostridium perfringens*- *Micrococcus* - *Escherichia coli*; in 3 cases, the association *Streptococcus* - *Staphylococcus* - *Fusobacterium necrophorum* - *Escherichia coli*; in 2 cases, the association *Streptococcus* - *Arcanobacterium pyogenes* - *Pseudomonas aeruginosa*, and, in one case, the association *Staphylococcus* - *Streptococcus* - *Escherichia coli*.

Starting on day 3 postpartum, no bacteria belonging to the genera *Micrococcus*, *Enterococcus* and *Proteus* were isolated, and the bacterial flora varied widely from one animal to another, being represented in 52 % of the samples by the association *Streptococcus*-*Staphylococcus*; in 38 % of the samples by the association *Streptococcus* - *Staphylococcus* - *Escherichia coli*; in 7% of the samples by the association *Clostridium perfringens* - *Micrococcus* - *Escherichia coli*; in 2% of the samples by the association *Streptococcus* - *Staphylococcus* - *Fusobacterium* - *Necrophorum* - *Escherichia coli* and 1 % of the samples by the association *Streptococcus* - *Arcanobacterium piogenes* - *Pseudomonas aeruginosa*.

In 6 of the 22 cows monitored during the 6-9 postpartum days, they found out, by clinical examination, the presence of endometritis. In the uterine secretions collected and examined, the isolated bacterial flora varied from one animal to another: 4 bacterial species (*Streptococcus*, *Staphylococcus*, *Fusobacterium necrophorum*) were isolated from 3 cows; 3 bacterial species (*Streptococcus*, *Arcanobacterium piogenes*, *Pseudomonas aeruginosa*) were isolated from 2 cows;

and 3 bacterial species (*Staphylococcus*, *Streptococcus*, *Echerichia coli*) were isolated from one cow.

### Conclusions

In the period 2012-2013, there were undertaken investigations on the cow puerperal uterine pathology. For this purpose, 22 cows were monitored over a period of 21 days after calving, and, after 1, 3, 6, 9, 12, 15 and 21 days, 132 samples of uterine secretions were collected and processed by complex bacteriological examination.

The investigations generally allow the following conclusions:

1. 197 bacterial strains were isolated of the 132 samples of uterine secretions, of which 145 belonged to the species Gram+ and 52 strains to the species Gram-.
2. Of the 145 Gram+ strains, 39 belonged to the genus *Streptococcus*, 27 to the genus *Staphylococcus*, 34 to the genus *Micococcus*, 27 to the genus *Enterococcus*, 12 to the genus *Clostridium* and 6 to the species *Arcanobacterium pyogenes*.
3. The 52 strains of Gram- were represented by the following species: 41 strains of *Escherichia coli*, 6 strains of *Proteus vulgaris*, 2 strains of *Pseudomonas aeruginosa* and 3 strains of *Fusobacterium necrophorum*.
4. Between days 6-9 after the calving, 6 of the 22 cows monitored by clinical examination were diagnosed with endometriosi: in 3 of them it was present the association *Streptococcus* - *Staphylococcus* - *Fusobacterium necrophorum* - *Escherichia coli*; in 2 animals the association *Streptococcus* - *Arcanobacterium pypogenes* - *Pseudomonas aeruginosa*, and in one cow the association *Staphylococcus* - *Streptococcus* - *Escherichia coli*.

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## MEDICAL MANAGEMENT IN A CAT WITH PYOTHORAX

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### Summary

A domestic 13-years old male cat presented due to, dyspnea, lethargy, coughing and anorexia. Radiographic examination revealed a bilateral pleural effusion, which was diagnosed as pyothorax based on macroscopic and cytological examination of the fluid drained from the pleural space. The first thoracocentesis was made with diagnostic and therapeutic purpose and it allowed the removal of 500 ml of purulent fluid. Resolution of clinical signs had occurred 5 days after the treatment started, although radiographic abnormalities were still present.

**Key words:** pyothorax, cat, medical management

Pyothorax or pleural empyema is a condition characterized by the accumulation of purulent septic inflammatory effusion within the pleural cavity and is a serious life-threatening condition in the cat (2, 3, 4). There are many potential causes of feline pyothorax, but the inciting cause remains undetermined in most cases. In cats, penetrating wounds to the thorax (e.g., bite wounds), extension of bacterial pneumonia and hematogenous spread of bacteria into the thorax are most commonly suspected (1, 2, 4). Common clinical signs include inappetance or anorexia, dyspnoea, lethargy, weight loss, pyrexia. Diagnosis is usually plain, based on clinical signs, thoracic radiography, thoracocentesis and cytology (5, 7). Treatment for pyothorax can be surgical and/or medical, although the most appropriate treatment protocol has not been established. Medical treatment involves administration of intravenous fluid, broad-spectrum antimicrobial therapy and thoracic drainage with or without thoracic lavage (4, 5). Thoracic drainage is usually provided by placement of thoracostomy tubes and it has been associated with an improved outcome, though in cases where drain cannot be placed (usually for financial reasons) one thoracocentesis and long term antibiotic therapy can still yield good survival rate (4, 7). There is controversy regarding the necessity for thoracic lavage as a treatment for pyothorax (4, 5).

This case report describes a cat with pyothorax treated with an aggressive medical treatment and repeated thoracocentesis with the drainage of the pleural space without pleural lavage.

### Materials and methods

A 13-year-old, 4,3 kg indoor - outdoor male neutered domestic cat presented to the University Veterinary Clinics, Faculty of Veterinary Medicine Timisoara, with a history of anorexia, dyspnoea, coughing and lethargy. The cat was reported to be anorexic and to have lost weight over the previous four days. The cat had been vaccinated annually against feline panleukopenia, feline calicivirus and feline herpesvirus since has been in the owner possession from birth. There was no history or clinical signs of recent trauma of the thoracic area. The radiographic images were achieved with Siemens Multix Swing unit, Carestream Vita CR system and procesed with Examion AQS Veterinary Software version 10.1. The sanguine hematological examination was performed with an automated analyzer (ADVIA 2120i) and the biochemical sangvine evaluation was performed with an automatic analyzer (Eos Bravo Forte).

### Results and discussions

Clinical examination revealed that the cat was lethargic and poorly responsive with an 8% degree of dehydration. The patient was tachycardic with a weak peripheral pulse and cold extremities. Also, tachypnoea and an inspiratory dyspnoea were observed and increased respiratory distress in lateral recumbency. Auscultation revealed decreased vesicular murmur bilaterally. The rectal temperature was 37.6°C and peripheral lymphnodes were normal.

Radiographic examination (figure 1) was taken without sedation and revealed in laterolateral view opacity in the medium and ventral thorax covering the cardiac silhouette and adjacent diaphragmatic margin.



Fig. 1. Laterolateral radiographic view (before pleural drainage) in a 13-years old cat with pyotorax

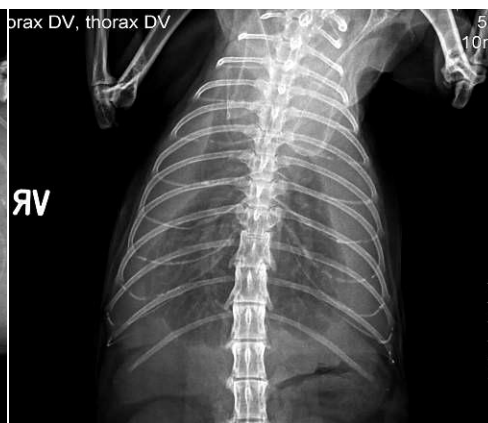


Fig. 2. Dorso-ventral radiographic view (before pleural drainage) in a 13-years old cat with pyotorax

In dorsoventral incidence the lungs were separated from the thoracic wall, indicating the presence of a pleural effusion (figure 2).

Bilaterally thoracocentesis was made with diagnostic and therapeutic purpose without sedation because the patient was severely dyspneic. The first thoracocentesis allowed draining about 500 ml of yellow - grey purulent material with a high turbidity. The fluid obtained was a septic exudates with total protein 3.9 g/dl, total nucleated cell count 8,400/ $\mu$ l and pH 6.7. Pyotorax was diagnosed based on macroscopic and citological examination of the pleural effusion. The presence of the high celularity was revealed by cytological exam (erythrocytes, normal and degenerated neutrophils, histiocytes, histiomaophages and a high number of plasmocytes without any atypical cells). It was concluded that a nonspecific mixt inflammatory process was present.

After that, a radiographic laterolateral view was made to assess the amount of the purulent effusion remain in pleural cavity (figure 3).



Fig.3. Laterolateral radiographic view (after pleural drainage) in a 13-years old cat with pyotorax

The sanguine hematological examination revealed only a mild leukocytosis (total white blood cell count 26800/ $\text{mm}^3$  with a reference range 5000 to - 19000/ $\text{mm}^3$ ), with moderate neutrophilia (19200/ $\text{mm}^3$ ). Regarding biochemical serum results the abnormal values were: slight increased blood urea nitrogen due to dehydration (49 mg/dl; reference range 7 to 32 mg/dl), moderate hypoproteinemia (4.43 g/dl; reference range 5.6 to 7.9 mg/dl) with hypoalbuminemia (1.72 g/dl; reference range 2.6 to 4.0 g/dl).

In one study performed on 26 cats with pyotorax, common historical findings were dyspnea (85%), lethargy (65%), and anorexia (62%), common clinical signs were dyspnea (89%), tachypnea (73%), fever (39%), and hypothermia (27%). Frequent laboratory abnormalities were leukocytosis (68%) with a left shift (100%), anemia (65%), hypoalbuminemia (91%), hyperglobulinemia (86%), hyperbilirubinemia (60%) as well as azotemia (52%) (6, 8).

For bacteriologic examination, samples of exudates collected from pleural cavity were cultured but the result was negative, furthermore the antibiotic therapy was instituted. The treatment was instituted with intravenous fluid and broad-spectrum antimicrobial therapy (ampicilin with sulbactam - Ampiplus; Antibiotice S.A.: 20 mg/kg i.v. q12 h and enrofloxacin - Baytril; Bayer: 5 mg/kg s.c. q12h).

The cat has resumed her food appetite after two days since the antibiotic therapy was initiated and also was observed an improvement in respiratory signs. The second bilateral thoracocentesis was performed after two days from the first thoracic drainage and it allowed the removal of 230 ml of purulent fluid. The macroscopic aspect of the fluid was improved but degenerate neutrophils were still presents in large number.

Another radiographic examination (figure 4) was taken after ten days of treatment and revealed opacity in the ventral thorax covering two-thirds of the cardiac silhouette and adjacent diaphragmatic margin.



Fig. 4. Laterolateral radiographic view (after 10 days of treatment) in a 13-years old cat with pyothorax

After 14 days of treatment, the clinical condition of the cat has improved considerably with significant remission of respiratory signs. The antimicrobial therapy with ampicilin and sulbactam was continued for another 14 days.

### **Conclusions**

In this case, repeated thoracocentesis with pleural effusions drainage (without thoracic lavage) and broad-spectrum antimicrobial therapy for four weeks was successful. After four weeks of treatment the patient was recovered completely with a good clinical condition and without the presence of respiratory signs.

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## **CASE REPORT: A DELAYED UNION CASE AFTER MIPO IN A CROSS-BREED DOG**

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### **Summary**

Many specialists cite a series of indication for MIPO (Minimally Invasive Plate Osteosynthesis), most times recommending this technique for highly comminuted fractures and more. Some of our recent studies compared simple fractures healing times after MIPO and ORIF and the results were similar. However, as it has been mentioned before the possible occurrence of nonunion or delayed union cases when simple transverse fracture are immobilized by bridging plating, therefore we would like to present a case of delayed union in an femur fracture remedied by MIPO, in a cross-breed dog.

**Key words:** MIPO, dog, delayed union

Delayed union refers to a fracture that has not healed in the usual time for that particular fracture (veterinary handbook, ao). Factors which negatively influence fracture healing are multiple and it is not always easy to identify them all. In general, the biological and mechanical environments largely determine the rate and extent to which healing progresses (4). Adequate vascularity of the bone as well as stability is essential for fracture healing (4).

In case of MIPO, the cause of delayed union may be the severity of the initial soft-tissue and bone injury (patient factor) which is uncontrollable. Another cause may be technical errors (surgeon factor) which can be avoided (malalignment, errors on the tunneling step, vascularity destruction).

### **Materials and methods**

The patient (canine, cross-breed, 27 kg, 8 years, male) was brought to the Clinic of Surgery, Faculty of Veterinary Medicine from Timisoara. For diagnostic and treatment all the facilities of the clinic were used.

For pre-, intra- and postoperative imaging we used Siremobil Compact L (Siemens) for intraoperative control images acquisition and the radiological facility type Multix Swing (Siemens) (computerized radiography system (CR) Direct View Vista CR (Carestream) for image processing and AQS Vet Standalone software (Arzt + Praxis GmbH)). The patient was monitored for several months.

### **Results and discussions**

Transverse diaphyseal long bone fracture, according to experts, can not be treated by bridging fixation, otherwise will lead to nonunion, delayed union or even pseudoarthrosis. In our last study, we presented cases of transverse simple fracture treated by MIPO that healed properly

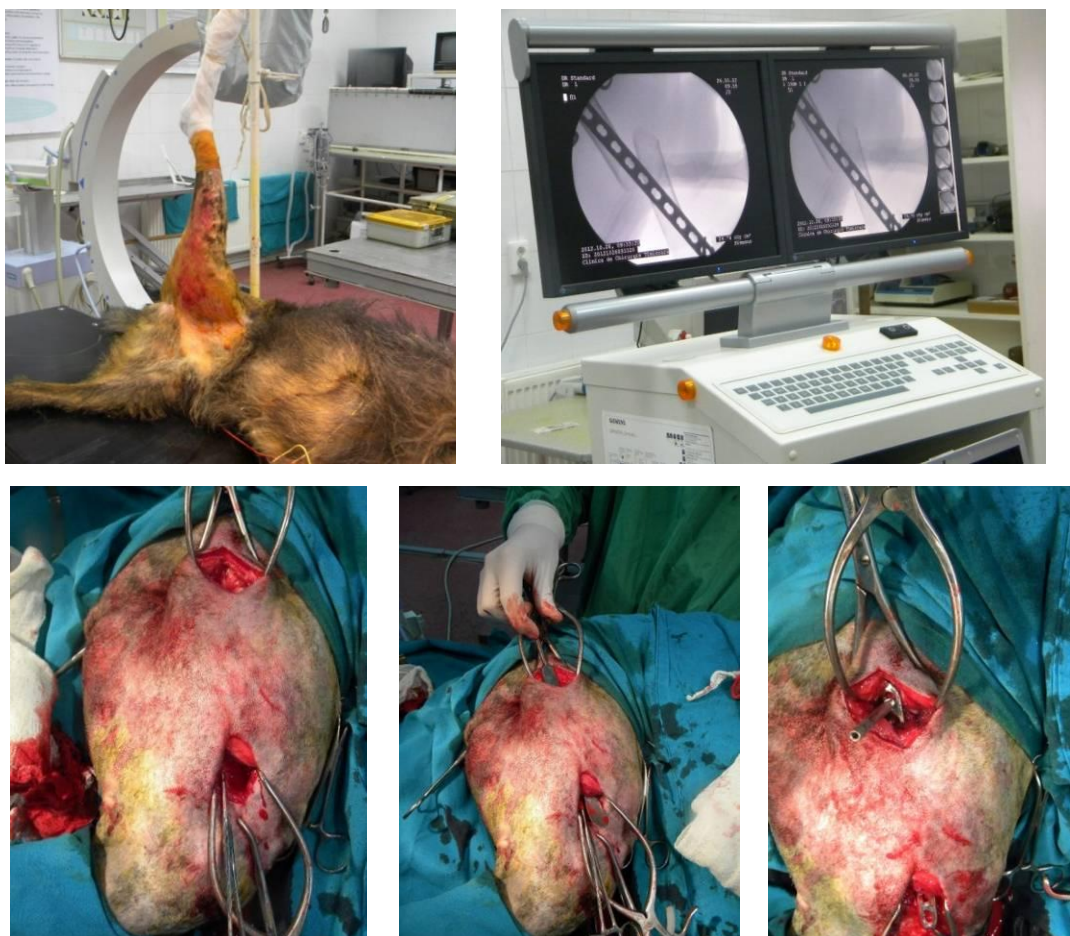


Fig. 1. Intraoperative images

Now, we present a case that exceeded expected healing time.

Our patients was diagnosed with a simple, diaphyseal, transverse fracture of the right femur on an 8 year old cross-breed. He underwent MIPO and was

followed up for 7 months, until radiographic healing was observed. In the figure 1, we present the dynamic of bone healing (Figure 2).



Fig. 2. Fracture healing assessment. Preoperative images (A1, A2). Postoperative image (B). 2 weeks PO (C). 4 weeks PO (D). 6 weeks PO (E). 8 weeks PO (F). 7 months PO (G)

Every two weeks for 8 weeks, the patient was X-ray exposed.

Starting four weeks after the surgery, it can be observed an intense periosteal reaction on the femur shaft, meaning that there is a bone activity. The fracture line is still present.

On the 8 weeks monitorization period we observed: long-standing persistent fracture line with a woolly appearance, incomplete callus, open medullary cavity, no signs of sclerosis, uneven bone fragments surfaces.

The term of delayed union is subjectiv and it applies when the healing time has overcome the expected period. In time the fracture can heal or evolve to nonunion. This complication can appear due to improper fixation, decrease of vascularization, increased gap between fragments, infection, sistemic/ chronic health condition (1).

At 8 weeks postoperative control, there were light signs of hypertrophic nonunion - callus on an elephant foot appereance (Figure 2F). Hypertrophic nonunion can be caused by insufficient fixation and of premature limb weight bearing (2, 3) or by the early removal of implant which was not the case.

### **Conclusions**

The fracture healing was achieved in 7 months, 4 month later than expected.

There were signs of nonunion, but the situation was overcome and healing had occurred.

Some of the possible causes were excluded, so we incriminated the age of the animal and also the severe soft tissues trauma which led to decreased vascularization and intense over-lasting postoperative oedema.

### **Acknowledgements**

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## **CASE REPORT: MIPO OF A TIBIA FRACTURE IN A CAT WITH SEVERE SOFT TISSUE TRAUMA**

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### **Summary**

MIPO (Minimally Invasive Plate Osteosynthesis) it is recommended in polytraumatized patients due to one of the main principles of the technique namely the reduction of iatrogenic trauma during surgery. Our patient, an European cat, presented a complete short oblique fracture of the right tibial shaft and a severe soft tissue trauma of the right limb, implying multiples wound and ecchymoses that further developed with signs of dry gangrene. In the period of monitoring, fracture healing developed along with soft tissue healing, including all stages of secondary healing.

**Key words:** MIPO, cat, healing.

The concept of "atraumatic surgical technique" has taken a particular interest into management strategies of osteosynthesis, especially when it is desired to preserve blood resources involving fracture fragments. Ensuring sufficient internal fixation to allow mobilization without pain has been and will continue to be a core goal in veterinary orthopedic surgery. Not only bone healing is favored but also soft tissue is better maintained and postoperative care becomes more easy (3).

Minimally invasive plate osteosynthesis (MIPO) in animals implies plating in a bridging fashion, without performing an open approach to expose the fracture site (1).

Treatment of a diaphyseal fracture with MIPO does not usually require the anatomic reduction of the fracture. Functional reduction is the goal; it restores bone length and correct alignment in the frontal, sagittal and axial planes. Indirect reduction is used to obtain functional fracture reduction without opening the fracture site. This method allows the fracture fragments to remain connected to the adjacent soft tissues. This is the key to improve bone healing because viable bone rapidly unites by callus formation (2).

Therefore, MIPO puts emphasis on preserving the integrity of the surrounding soft tissues. One of the most important indications of this technique is represented by polytrauma cases where it is desired not to overload the organism through the opening of the fracture, thus compromising the blood resources essential in the healing process of not only the bone but also soft tissues.

Thus, we want to present a case of multiple trauma with extensive destruction of soft tissue in the right lower limb and tibial diaphyseal fracture in a male cat.

### **Materials and methods**

The patient (feline, European breed, male, 4 kg, 1 year ) was brought to the Clinic of Surgery, Faculty of Veterinary Medicine from Timisoara. For diagnostic and treatment all facilities of the clinic were used.

For pre-, intra-and postoperative imaging we used Siremobil Compact L (Siemens) for intraoperative control images acquisition and the radiological facility type Multix Swing (Siemens) (computerized radiography system (CR) Direct View Vista CR (Carestream) for image processing and AQS Vet Standalone software (Arzt + Praxis GmbH)).

### **Results and discussions**

The diagnosis was complex and consisted of 3rd degree concussion of the lower limb region and metatarsal region (Fig. 1) with simple, complete, oblique with short path, middle diaphyseal, right tibia fracture and fibula fracture, and septic complicated multiple superficial wounds.

For fracture repair MIPO was performed using an LCP plate and four threaded head screws (two proximal and two distal). Operating time was summarized as following: two incisions, one proximal and one distal to the fracture without disturbing, creating an epiperiosteal tunnel between the two incisions, plate sliding through the created tunnel and plate fixation; first screw applied was at the proximal fragment to prevent rotation due to the triangular shape of the proximal end of the tibia) (Fig. 2).

Fracture reduction was assessed by fluoroscopy (Fig. 3). There was no absolute reduction obtained nor was intended. Bone length was restored and proper alignment on frontal, sagittal and axial planes was achieved.



Fig. 1. Diagnosis was established clinical and by X-ray imaging



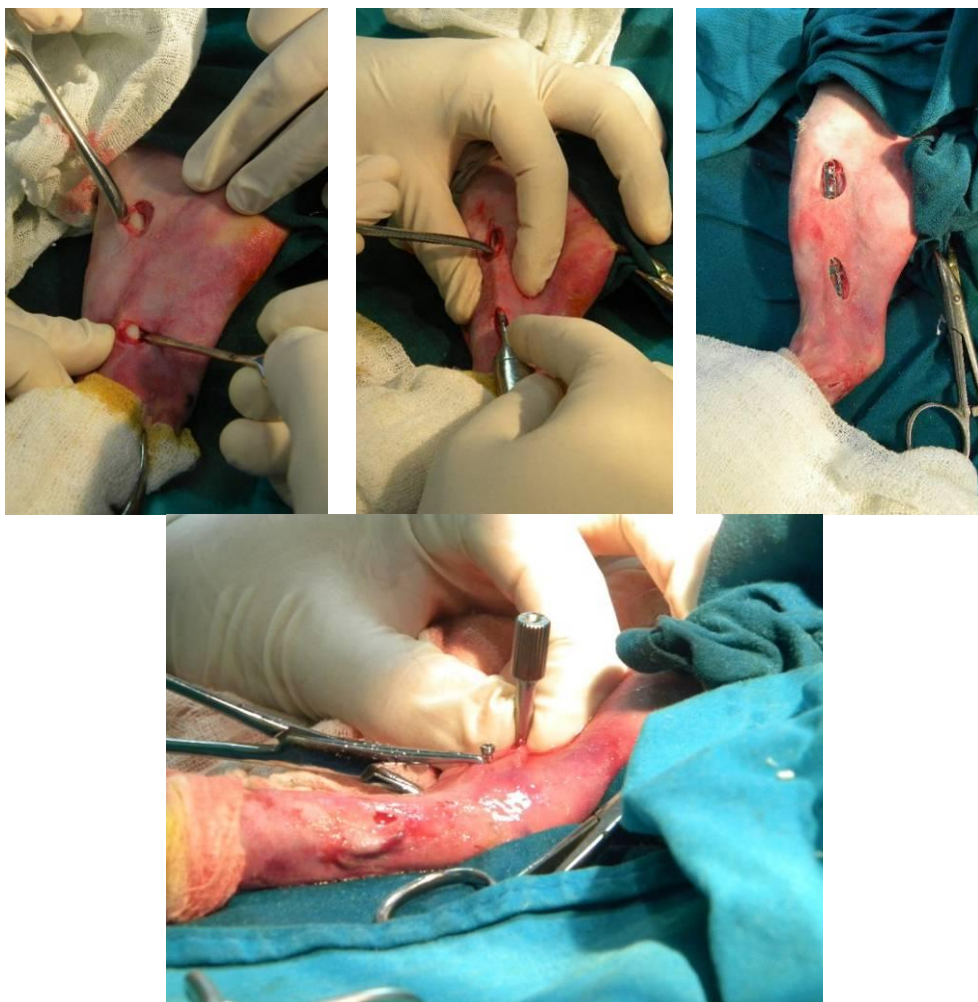


Fig. 2. Minimally invasive plate osteosynthesis – description of the technique.

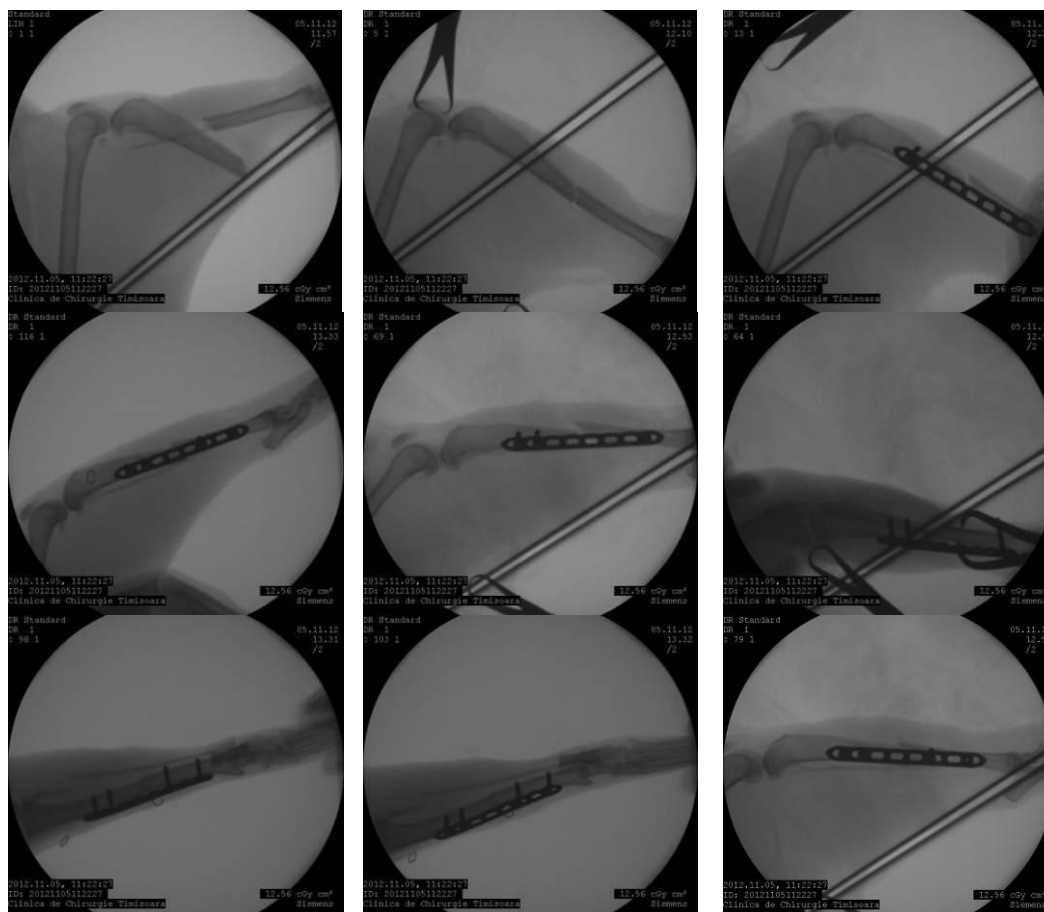


Fig. 3. Fracture reduction under fluoroscopy

Postoperatively, patient was hospitalized. Limb functional rest was assured and it was applied treatment for soft tissue injuries. Surgical wound healed without any complication. Skin sutures were removed at one week.

During monitorization, healing of skin tissue of the metatarsal region progressed to mummified necrosis. Thus, we were able to capture all dry gangrene stages:

1. desiccation - skin contusion, hypersensitivity, devitalized aspect
2. mortification - gray brown skin, reduced volume, insensitive surface
3. delimitation - 6 - 8 days separation groove breaker, dry, cold, hard, blackish, hypersonic tissue. Gangrenous tissue is deleted.
4. scarring (budding and epithelialization).



Fig. 4. Dry gangrene stades. Desiccation (A). Mortification (B). Delimitation (C). Scarring (D). Final aspect (E).

Throughout the monitoring period, the patient was treated prophylactically with antibiotics (amoxicillin with clavulanic acid - Synulox – Pfizer, in a dose of 12.5 mg/kg b.i.d.) the patient showed no sign of hyperthermia and the general condition was good. Traumatized area was initially protected by a gauze and kept clean, dry

and antiseptic. During mortification, we applied emollient ointment to facilitate the separation of eschar (Epitelin - Calendula officinalis - Exhelios). In the next phase, in addition to the already implemented treatment at the boundary of the delimitation groove we was applied a solution of scarring (Cicatrisol - Romvac). The rest of the wounds went secondary healing.



Fig. 5. Bone healing. Postoperative aspect (A). Control 2 weeks (B). Control 4 weeks (C). Control 6 weeks (D). Control 4 months - healed bone (E).

Bone healing was achieved in 4 months. MIPO relies on an decreased time of recuperation. In this case, the delay may be due to the severe trauma of the soft tissue. We present the healing on progress (Fig. 5). The bone healing consisted in the absence of the fracture line, the presence of callus (in this case a small quantity, although after MIPO we should have had a massive callus) and the medullary cavity becomes joint to the two fragments.

### Conclusions

Our patient was completely recovered in 4 months.

Even if the secondary bone healing by massive callus formation wasn't achieved, the pain was decreased by performing small skin and muscle incisions. This fact promotes early recovery.

Severe soft tissue injury cases may develop an early resumption of function if all iatrogenic overload of the organism are minimized.

### **Acknowledgements**

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## **SYNTHETIC PROGESTAGENS AND EXOGENOUS GLUCOCORTICOIDS: ARE THEY EXERTING A REAL IMPACT ON INCIDENCE OF FELINE DIABETES MELLITUS?**

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### **Summary**

Endogenous glucocorticoids and progesterone exert an anti-insulinic effect by increasing hepatic gluconeogenesis, glycogenolysis and by reducing the availability of insulin receptors and insulin dependent GLUT-4 glucose transporters.

The current study is a retrospective analytic interpretive epidemiological inquiry of diabetes mellitus in feline pets which are documented with synthetic progestagen and steroidal anti-inflammatory drugs in their recent anamnesis.

The frequency of occurrence in the group of progestagen treated cats was 2.51%, with a cumulative incidence of 25.16 cases per 1000. For the corticosteroids treated cats, the frequency of occurrence was 1.9% with a cumulative incidence of 19.35 cats, per 1000. Univariate analysis indicated a high statistical implication of progestagens ( $p < 0.001$ ) in diabetes mellitus, while multivariable risk analysis revealed an association of progestagens with the unneutered females ( $p < 0.01$ ), corticosteroids were correlated with the body weight ( $p < 0.05$ ).

This study shows a real implication of glucocorticoids and progestagens administration on the increased incidence and prevalence diabetes mellitus cats.

**Key words:** diabetogenic potential, glucocorticoid, insulin resistance, megestrol acetate

Insulin is the main counter-regulatory anabolic hormone that controls glucose metabolism and is a direct participant on the maintenance of euglycemia state (4,6,21,22). Plasma insulin concentration varies with blood glucose (BG) fluctuations and reduces the glucose by tissue uptake, glycogen synthesis and gluconeogenesis suppression. Insulin's secretion is reduced between meals, increased after each caloric intake, is a mediator of glucose utilization as energy support and promotes the storing of excess glucose in the form of glycogen deposits in the liver (21, 22).

Administration of medication with diabetogenic potential has a high impact over the glucose homeostasis and exerts a direct influence over the insulin activity (7, 18). Synthetic progestagens (SP) such as medroxyprogesteron acetate or megestrol acetate, as well as steroidal anti-inflammatory drugs (SAID), were demonstrated to act as strong insulin antagonists and to be contributing factors in the physiopathogenesis of diabetes mellitus (DM) in felines (2, 12, 13, 14, 20). The

diabetogenic potential of SP is caused by a reduced migration of GLUT-4 transporters to the membrane and a reduced glucose uptake in the intracellular space. Also progestagens stimulate the secretion of growth hormones, which are also important contributors to the insulin resistance. Concentrations of progesterone within the normal range were demonstrated to induce glucose intolerance and increased insulin resistance, during pregnancy in female mice (11). Glucocorticoids are involved in the process of sustained hyperglycemia through their contribution to the increased gluconeogenesis and glycogenolysis. In the conditions of a high concentration of insulin antagonists, the pancreas usually responds with insulin overproduction, to the exhaustion and shutdown of  $\beta$ -cells (2, 13, 23). Although there are still uncertainties about the promoter of diabetes mellitus, direct drug activity is highly associated with disturbances on insulin secretion and glucose homeostasis (5, 7, 12, 20). Both types of drugs induce: a disturbance on the insulin production and path-way, (13, 14, 20), fasting hyperglycemia, decreased glucose excretion rate and reduced clearance of intravenous glucose load (1, 3, 6, 13, 20).

The current study is a descriptive analytic epidemiological inquire of feline insulin dependent diabetes mellitus, associated with synthetic progestagens and steroidal anti-inflammatory drugs. The main objective is to describe the characteristics of the risk population and to determine if indeed corticosteroid and progestagens share a direct implication in the incidence of feline insulin dependent diabetes mellitus.

### **Materials and methods**

All cats registered with DM were selected retrospectively from the database of the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine from Iasi, Romania and from local private practice. Clinics were designed for both cats and dogs and the percentages of species addressed were the same. Case retrospective registration started with January 2005 and was carried up until December 2011. Records of a total of 4009 cases summed in the clinics, both male and female cats, age 4 weeks to 18 years were consulted.

Revised medical records provided complete information regarding age, gender, breed, body weight and reproductive integrity. Individuals were considered eligible for inclusion if they were registered with a clear diagnosis of diabetes mellitus and characteristic clinical signs. For all cases, diabetes mellitus was diagnosed on the basis of persistent fasting hyperglycemia over 270 mg/dl (80-120 mg/dl), glycosuria and characteristic clinical signs (polyuria-polydipsia, polyphagia and weight loss) and general serum biochemistry (3, 18).

For each selected case, additional data regarding SP and SAID treatment, dose and number of days on which they were administered and the reason of administration, were documented.

### Data analysis

Data analysis was performed with the statistical package for the social sciences (SPSS, version 20). To identify risk factors, univariate and multivariate analysis were performed for all diabetic cats with the following dichotomous variables: gender (male/female), reproductive integrity (neutered/unneutered), corticosteroid and megestrol acetate administration, breed (Burmese/other breeds) and weight (<5kg/ ≥5 kg). Spearman's rank correlation coefficient was used to test variable association and dependencies and  $p \leq 0.05$  was considered statistically significant.

A cumulative incidence was calculated for both SP and SAID treated cats and translated as the proportion or number of affected individuals on each 1000 evaluated cases (10, 19). All data was reported as frequencies, percentages, ranges, mean values and standard deviations.

### Results and discussions

Diabetes mellitus was diagnosed in 50 out of 4009 cats revealing a prevalence of 1.25%, average age of 11.1 years (range: 3 to 18) and average weight of 4.8 (range: 2.8 – 7.2 kg). Studied cats included 23 females (46%), and 27 males (54%). In what regards the reproductive integrity, 26 cats (52%) were neutered (19 males-38%; 7 females-14%) and 24 (48%) were entire individuals (8 males-16%; 16 females-32%). Cats were separated according to breed into 4 groups and included 24 Burmese (48%), 17 DSH (34%) and 6 Siamese (12%). The 4<sup>th</sup> group consisted of 3 cats (6%), belonging to different breeds (1 Snow-shoe, 1 British shorthair and 1 Persian) (Table 2).

Analytic results for SP treatments indicated that out of the total cat population ( $n=4009$ ), 596 entire individuals (14.86%) were treated as least two times a year with synthetic progestagens. Out of the SP population ( $n=596$ ), a number of 15 cats were diagnosed with DM revealing a prevalence of 0.37% for the total 4009 non-diabetic and diabetic cats. Progestagen treated individuals ( $n=15$ ) represented 30% of the diabetic patients. Diabetic SP cats comprised of 13 entire females (26% of the 50 DM cases) with an average age of 11.06 years and average weight of 3.2 Kg. Two entire males (4% of the 50 cases) with a median of 9.5 years and an average weight of 4.5 Kg were treated with SP. Entire female cats received SP on a mean time period of 7.7 days (range: 6-10; SD-1.3) in order to prevent estrus manifestations and unwanted litter in outdoor cats. In the male cats, the drugs were administered for 8 and 10 days respectively on an average period of 9 days (range: 8-10; SD-1.4), to alleviate behavioral issues, such as indoors spraying. All cases received the drug as least twice a year and recent administration prior to diabetes diagnosis was documented. If related only to the SP treated individuals ( $n=596$ ), the frequency of occurrence was 2.51%.

Steroidal anti-inflammatory drugs were administrated in 620 cats (15.46%), both entire and neutered. Twelve were diagnosed with diabetes and showed a



prevalence of 0.29% for the total population (n=4009) and represented 24% of the diabetic cats (n=50). All 12 cats, 8 males (16%) and 4 females (8%) diagnosed with different types of inflammations, dermatitis and otitis had a recent history of SAID administration prior to diabetes mellitus diagnosis. Corticosteroids were administrated on an average 51 days in males (range: 25-71; SD 19.9) and 61.7 days in females (range: 62-75; SD 12.4). Frequency of occurrence in corticosteroid treated individuals (n=620) was 1.9%.

#### Data analysis

Univariate analysis of risk factors revealed a high dependency of diabetes mellitus and megestrol acetate treatment ( $p<0.01$ ). Also diabetes occurrence was highly associated with the Burmese breed ( $p<0.01$ ), with a probability of occurrence of 2.08 times higher compared to the other breeds. Multivariate risk analysis revealed a high statistical association of SP treated diabetic individuals and females if unneutered ( $p<0.01$ ), while males were considered under a higher risk if  $>5$  kg ( $p<0.05$ ). No other significant correlations regarding age, gender or other breeds, were associated with SP and SAID and diabetes mellitus in cats.

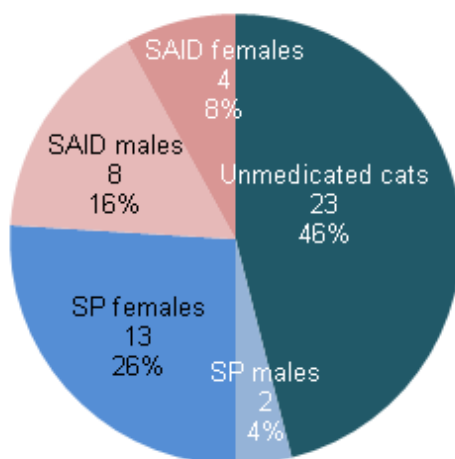


Fig. 1. Percentual dispersion of SP and SAID treated individuals, within the total diabetic population.

To the authors knowledge this is the first retrospective analytic interpretive epidemiological report of diabetes mellitus in association with megestrol acetate and corticosteroid administration in a well defined population of cats. Steroidal anti-inflammatory drugs were reported to act by increasing hepatic gluconeogenesis, decreasing tissue glucose utilization, reducing affinity and number of GLUT-4 glucose transporters, also reducing affinity of insulin receptors. Progestagens,

reduce the processes of insulin binding, glucose transport in the intracellular space (20).

Cumulative incidence for the SP treated individuals was 25.14 diabetic cats per each 1000 observed cases, or 1 case of DM for each 39.73. For the SAID treated individuals, cumulative incidence was 19.35 on each 1000 cases, or 1 case of DM on each 51.6 cases. Results of cumulative incidence on groups, could be one of the first indicatives that progestagens and glucocorticoids are important contributors in diabetes mellitus epidemiology, as the prevalence for the cats that did not received either of drugs had a considerably lower incidence, of 1 on each 121.4 cases or 8.23 cases per 1000 cats (Table 1).

Table 1

**Prevalence/cumulative incidence of DM in SAID and SP treated cats**

Total 4009	SP		SAID		Undedicated	
	No.	%	No.	%	No.	%
No. of cats out of 4009	596	14.86%	620	15.46	2793	69.6
DM Prevalence (n=50)	15	30	12	24	23	46
Prevalence in females	13	26	4	8	6	12
Prevalence in males	2	4	8	16	17	34
Cumulative incidence per 1000	25.14	2.51	19.35	1.93	8.23	0.83

An important risk factor was body weight over 5 Kg. All individuals treated with progestagens and corticosteroids presented a higher average weight (5.6 kg) compared with the non-diabetic population (4.8 kg). Although in some cats the weight was close to normal, small increase of body index was reported as a risk factor for fasting hyperinsulinemia, impaired glucose tolerance (17) and decreased insulin sensitivity by up to 50% in obese cats(20).

Burmese cats were overrepresented in the present report (48%) and as reported in other studies (8, 9, 15), this breed was demonstrated to be an important predisposing risk factor. Burmese cats, represented with 13 males (26%) and 11 females (22%) indicated a probability of developing diabetes of 2.08 times higher compared to the total population.

Due to the marked insulin resistance, hyperglycemia and deleterious effect exerted on the natural secretion of insulin, (1, 12, 20) knowing wither or not the patient was treated with such medicine should be one of the main concerns when performing the anamnesis of diabetic cats (12, 23). A study developed by Middleton and Watson demonstrated that prednisolone and megestrol given to six cats on different occasions for 8 days, leads to increased fasting glucose, decreased glucose excretion and reduced glucose tolerance (13), suggesting

immediate disturbance on glucose homeostasis. Another study developed by Peterson, indicated that, insulin resistance and clinical signs resolution can be achieved after SP treatment cessation, even after long term high therapeutic dosage administration (14).

Progestagens and corticosteroids play an important role in the recent history of insulin resistant cats prior to diabetes diagnosis, especially in Burmese cats (15), thus a first step in reducing diabetes incidence could lie in the veterinarians decision of prescribing such drugs. Still, a sum of underlying conditions that interfere with the insulin pathway, such as chronic medical issues, dental problems, reduced physical activity, obesity, diet (9, 17) associated with the use of diabetogenic drugs might spur diabetes mellitus occurrence (16). Administration of this type of drugs in cats that are obese, especially males, belong to the Burmese breed, have undiagnosed/untreated infections, thus already hold a degree of insulin resistance, increase significantly the risk of developing diabetes mellitus (20). In cases that enclose different risk factors, insulin resistance is aggravated and generally followed by beta cell exhaustion and shutdown (9, 17).

In the present study the group of medicated cats comprised 54% (n=27) of the diabetic cats, compared to the cats that never received nor glucocorticoids or progestagens, represented by 46% (n=23) of individuals. The 8% difference could be an obvious indicator that corticosteroids and progestagens entail a true implication in diabetes mellitus occurrence in the studied population. Results prove an important epidemiological implication of synthetic progestagens and glucocorticoids in diabetes mellitus occurrence in cats. High differences of cumulative incidence and prevalence in the groups of cats that have been treated with drugs with diabetogenic potential and the group of cats that were never treated with such drugs could raise awareness in what concerns their inclusion and dosages in treatment protocols.

### **Conclusions**

The present study shows a real increase of diabetes mellitus incidence and prevalence after glucocorticoids and progestagens administration in cats. Weight, gender and reproductive integrity were confirmed as important risk factors in the general population and proved to be different for females when compared with males. A better understanding of risk factors could promote early implementation of prevention and control measures for diabetes mellitus and also could bring veterinarians to second thoughts before prescribing such medicines. Both progestagens and corticosteroids hold a high diabetogenic potential and it would be advisable to thoroughly evaluate all treatment options before initiating a therapy protocol. Also it would be imperious to educate and inform pet owners about the side effects associated with synthetic progestagens in cats and consider neutering as an alternative solution to behavior control. This type of analytic analysis could

provide framework for future studies in regard to glucocorticoid and progestagen induced diabetes mellitus.

### **Acknowledgements**

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## THE DIAGNOSTIC OF CENTRAL NERVOUS SYSTEM DISORDERS IN DOGS WITH NEPHROCALCINOSIS

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### Summary

Nephrocalcinosis is a disorder characterized by the deposition of calcium salts in the renal parenchyma secondary to chronic nephritis or antifreeze poisoning. Besides the symptoms of renal failure, the neurological signs are characterized by seizures which can evolve into *status epilepticus*. In such situations it is difficult to clinically differentiate the seizures due to chronic renal failure by those from idiopathic epilepsy (IE). In this study we analyze the electroencephalographic changes in dogs suffering from nephrocalcinosis using a short time EEG recording.

The electroencephalogram (EEG) is a method used to measure the electrical activity of the brain, which was originally described on animal studies being the most important diagnostic tool in epilepsy.

On EEG, nephrocalcinosis it was characterized by a nonspecific trace which allows the differentiation from idiopathic epilepsy. EEG trace is a reliable paraclinical tool useful to discriminate IE from other secondary conditions that have the same clinical appearance.

**Key words:** visual electroencephalography, nephrocalcinosis, dog, epilepsy

Nephrocalcinosis is a tubulointerstitial nephropathy that can be the result of a primary metabolic disorder involving calcium and phosphorus or may be due to chronic scarring and severe tissue damage caused by glomerulonephritis, pielonephritis, renal amyloidosis, chronic nephritis, renal neoplasia, urinary tract infections not treated properly, antifreeze poisoning, and primary or secondary hyperparathyroidism (7).

The symptoms of antifreeze poisoning are usually very severe, most of the pets that consume ethylene glycol do not survive because of the breakdown products such as oxalic acid that combined with calcium forms calcium oxalate which is deposited in the kidney (1).

Beside the typical symptoms due to the renal impaired functions such as polydipsia, polyuria and gastrointestinal signs caused by the uremic intoxication, the neurological signs can be quite important, being characterized by anorexia, nausea, vomiting, depression, reduced or exaggerated reflexes, and seizures that usually appear in the final stages of uremia. The neurologic disorders are caused by the toxins that are present in high amounts and by the metabolic disturbances when the kidney function is decreased and can affect the brain, one of the most

blamed toxins is considered to be the parathyroid hormone that it is found in excess in chronic kidney failure (2, 3).

Neurological complication due to the uremic state can be diagnosed using electroencephalography, the findings are non-specific but can be related to the symptoms, electroencephalography can also help us to exclude idiopathic epilepsy as the cause of the seizures (3, 5).

Advanced cerebral imaging such as CT or MRI can be useful to discard other underlying cause of the central nervous system disorders, but in the case of uremic encephalopathy does not show any changes (2).

The aim of this study was to describe electroencephalographic changes in dogs suffering from nephrocalcinosis using a short time EEG recording.

### **Materials and methods**

EEGs were performed on 5 dogs of mixed breeds, with ages between seven and nine years, suffering from renal calcinosis caused by antifreeze poisoning and glomerulonephritis, the dogs had no history of previous seizures or other neurologic disorders. The research was conducted in the Internal Medicine Clinic of the Faculty of Veterinary Medicine Iasi.

Nephrocalcinosis was diagnosed based on clinical examination, blood work using Cormay Accent chemistry analyzer, complete blood cell count was done using vet ABC Hematology Analyzer, ultrasound imaging was obtained using Aquila Pro ultrasound machine from Esaote Piemedical.

All patients were sedated before EEG recording, using medetomidine hydrochloride 0.03 mg/kg inj. i.m., after the electroencephalographic examination saline solution was administered IV to eliminate the sedative.

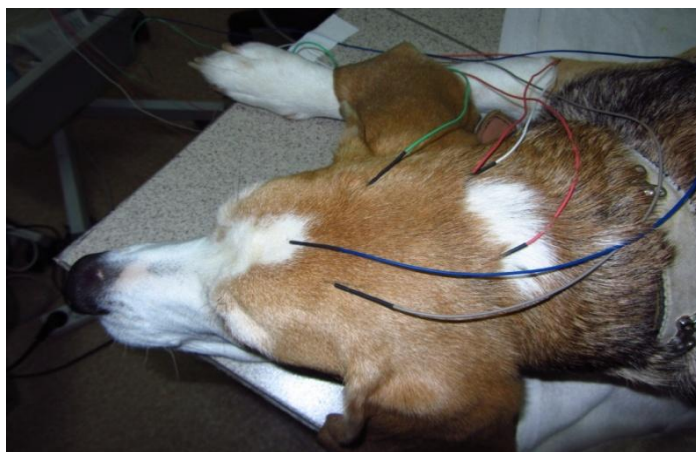


Fig. 1. The Redding model of placement of subdermal needle electrodes

Electroencephalographic recording was obtained using five subdermal needle electrodes (F3, F4, O1, O2 and Cz; disposable subdermal stainless steel EEG needle) placed using the Redding model, the area where the electrodes were placed was degreased with alcohol.

The reference electrode was placed on the bridge of the nose and the ground electrode right caudal to the occipital protuberance. The EEG (the Neurofax S, MEB 9400K Nihon Kohden) was recorded with sensitivity = 70 $\mu$ V/cm; time constant = 0.3 seconds; Hf = 70 Hz; Lf = 0.5 Hz; notch filter inserted; impedance of all electrodes < 10 k $\Omega$ .

All EEGs recordings were visually analyzed with monopolar montages, the discharges and the artifact were determined by direct following of the recordings, background activity being analyzed during the recording to identify any detectable changes.

Recording section was 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.

### **Results and discussions**

The blood work done on all the patients revealed high levels of creatinine and urea; normochromic, normocytic, non-regenerative anemia, leukocytosis and lymphopenia.

Neurological examination showed muscle twitching, impaired cognitive processing, normal or exaggerated reflexes and seizures.

Ultrasound imaging was consisting, showing an increased echogenic aspect of the renal parenchyma (Fig. 2).

For all patients brain activity was characterized by non-specific electroencephalographic abnormalities: increased percentage of slow wave and high amplitude bursts of delta activity. No interictal epileptic discharges (characteristic for IE condition) were observed.

In the early stages of nephrocalcinosis there was found a normal bioelectrical brain activity. When nephrocalcinosis reached a moderate level; the frequency of spontaneous rhythms decreased and the amplitude increased.



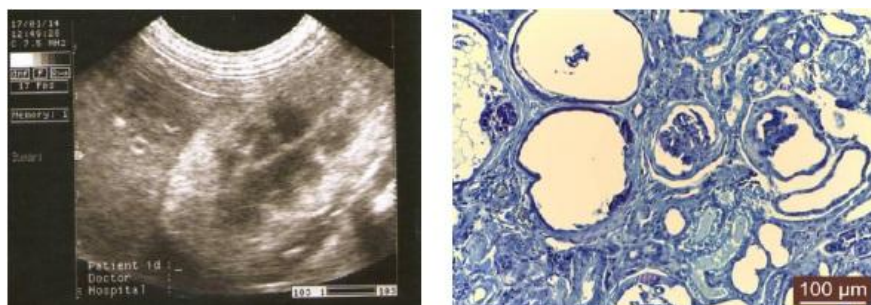


Fig. 2. Ultrasound image of nephrocalcinosis and its histological substrat (glomerulosclerosis, X 400, scale bar 100  $\mu$ m )

The EEG routes slightly voltated presented an initially slowed background activity of 5-7 cycles/second, above which synchronous bilateral diffuse delta waves where registered, the expression of cortical or subcortical neuronal hyperexcitability state.

In advanced stages of nephrocalcinosis the EEG recording route became irregular and arrhythmic with epileptic abnormalities (Fig. 2), clinically manifested. Their occurrence is given by the metabolic and waste products that appear as a result of reduced filtration capacity of the kidney.

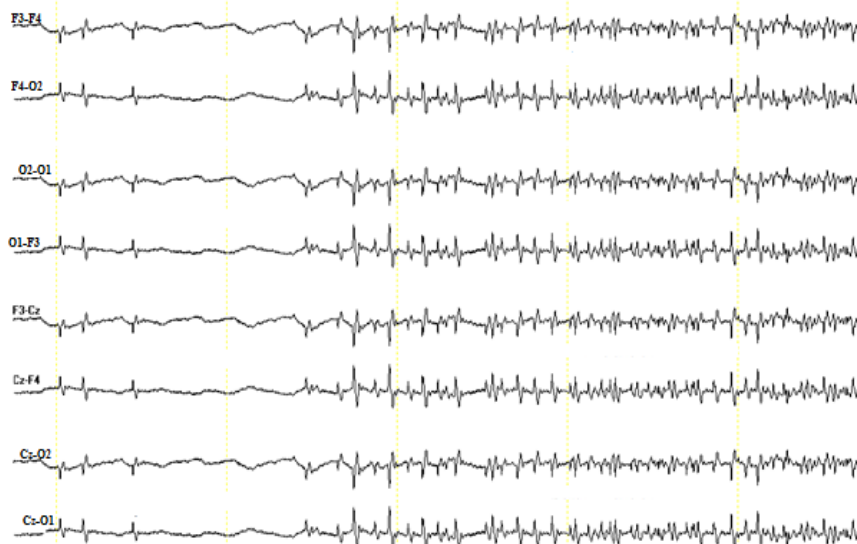


Fig. 3. Electroencephalography recording of a eight years old Beagle female with moderate nephrocalcinosis characterized by excess of delta waves and bilateral spike-wave complexes

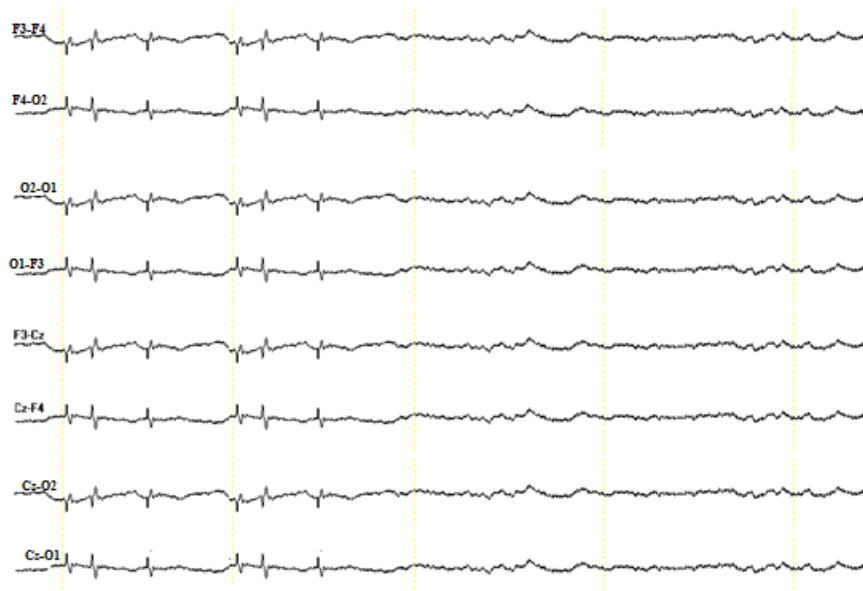


Fig. 4. Slow spike waves and reduced amplitude of EEG recording route in a six years old Pekingese male dog

Sharp waves are monophasic, and have a particular morphology with steep upward slope, and the downward slope more inclined which are self-sustaining expressions of highly synchronized neuronal processes with different synaptic delays.

The abnormalities that appear on EEG recordings of uremic patients are very similar with the findings of Guisado et al. who performed a study on five groups of dogs: seven normal dogs; six dogs with uremia of 3.5 days duration; seven normal dogs which received intramuscular injections of parathyroid extract, 80 U twice a day for 3.5 days; seven normal dogs which were subjected to thyroparathyroidectomy and received vitamin D3 in adequate doses to maintain a serum calcium level of 9-11 mg/dl for 7-10 days and were then made uremic for 3.5 days; and six normal dogs which were first subjected to parathyroidectomy, then made uremic, and received parathyroid extract 100 U four times a day, performing EEG recordings obtained slow wave activity with slow and disrupted of background activity (5).

The findings are also similar with the human model of EEG in uremic encephalopathy: generalised slowing with an excess of delta waves and sometimes bilateral spike-wave complexes, with the progression of the illness the EEG becomes slower (3).

Due to advanced renal failure all patients were euthanized, necropsy was performed on the bodies followed by histological examination of specimens from all the major organs.

The kidneys show specific structural changes such as glomerulosclerosis, diffuse tubular injury and atrophy as well as deposition of calcium salts along the tubular basement membrane, interstitial fibrosis (Fig. 2).

### **Conclusions**

On EEG, nephrocalcinosis is characterized by a nonspecific trace which allows the differentiation from idiopathic epilepsy. EEG trace is a reliable paraclinical tool useful to discriminate IE from other secondary conditions that have the same clinical appearance.

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## **MEDICAL MANAGEMENT OF CORNEAL ULCERS IN DOG: A RETROSPECTIVE STUDY IN 20 CASES**

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### **Summary**

Nine dogs with superficial and eleven deeper corneal ulcers less than one half thickness were medical treated. The recovery rate was 95%. The mean time to complete healing of superficial ulcers was 8 days and minimal or no more macroscopically corneal opacities remain at ulcers location in those cases. The mean time to healing of deeper ulcerative lesion was 23 days and a permanent scar was still present at ulcers place but not affect normal vision.

**Key words:** dog, corneal ulcer, medical therapy

Corneal ulcers are probably the most common problem of the canine eye in general veterinary practice (3, 4). The results of a comprehensive ophthalmic examination as well as the dog's signalment and history are important factors to take into consideration when determining the underlying cause and instituting appropriate therapy. Most of these ulcers result from a variety of traumatic and irritating conditions and usually respond well to medical therapy alone, provided they are not too deep (3, 7).

The epithelium has a remarkable capacity of regeneration and usually heals within approximately seven days. Healing of an uncomplicated superficial corneal ulcer is rapid, and most heal in three to five days (1, 2, 4, 6).

Topical antibiotics are indicated in nearly all cases of corneal ulceration and oral doxycycline may assist the healing of deeper ulcers, as well as reducing the risk of keratomalacia. Atropine may be needed to dilate the pupil. Systemic antiinflammatories are indicated to control secondary uveitis associated with the corneal ulceration. Third eyelid flaps or contact lenses may help protect the cornea blepharospasm, and other sources of ocular irritation. In most cases of superficial ulceration healing should take place within three to five days. Deeper ulcers need to slough and replace the necrotic tissue. For this reason surgery is nearly always indicated in deeper ulcers to allow the cornea to heal quickly with the least amount of corneal scarring. Without surgery many of the deeper corneal ulcers require corneal vascularisation to fully heal. This will result in a lot more corneal scarring (2, 4, 6, 7).

Inability of the owner and clinician to monitor progress or, more important, worsening of the ulcer behind the third eyelid is another serious limitation of this technique. Some of the more serious, progressive ulcers presented to veterinary ophthalmologists have developed behind a third eyelid flap (4). The objective of the study was to make an analysis of results of medical corneal ulcers treatment in dogs.

### **Materials and methods**

The studies were performed in 20 clinical cases of different dogs breeds, Pekinese (n=7), Tosa Inu (n=1), Rotweiler (n=1), rumanien Shepherd (n=1), Pug (n=1), Shi-tzu (n=4), crossbreed dogs (n=5), without systemic diseases and with corneal ulcerative lesions managed with medical therapy.

Only the cases with less than about one half thickness of cornea affected were included in this study. Corneal lesions were evaluated regarding location, size and depth. In all cases a fluorescein test was made. Lesions were classified as superficial corneal (n=9) ulceration, deep corneal ulcers (n=6), and keratomalacia (n=5).

Corneal edema, vascularization and white or yellowish infiltrate at the ulcer site were registered. The ulcer margins were carefully assess to epithelial adherence. In four cases the loose epithelial and stromal tissue was debrided with a cotton-tipped applicator.

All cases with superficial small lesions were treated ambulatory and reevaluated at to days interval. Topical antibiotics were applied 4 times daily. Topical ciprofloxacin or ofloxacin, fluoroquinolones that demonstrate efficacy against a wide range of Gram positive and Gram negative organisms and fortified gentamicin were used.

A topical one daily application of atropine 1% eye drops was used to control the anterior uveitis for three days. EDTA protease inhibitors were applied topically. Adjuvant therapy to help the healing of corneal lesions consists from corneregel application.

In dogs with deeper corneal ulcers an aggressive medical regimen at 1 hour intervals was followed with minimal 8 time daily application for antibiotics and EDTA and was provided by hospitalization. Doxycycline by oral dosage was also used in those cases.

Systemic carprofen antiinflammatories are administrated to control secondary uveitis.

An Elizabethan collars were used for prevention of self-trauma in dogs with deep ulcers.

The frequency of topical medication has been reduced to 4 times daily when edema and cellular infiltrates in the adjacent stroma decrease and epithelization occurs.

The healing of corneal epithelium was considered when the uptake of fluorescein was absent. Topical corticosteroids were used under strict control after epithelium repaired to reduce the scar dimension and decreasing of vascularization. Antibiotic and corneregel has been continued two times daily for one week.

### **Results and discussions**

Brachiocephalic dogs were the most affected dog breeds (12/20). The axial location (n=11) was followed by paracentral (n=4), inferior temporal (n=3) and perilimbal (n=2).

No signs of entropion or ectropion, ectopic cilia or foreign body were identified. The traumatic cause remains the most plausible.

The shape of the lesions was circular except one cases with perilimbal location what presented a linear one.

The mean time to complete healing of superficial ulcers was 8 days. In all cases a minimal corneal opacities remain at ulcers location.

In two cases dogs with deep corneal ulcers and keratomalacia the formation of small vesicles in the epithelium and stroma of an edematous cornea (bullous keratopathy) was found in healing evolution. The mean time to healing of deeper ulcerative lesion was 23 days.

A large corneal scare similar to ulcer dimension result in this dogs and corneal edema and vascularization persists after epithelial healing.

In two cases the ulcer progresses and a staphyloma and respectively descemetocoeles had developed. Third eyelid flaps have been used in this cases and medication was continued. Initial treatment failure was considered in boot cases.

All superficial corneal ulceration has had a favorable outcome. Similar to the literature dates (1, 4, 6, 7) simple ulcers heals without stromal involvement in about one week.

In deeper defects the longest time necessary to complete epithelial healing was 36 days. It is know that, healing of the stroma takes several weeks to month (1, 4). The scar was denser than in superficial corneal ulceration but not affect seriously normal vision. Some blood vassels still remain visible despite antiinflammatory medication. They can remain as ghost vessels and if inflammation recurs may rapidly become perfused (4).

In this study the recovery rate for deeper corneal ulceration was 81.81%. A high success rate (93.18%) of conjunctival pedicle graft technique for the treatment of deep corneal ulcers was reported by Soontornvipart et al. (2003). They found that the success rate depended significantly only on surgical techniques and type of technique performed. Similar to those reports we also found a predisposition to permanent scar and leukoma when uveitis prior to treatment is present.

### **Conclusions**

The recovery rate after medical treatment, in dog with corneal ulcers less than one half thickness was 95%. The mean time to complete healing of superficial ulcers was 8 days and minimal or no more macroscopically corneal opacities remain at ulcers location in those cases. The mean time to healing of deeper ulcerative lesion was 23 days and a permanent scar was still present at ulcers place.

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## **OBSERVATIONS REGARDING ISOFLURANE AND EXPIRATORY CONCENTRATION VALUES IN DOGS**

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### **Summary**

At patient to which muscle relaxants were used and CNS inhibition monitoring through medical devices are not available, to maintain unconscious with appropriate concentration values of inhaled anesthetics becomes vital. Surgical anesthesia is maintained by multiples of minimum alveolar concentration, which has specific values for each inhalation anesthetic. This value is influenced by a number of factors among which the premedication. In a total of 30 cases to which were used in premedication three different drug combinations has been monitored end-expiratory isoflurane concentration. Maintenance of surgical anesthesia was achieved at isoflurane alveolar concentration values between 0.52-1.4% which represents 1 MAC x 0.4-1.09. No significant differences of Et-Iso minimum values were found between groups. Intra anesthetic hypotension, advanced age or gender did not significantly affect Et-Iso concentration; low values which have been recorded are the result of anesthetic associations used in premedication.

**Key words:** dog, isoflurane, end-expiratory.

The potency of inhaled anesthetics is described by the minimum alveolar concentration, MAC, which is the concentration at which in 50% of the patient motor response to the application of the standard stimulus is abolished (7).

Each inhalation anesthetic has its own MAC value, value which is influenced by many factors.

Inhalation anesthetics are taken by blood from alveoli and carried to tissues where diffuses by virtue of partial pressure difference. Uptake by the blood and tissues occurs until the equalization of the partial pressures among alveoli-blood-tissue (brain) (2). Therefore it can be considered that the value of end-expiratory alveolar concentration estimate the value of the central nervous system compartment.

Changing the setting on the vaporizer, thus variation of anesthetic concentration in the inspired gas mixture will cause anesthesia lightening or deepening due to changes of partial pressures within compartments mentioned above. In order to maintain surgical plane of anesthesia 1 MAC x 1.2 to 1.5 is usually required (4).



In the conditions of a patient to which muscle relaxants were used and monitoring CNS inhibition depth is not possible by monitoring the electrical activity of the brain, respectively by bispectral index, or evoked potentials, maintaining the unconscious state through appropriate concentration values of inhaled anesthetic becomes vital. Knowledge of brain concentration of the anesthetic, ultimately, may be a better guide of the presence or absence of anesthesia status than other methods of determining of its depth (8). The purpose of this paper work is to notice to what extent different drug combinations used in premedication influences the values of end-expiratory isoflurane, respectively MAC, in dogs.

### Materials and methods

In the study were involved 30 clinical cases, dogs with soft tissues surgery (Table 1) at which inhaled anesthesia was performed in semi-closed rebreathing circuit.

Table 1

Cases distribution

Surgical intervention	Age group				Gender	
	Puppy under 6 months	Young under 1 year	Adult	Geriatric*	♀	♂
Intra-abdominal tumors	-	-	7	3	4	6
Mammary tumors and ovariectomy	-	-	2	4	6	-
Ovariectomy	-	3	2	-	5	-
Mandibular gland cysts	-	-	3	1	2	2
Complicated hernias (inguinal, umbilical)	3	-	-	2	2	3

In these patients for premedication the following intravenous anesthetics combinations were used:

- Diazepam/Ketamine (group 1) in all geriatric patients and under one year of age;
- Acepromazine/Ketamine (group 2) or
- Xylazine/Ketamine (group 3) in adult patients.

Induction was performed with propofol administered IV followed by endotracheal intubation. Maintenance was done with isoflurane in oxygen (Fig. 1). Spontaneous ventilation was manually assisted to assure ventilatory exchange and to maintain effective and end-tidal carbon dioxide (Et CO<sub>2</sub>) concentration between 35-45 mmHg.

Intraanesthetic standard monitoring was done watching the following parameters: oxihemoglobin saturation, FiO<sub>2</sub>, EtCO<sub>2</sub>, respiratory rate, arterial

pressure, pulse rate, DII electrocardioscopy (Cardell Veterinary Monitor 9405 and Datex Capnomac Ultima). Particular emphasis was placed on monitoring the depth of anesthesia aiming at the nociceptive stimuli reaction, the degree of reflexes inhibition, pupillary size and reaction, changes in hemodynamic parameters as a reflection of the capacity to respond at nociceptive stimulation. Also, inspiratory and end-tidal isoflurane (Et-Iso) concentrations were continuously monitored using a gas analyzer (Datex Capnomac Ultima) connected to the endotracheal tube, and Et-Iso minimal and maximal values were noted every five minutes (Fig. 2)



Fig. 1. Inhalation isoflurane maintenance of anesthesia

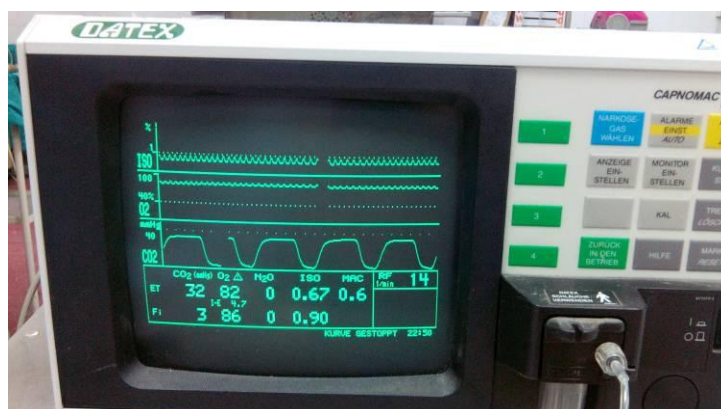


Fig.2. Continuously anesthetic gas monitoring

Statistical analysis was performed with ANOVA test, for values of  $p \leq 0.05$  considering significant differences.

### Results and discussions

In patients included in the study Et-Iso values are shown in the table below (Table 2).

Table 2

#### Isoflurane end-expiratory concentrations and MAC values

Group - Premedication	Surgical intervention (n=30)	Gender	Et-Iso concentration (%)	Percentage of the 1 MAC
			Min/max	Min/max
1 - Diazepam/ Ketamine (n=16)	Ovariohysterectomy	♀	1.20/1.40	0.93/1.09
	Ovariohysterectomy	♀	0.91/0.93	0.71/0.72
	Ovariohysterectomy	♀	0.74/0.88	0.57/0.68
	Mammary tumors and ovariohysterectomy	♀	0.68/0.78*	0.53/0.60
	Mammary tumors and ovariohysterectomy	♀	0.72/0.84	0.56/0.65
	Mammary tumors and ovariohysterectomy	♀	0.83/0.85	0.64/0.66
	Mammary tumors and ovariohysterectomy	♀	0.77/0.83*	0.60/0.64
	Complicated umbilical hernia	♂	0.78/0.84	0.60/0.65
	Complicated umbilical hernia	♂	0.89/0.97	0.69/0.75
	Complicated umbilical hernia	♂	1.00/1.14	0.78/0.89
	Complicated inguinal hernia	♀	0.97/1.13	0.75/0.88
	Complicated inguinal hernia	♀	0.76/0.95	0.59/0.74
	Mandibular gland cyst	♂	0.72/0.79	0.56/0.61
	Intra-abdominal tumor	♂	0.66/0.84	0.51/0.65
	Intra-abdominal tumor	♂	0.70/0.89	0.54/0.69
	Intra-abdominal tumor	♀	0.65/0.98	0.50/0.76
2 - Xylazine/ Ketamine (n=5)	Intra-abdominal tumor	♂	0.71/0.94	0.55/0.73
	Intra-abdominal tumor	♂	0.52/0.86	0.40/0.67
	Mammary tumors and ovariohysterectomy	♀	0.64/0.87	0.50/0.68
	Ovariohysterectomy	♀	0.67/0.94*	0.52/0.73
	Ovariohysterectomy	♀	0.69/0.89	0.53/0.69
3 - Acepromazine/ Ketamine (n=9)	Intra-abdominal tumor	♂	0.81/1.10	0.63/0.85
	Intra-abdominal tumor	♂	0.84/1.20	0.65/0.93
	Intra-abdominal tumor	♀	0.93/1.11	0.72/0.86
	Intra-abdominal tumor	♀	0.64/0.91	0.50/0.71
	Intra-abdominal tumor	♀	0.59/0.86	0.46/0.67
	Mammary tumors and ovariohysterectomy	♀	0.73/0.95	0.57/0.74
	Mandibular gland cyst	♂	0.97/1.02	0.75/0.79
	Mandibular gland cyst	♀	0.79/0.88	0.61/0.68
	Mandibular gland cyst	♀	0.73/0.93	0.57/0.72

\*intraanesthetic hypotension

In a number of six patients intraanesthetic monitoring signaled hypotension with mean arterial pressure values maintained less than 60 mmHg for more than 15 minutes. This has corrected by lowering the concentration of inhaled anesthetic and by bolus administration of 10 ml/kg of saline solution.

Isoflurane is a potent inhalation anesthetic with very quickly induction and recovery due to very small blood-gas partition coefficient, 1.4 (1). MAC value for isoflurane in dogs is 1.28% (6). MAC values do not take into consideration any pre-medications that may have been given. Advanced age, bleeding, metabolic acidosis, hypoxia, hypotension, pregnancy, alpha-2 adrenergic agonists, opioids, ketamine, barbiturates, diazepam, pancuronium, decreased concentration of neurotransmitters in the CNS, are factors which reduce the MAC (3, 7). Information on the isoflurane use in veterinary medicine show that for induction of surgical anesthesia, which lasts about 5-10 minutes, inspired isoflurane concentration is between 2 and 4% (10). Following a barbiturate, values of 2.0 to 2.5% isoflurane alone with oxygen are usually employed (9). Surgical levels of anesthesia may be sustained with a 1.5 to 1.8% concentration of isoflurane in oxygen, therefore the concentration of vapor is much less than that required to induce surgical anesthesia (9). In practice, vaporizer settings of 1.5 to 2.5% are used, but premedication and/or concurrent use of nitrous oxide or the use of sedatives and/or analgesics during anaesthesia reduces the concentration of isoflurane required (10).

From the analysis of data obtained during surgery maintenance stage, results that surgical anesthesia was performed at minimum Et-Iso values between 0.52-1.20%, which is 1 MAC x 0.4-0.93, and maximum Et-Iso values between 0.78-1.40%, which is 1 MAC x 0.6-1.09. These results confirm the literature data indicating that for anesthesia maintaining are used concentrations below 1 MAC because of anesthetics associations (1). Significantly lowered of MAC by 30, 55, and 90% were noted after infusion of dexmedetomidine, a co-infusion of morphine-lidocaine-ketamine, and a co-infusion of dexmedetomidine-morphine-lidocaine-ketamine respectively (5).

Minimum values recorded, represents a significant reduction ( $p = 3.7 \times 10^{-16}$ ) reported to the MAC value of 1.28%. Analyzing the effect of premedication on minimum Et-Iso, results that the lowest values (average of minimum value was 0.646, compared to 0.811 for the Diazepam/Ketamine association and 0.781 for Acepromazine/Ketamine association) were required in Xylazine/Ketamine combination, which show a more intense inhibition of CNS and a lower need for inhalatory narcotic. Unfortunately this reduction is not significant, differences between the three groups being statistically insignificant.

In cases in which hypotension was found compared with the remainder cases, the statistical analysis show not significant differences. In geriatric patients Et-Iso values were not significantly different compared to other age groups. In this study the lowering of Et-Iso cannot be attributed to old age criterion but is the result

of premedication. Also were noted no significant differences of minimal Et-Iso to the two genders, literature neither in fact notifies any difference in this sense.

### **Conclusions**

In this study the maintenance of surgical anesthesia was achieved at minimum Et-Iso values between 0.52 and 1.20%, which represents one MAC x 0.4-0.93.

No significant differences of minimum Et-Iso values were found in the three groups.

Intraanesthetic hypotension, age or gender, did not significantly influence the alveolar concentration of isoflurane, low values of Et-Iso being the result of anesthetic associations used in premedication.

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## **REVIEW OF THE IMPORTANCE OF FRACTURE ASSESSMENT SCORE (FAS) IN THERAPEUTIC MANAGEMENT OF LONG BONE FRACTURES IN DOG AND CAT**

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### **Summary**

The objectives in treating fractures are bone union and the patient's return to normal function. Fracture planning should focus on the repair and also on all potential problems that might occur during the surgery.

Fracture-assessment scores are assigned on a scale of 1 to 10 but generally are grouped as high (8-10), moderate (4-7), and low (0-3).

Fractures with high scores have a short healing period of time and less rigid fixation and mechanical demand on the fixation are required. Instead, low score fracture have an extended healing time and require rigid fixation because of the great mechanical demand upon the fixation.

**Key words:** Fracture Assessment Score, examination, fracture management.

Making a decision in a final treatment plan for a fracture involves some factors and each factor has characteristics that support easy/rapid fracture healing and characteristics that result in slow/complicated fracture healing.

The objectives in treating fractures are bone union and the patient's return to normal function. (1, 2, 3, 4, 5). The fracture, the patient, and the client must be evaluated in order to identify which implants will achieve the necessary stability for the appropriate time to satisfy the objectives (i.e., develop a fractureassessment score) (1, 2).

Additionally, the selected fixation must be able to counteract the compressive, bending, and torsional forces applied to the stabilized bone by weight bearing and adjacent muscular contraction (1, 2).

It is always important to obtain an accurate history prior to stabilizing fractures. Failure to plan and anticipate problems associated with fracture repair consistently results in prolonged operating time, excessive soft-tissue trauma, and technical errors (3).

### **Fracture planning – general considerations**

After the complete examination of the patient, preoperative data must be analyzed to start the decision-making process. Patient information such as age,

weight, general health, activity level, and presence of other orthopedic pathology, radiographs of the fractured and corresponding contralateral intact bones, and client information, such as their expectations and ability to perform postoperative care represent the postoperative data (1, 2, 3, 5). All these data can be summarized as a fracture-assessment score that reflects the mechanical, biologic, and clinical environment in which the implants must function and guides the types of implants chosen (1, 2, 3).

Fracture planning should not only focus on the repair but also on all potential problems that might occur during the surgery, therefore, there should always exist a second plan if the initial plan is not working.

An optimal fracture plan is based on the radiographs to evaluate the fractured bone. Assessment of the limb and the patient is also important to predict the healing potential of the fracture and the risk of complications.

Improper planning outcomes are implant failure, delayed healing, infection, and nonunion.

Mechanical factors such as fracture configuration, potential for reconstruction and presence of concurrent musculoskeletal injuries affect the load the implant will bear (3).

Biological factors such as, fracture location, age and soft tissue injury affect the length of time an implant must function (3).

Clinical factors include a patient's and a client's compliance, desired postoperative limb function and surgeon's experience (5). The method of fixation is chosen by radiographic evaluation and by determining the areas of tensile and shear deformation and principles of application of each implant (5).

### Mechanical factors

An accurate mechanical evaluation indicates how strong the fixation must be for the patient. Mechanical factors (Table 1) include the number of limbs injured, patient size and activity, and ability to achieve load-sharing fixation between the bony column and the implant (1, 2, 7).

The selected fixation must be able to counteract the compressive, bending, and torsional forces applied to the stabilized bone by weight bearing and adjacent muscular contraction (1, 2).

Table 1

Assessment of mechanical factors			
Mechanical factors	1 (caution) ←-----→ 10 (little risk)		
Patient size	Large	Medium	Small
Load sharing	None	Partial	Ideal
Disability	More than a single limb	Preexisting clinical disease	Single limb

Reducibility of the fracture must be determined. In general, two-piece fractures and fractures with large butterfly fragments are considered reducible, allowing the reconstructed cortex to share the load of weight bearing with the implants. Fractures with multiple large fragments or multiple small fragments that cannot be secured with implants are considered nonreducible; in this case the implants will carry the load of weight bearing until callus is formed (1, 2).

Because of this implants are at risk to fail, therefore, a more rigid type of fixation with stronger implants is mandatory.

Restoration of the bone permits sharing of the weight-bearing load with the implant and will protect the implant from fatigue and early failure (3).

Complication rates are influenced by the degree of load sharing between implants and the bony column also. Ideal load sharing occurs when a transverse fracture is repaired because much of the force is transmitted axially through the limb (1, 2).

Animals with multiple orthopedic injuries will place greater demands on implants as they may be forced, prematurely, to take weight on an injured limb (3).

The transmission of loads from bone segment to bone segment through implants rather than through the bony column (e.g., highly comminuted fractures) implant loosening and fatigue failure are more common (1).

### **Biologic factors**

An accurate biologic evaluation (Table 2) determines how long the implants need to function to support the bone (1, 2, 7).

Usually, young animals (less than 6 months of age) have an active periosteum and the fractures are quick to heal because of the presence of an abundant cancellous bone; they need functional fixation devices only for a limited time (1, 2, 3).

The same fracture in an older animal will require stable fixation for a longer time. Geriatric or debilitated animals, or animals that have sustained substantial soft-tissue injury, will experience prolonged healing times which necessitate the need for stable implants for extended periods of time (1, 2, 3).

Other biologic factors to consider are whether the fracture is open or closed and if it resulted from a low energy or high-energy injury (1, 2).

Injury location and the bone injured influences biologic assessment because the soft tissue surrounding various long bones differs. Fractures located in areas with spare soft tissue envelope have, more often, delayed unions or other complications than fractures that are located in areas with more soft tissue envelope (1).



Table 2

**Assessment of biologic factors**

Biologic factors	1 (caution) ←-----→ 10 (little risk)		
Tissue injury	Severe	Moderat	Mild
Age	Geriatric	Mature	Young
Health	Debilitated	Concurrent diseases	Healthy
Trauma intensity	High velocity injury		Low velocity injury
Time of traumatic factor's action	Prolonged	Moderate	Short
Approach	Extensive/open	Mini	Closed

**Clinical factors**

Clinical factors (Table 3) are patient and client factors that affect healing during the postoperative period and thus has an influence on fracture-assessment score (1, 2, 6, 7).

Factors include the willingness and ability of clients to attend to their pet's postoperative needs, anticipated patient cooperation after surgery, and anticipated postoperative limb function. Regarding some of the clinical factors, there are unwilling clients or clients that are unable to commit the time needed to care for stabilization systems (e.g., external skeletal fixations and external coaptation); in this situation, bone plates and screws would be more appropriate (1).

Table 3

**Assessment of clinical factors**

Clinical factors	1 (caution) ←-----→ 10 (little risk)		
Patient/Client compliance	Poor	Suspect	Good
Patient's comfort level required	High		Not a consideration

Active and hyperactive patients are not good candidates for external coaptation because casts or splints are difficult to maintain without shifting or sliding. Also, external skeletal fixators may be poor choices because these patients may continuously bump the external bar against objects, causing premature transfixation pin loosening (1).

Postoperative limb function must be considered and anticipated when assessing a fracture, return to normal limb function being a goal of fracture management. Also, patient comfort during healing must be considered when selecting implants, including the patient's ability to cope with discomfort (i.e., a stoic animal) and estimated time to bone union (1).

The client education is very important (Table 4) (7).

Table 4

**Client education**

	1 (caution) ←-----→ 10 (little risk)		
Complications	Likely	Not unusual	Rare
Postop care	Critical	Important	Less critical
Surgeon	Expert	Experienced	Enthusiast

Implant systems vary in degree of comfort, depending on the involved bone and individual patient tolerance. As a general rule, bone plates provide the greatest level of postoperative comfort (1).

**Fracture Assessment Score interpretation**

Fracture-assessment scores are assigned on a scale of 1 to 10 but generally are grouped as high (8-10), moderate (4-7), and low (0-3). Fractures with high scores generally heal successfully with few complications, whereas fractures at the lower end of the scale are potentially less successful and have more complications (1).

Higher fracture-assessment scores mean less stress on the fixation system and less time required for bone healing (1).

Once information regarding the patient has been obtained and the mechanical and biological environment of the fracture is known, a decision regarding the appropriate type of fixation can be made (Table 5) (1, 2, 3, 4).

Table 5

**Implant requirements**

FAS (Fracture Assessment Score)	Type of implant
0 – 3	-plate – fixation combination -plate – rod combination -lengthening plate -type II fixator – pin combination with tie in - type III fixator – pin combination with tie in -interlocking nail
4 – 7	-type I/type II fixator -bone plate -pin + cerclage -fixator – pin combination with/without tie-in
8 – 10	-pin + cerclage -type I fixators (small/smooth pins) -external coaptation (splint/cast)

The implants used in fractures from group 0 – 3 must be strong enough to prevent bending or breaking. They must bridge the fracture and also prevent the excess motion at the fracture site (1, 2, 3, 4). These patients are not candidates for IM pin and cerclage wire fixation.

Implants used in fractures from group 4 – 7 intermediate load sharing and healing time.

Implants used in fractures from group 8 – 10 intermediate load sharing; very strong or stiff implants are not needed and they do not need to function prolonged time. Immediate load sharing between the bone-implant construct and rapid bone union are expected (1, 2, 3, 4).

### **Conclusions**

In fractures with high FAS (Fracture Assessment Score), the time necessary for a fracture to heal is shorter and less rigid fixation and mechanical demand on the fixation are required. These cases represent an opportunity for building surgical experience.

In fractures with low FAS (Fracture Assessment Score), rigid fixation is required and there is a great mechanical demand upon the fixation. Also, the fracture healing time is extended.

Usually casts, splints and external fixators require an intensive postoperative management.

Bone plates provide the greatest postoperative comfort and require less/no postoperative management.

**As surgeons, we have to always give the patient and the client the best treatment option for a given fracture. If the client cannot afford it or is not willing to pay, alternative repairs can be found.**

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## **ELECTROCARDIOGRAPHIC INVESTIGATIONS IN DOG BEFORE AND AFTER DIGOXIN THERAPY**

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### **Summary**

The electrocardiographic heart investigations, ensure not only an accurate diagnosis and help establishing a certain etiologic treatment, but also allow checking of its effectiveness by the high degree of precision that can follow certain heart parameters.

The research was conducted on a total of four crossbred dogs, two males and two females of different sizes and ages diagnosed with different degrees of compensated heart failure over 4 weeks. We have investigated the electrocardiographic parameters before and after the Digoxin treatment.

After having established the diagnosis and the stage of the heart failure, corroborating the objective symptoms with the electrocardiographic parameters, we have repeated the same investigation protocol after two and four weeks of Digoxin administration in therapeutically doses.

We concluded that in all cases Digoxin had favorable effects on the myocardial work, increasing also the atrioventricular conduction time.

**Key words:** dog, heart, electrocardiography, Digoxin

The increased incidence of cardiomyopathy in dogs required the updating means of diagnosis using modern noninvasive methods. This is why we may use heart electrocardiographic and echocardiographic investigations which ensure not only an accurate diagnosis and help establishing a certain etiologic treatment, but also allow checking of its effectiveness by the high degree of precision that can follow certain heart parameters (the cardiac dynamics, the wall thickness, the aspect of the valvular apparatus) (2, 5).

Considering the above, this paper investigates some electrocardiographic, echocardiographic and hematology parameters before and after Digoxin treatment in dogs that have been diagnosed with different degrees of compensated heart failure (7).

### **Materials and methods**

The research was conducted on a total of four crossbred dogs, two males and two females of different sizes and ages (7-20 kg, 12-16 years old) over 4 weeks. The dogs were clinically examined by using well known semiological methods and paraclinically (electrocardography) before and after the Digoxin administration (0.25 mg tablets).

The medicine was administered as follows:

- during the first 3 days we used a dose of 0.01 mg / kg body weight divided in two doses (in the morning and in the evening) in order to obtain the effect of digitalization;

- after having obtained the desired effect, the dose was halved, i.e. 0,005 mg / kg body weight also divided in two doses (in the morning and in the evening) for the rest of the treatment period.

We prepared an oral administration solution by using 0.5 mg Digoxin in 2 ml vials and 100 ml syrup to obtain a concentration of 0,005 mg active ingredient/ml for a more accurate dose administration to dogs under 10 kg body weight

During this research we used a Delta 1 CARDIOLINE ECG, the digital 12-lead pattern fitted with a high resolution thermal printer that uses 60 mm paper roles. The derivations and speed (25-50 mm / s) are displayed on the LCD screen. A standard ECG cable with four-electrodes: red (right thoracic limb), black (right pelvic limb), yellow (left thoracic limb), green (left pelvic limb) and six electrodes for recording the precordial derivations V1-V6 has been used. The electrodes were fixed in direct contact with the skin over the olecranon proximal thoracic limb and over the patellar ligament to the pelvic limbs. After application, the electrodes have been moistened with 70% isopropyl alcohol, to increase the electrical resistance. The ECG used: speed: 25 mm/sec; calibration curve etalon: 1 mV = 10 mm.

### **Results and discussions**

There has been registered an increased frequency of cardiomyopathy in the canine population, lately. These diseases are more often encountered even at a young age, on the one hand due to the more artificial living conditions, and on the other hand due to the stressful environment we work in.

The first case has been a 16 years old uncastrated crossbreed female of 7 kg. Clinically, it showed mild exertion fatigue, dyspepsia, nocturnal cough especially during periods of inclement weather (rain, fog, hot or cold temperatures).

Clinical examination revealed a reddish-purplish conjunctival mucosa, low intensity shock apex of high frequency, the heart projection area being slightly enlarged (1).

The following parameters have been found during the ECG examination (fig. 1): heart rate (HR) 136 beats/minute; sinus rhythm; vertical heart; P wave 0.04 s / 0.3 mV; 0.08 s P-Q interval; QRS 0.04 s / 1.3 mV; 0.18 Q-T interval; isoelectric S-T segment. There have not been found any changes, the electrocardiogram having a normal aspect with the exception of a sinus arrhythmia.

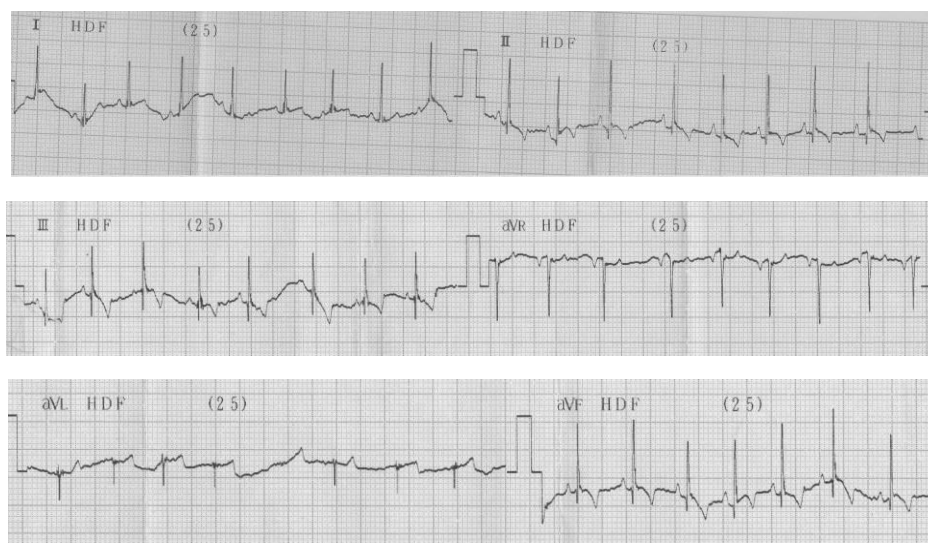


Fig. 1. Dog sinus arrhythmia

After two weeks of Digoxin administration new electrocardiographic recordings were carried out observing the following (fig. 2): decreased heart rate (HR) from 136 beats / minute to 107 beats / minute; regularization of the sinus rhythm; vertical heart; increased P wave duration from 0.04 s / 0.3 mV to 0.1 s / 0.3 mV, characteristic to left atrial cardiomegaly; a doubling of the PQ interval value from 0.08 to 0.16, which indicates a first-degree AV block; QRS complex remained unchanged at 0.04 s / 1.3 mV; increasing of the QT interval value from 0.18 s to 0.24 s, a value that reaches the upper limit of the species; isoelectric S-T segment.



Fig. 2. ECG image characteristic to left atrial cardiomegaly (CM<sub>AS</sub>) and first-degree AV block

The symptoms improvement was due to a slight change in the cardiac tone, lower heart rate, myocardial oxygen consumption, thereby increasing the myocardial performance. The delayed transmission of the electrical impulse from the sinoatrial node to the atrioventricular node has also been noticed, having adverse effects on the cardiac performance, known as the first-degree AV block in the literature (3).

By analysing the performed electrocardiogram, the following values have been revealed after a month of treatment (fig. 3):

- decreased heart rate from 109 beats / min to 79 beats / min, observing a tendency to bradycardia due to the Digoxin administration;
- maintaining increased P wave duration 0.08 s / 0.2 mV, characteristic to left atrial cardiomegaly;
- return to the physiological limits of the PQ (PQ int = 0.12 s) and QT (QT int = 0.16s) intervals.

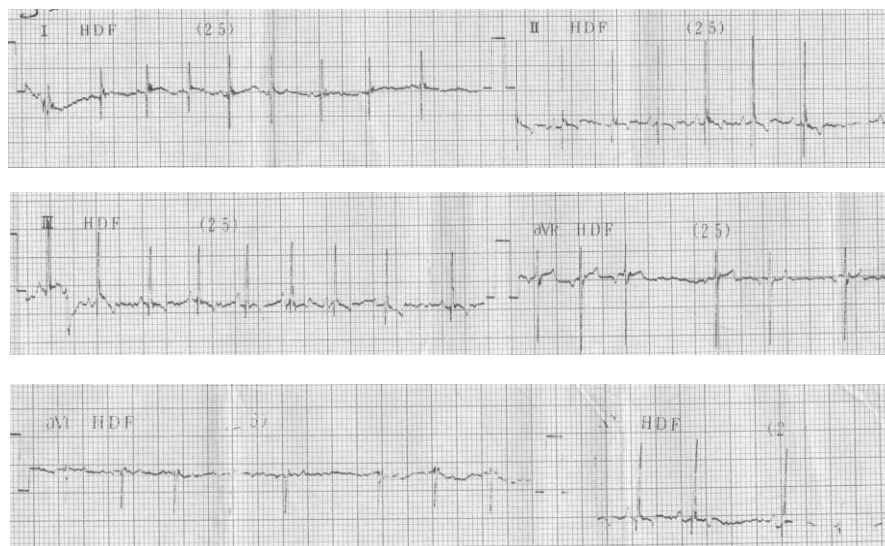


Fig. 3. Dog ECG – maintaining an increased P wave duration

From these data we can conclude that despite the positive effects of cardiac tonics, Digoxin maintains an increased capacity to influence the excito-conducting system, while reducing the heart rate and myocardial oxygen consumption level, with positive effects on the contractile force.

Another study performed on 62 dogs with congestive heart failure due to dilated cardiomyopathy revealed that Digoxin, Propranolol and Furosemide associated therapy has more positive effects on cardiac performance compared with their use as monotherapy (8).

The next studied animal (a castrated crossbreed female), radiologically diagnosed with a tumor in the left cranial mediastinum, presented clinically paroxysmic nocturnal cough and fatigue to average effort.

The ECG examination revealed the following parameters (fig. 4): heart rate (HR) of 150 beats / minute; sinus rhythm; vertical heart; P wave 0.08 s / 0.5 mV, characteristic to left atrial cardiomegaly; P-Q interval 0.12 s; QRS 0.04 s / 2.1 mV; Q-T interval 0.16; isoelectric S-T segment.

By analysing the ECG examination, the following values have been revealed after two weeks of treatment (fig. 5): decreased heart rate from 150 beats / min to 136 beats / min; maintaining the physiological sinus rhythm; decreased Q-T interval from 0.16 to 0.08 sec.

By interpreting the electrocardiogram, the following values have been revealed after four weeks of treatment (fig. 6): decreased heart rate from 136 beats / min to 108 beats / min, observing a tendency to bradycardia due to the Digoxin administration; maintaining the sinus rhythm; increased P wave duration from 0.08



s / 0.5 mV to 0.12 s / 0.6 mV, characteristic to left atrial cardiomegaly; increased PQ interval value from 0.12 to 0.16, which indicates a first-degree AV block.



Fig. 4. Dog ECG – tachycardia and left atrial cardiomegaly

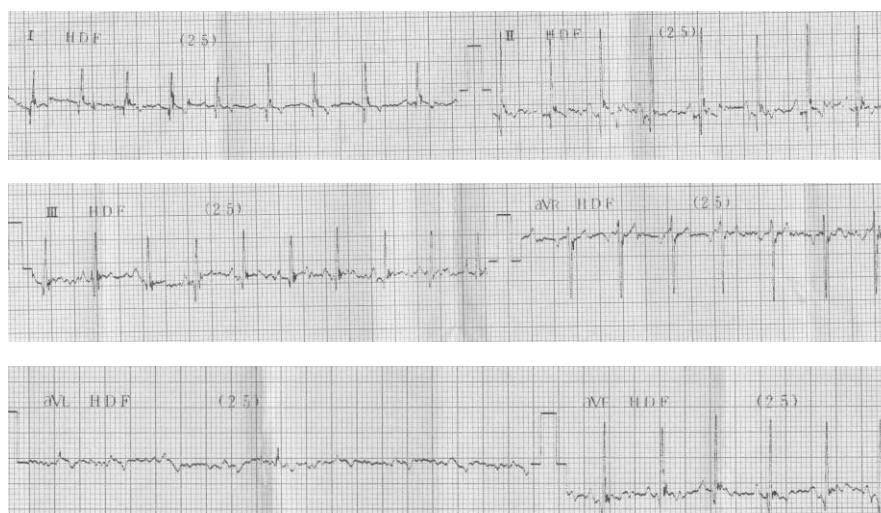


Fig. 5. Dog ECG– maintaining the aspect of a left atrial cardiomegaly

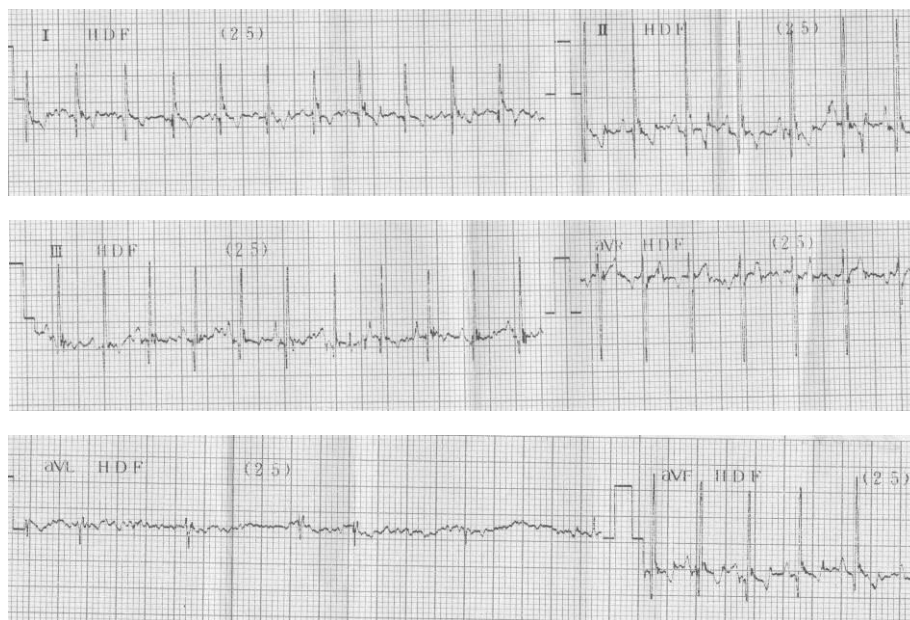


Fig. 6. Left atrial cardiomegaly and first-degree AV block

After a study performed on 18 dogs with atrial fibrillation was concluded that Digoxin and Diltiazem associated therapy has more benefic effects on cardiac frequency compared with monotherapy (6).

The next case has been a 13 years old intact crossbreed male of 13 kg. Clinically, it showed mild exertion fatigue, dyspepsia, nocturnal cough, the heart projection area being slightly enlarged, the heart sounds extending up to the 8th intercostal space (4).

The following parameters have been found during the ECG examination (fig. 7): heart rate (HR) 115 beats / min; sinus rhythm; horizontal heart; P wave 0.08 s / 0.3 mV, characteristic to left atrial cardiomegaly; 0.08 s P-Q interval; QRS 0.04 s / 2.9 mV; 0.16 Q-T interval; isoelectric S-T segment.

The following parameters have been found during the ECG examination after two weeks of treatment: decreased heart rate (HR) from 115 beats / min to 107 beats / min; increased P-Q interval from 0.08 s to 0,12 s, the other parameters being unchanged (fig. 8).



Fig.7. Left atrial cardiomegaly

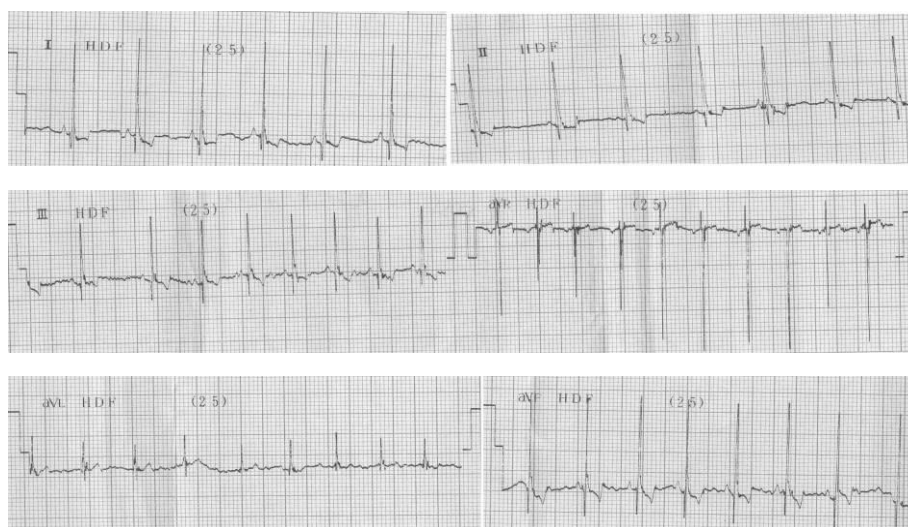


Fig. 8. First-degree AV block

At the end of the investigation period, after four weeks of treatment the following changes have been registered during the ECG examination (fig. 9):

- decreased heart rate (HR) from 107 beats / min to 100 beats / min;
- maintaining the sinus rhythm;

- increased P wave duration from 0.08 s / 0.3 mV to 0.10 s / 0.3 mV;
- increased complex QRS duration from 0.04 s / 2.9 mV to 0.06 s / 2.9 mV.

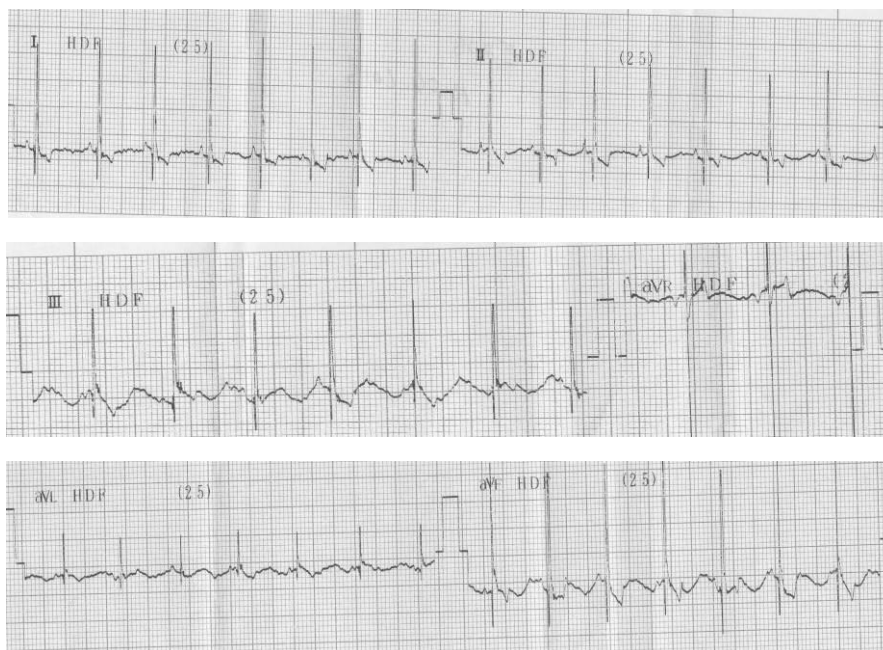


Fig. 9. AV cardiomegaly and first-degree AV block

The last case has been a 12 years old intact crossbreed male of 7 kg. Clinically, it showed fatigue to average effort, not only nocturnal cough, but also during periods of inclement weather (rain, fog, too low or too high temperatures).

The ECG examination revealed the following parameters: heart rate (HR) of 150 beats / minute; sinus rhythm; vertical heart; P wave 0.04 s / 0.5 mV, characteristic to left atrial cardiomegaly; P-Q interval 0.12 s; QRS 0.04 s / 2.9 mV; Q-T interval 0.08; isoelectric S-T segment; Q wave being  $> 1/3$  compared to R wave (fig. 10).

Repeating the ECG examination after two weeks of treatment, we have registered the decreased heart rate (HR) from 150 beats / min to 136 beats / min and the increased P wave duration from 0.04 s / 0.5 mV to 0.06 s / 0.6 mV.



Fig. 10. Sinus tachycardia, ventricular septal hypertrophy, myocardial ischemia

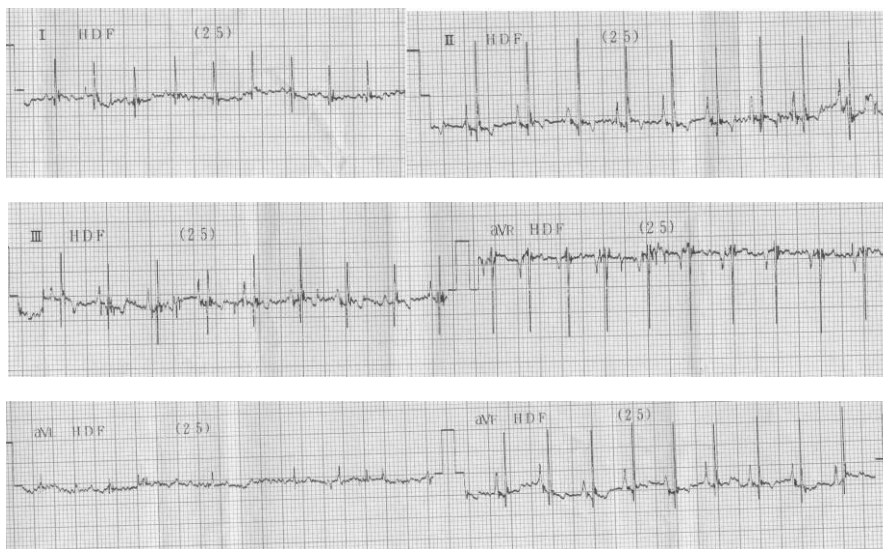


Fig.11. P wave duration and P-Q interval returned to physiological limits

The ECG examination has been repeated after four weeks of treatment revealing the following aspects: decreased heart rate (HR) from 136 beats/min to

125 beats / min); sinus rhythm within normal limits; P wave duration returned to physiological limits (0.04 s / 0.5 mV); decreased P-Q interval from 0.12 s to 0.08 s, the other parameters being within the normal limits (fig. 11).

### **Conclusions**

The use of Digoxin in therapeutic doses recommended by the literature may determine a reduction in heart rate with favorable effects on the myocardial contractile force reducing the oxygen consumption at this level.

The Digoxin administration used in special doses to "charge" the myocardium, caused an increased atrial complex duration by reducing the impulse discharge at the level of the sinoatrial node.

The administration of Digoxin in therapeutic doses may cause a first-degree AV block.

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## CLINICAL, ELECTROCARDIOGRAPHIC, ECHOCARDIOGRAPHIC AND THERAPEUTIC INVESTIGATIONS OF THE DOGS WITH HEART FAILURE

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### Summary

Cardiovascular diseases in mammals are almost identical, the difference being in their incidence and depend on the species, the breed, the age and the sex of the animal.

In the present study, we have established the diagnosis not only by using clinical, electrocardiographic and echocardiographic investigations, but also by a therapeutic approach of the heart failure in dogs, a complementary disease to degenerative lesions in the valve apparatus and/or myocardium.

We have examined a number of 5 dogs from different breeds, of both sexes, aged between 11 and 19 years old suffering from different cardiovascular diseases. After the physical examination, we detected insufficiency murmurs at the heart level while the paraclinical investigations highlighted cardiomegaly, subendocardial ischemia, the presence of fluid at the pleural and pericardial level and valvular degeneration. The treatment with Digoxin, Pimobendan, Benazepril, Furosemide and Panangin led to a visible improvement in the dogs' health condition.

**Key words:** dog, cardiomyopathy, electrocardiography, echocardiography

The dogs' heart diseases have increased lately, the most frequent being degenerative mitral valvulopathy, dilated cardiomyopathy, which can lead to heart failure. It is impossible to determine the heart failure in dogs, especially the degree of the myocardial dysfunction, without performing paraclinical investigations which provide the opportunity to elucidate the mechanisms that trigger the symptoms associated with heart disease (7, 8).

Through this paper we proposed to establish clinical, electrocardiographic, echocardiographic and therapeutic diagnosis of heart failure in dogs, as a secondary disease associated to degenerative condition of the valvular system and/or of the myocardium.

### **Materials and methods**

Five dogs from different races, of both sexes, aged between 11 and 19 years old, suffering from various diseases of the cardiovascular system have been examined in the Clinical Pathology and Medical Faculty of Veterinary Medicine in Timisoara.

The electrocardiographic (ECG) examinations have been performed using Cardiofax electrocardiographs with three channels monitors and 7.5 mm thermo sensitive paper and Delta 1 CARDIOLINE electrocardiographs with one channel monitor, the digital 12 - lead pattern and 60 mm paper.

The working parameters have been: unipolar and bipolar limb derivations; the mV amplitude of 10 mm, and the running speed of the paper of 25 mm/s. In some cases, the mV amplitude was of 5 or 20 mm and the speed of paper of 5 or 50 mm/s.

An ultrasound VIVID ECG has been used for echocardiography having the following technical characteristics: bi-dimensional module, M, color Doppler, pulsed, tissue, ECG channel that used a micro-convex probe of 5.0 to 6.5 MHz and the left parasternal window. In some cases, for the diagnostic accuracy the right parasternal window has also been used.

The sections were made as follows: longitudinal, transverse (to the mitral) and "4-rooms", working in particular in the 2-D cross mode, and the module M was used in order to calculate the thickness of the heart structure and the ejection or the shortening fraction.

### **Results and discussions**

A 16 years old, intact, Pekingese male of 5 kg showed fatigue to usual efforts, dyspnea with orthopnea, nocturnal dry cough, without fever. The auscultation revealed a mitral ejection systolic murmur of V/VI intensity and the ECG proved the sinus tachycardia (5).

The sinus tachycardia (HR = 250 beats/min.) characteristic to the systolic deficiency due to ventricular cardiomegaly has been discovered after the ECG examination using the following parameters: a 25 mm / s scrolling speed and the millivolts amplitude (mV) of 5 mm = 1 mV (fig. 1).



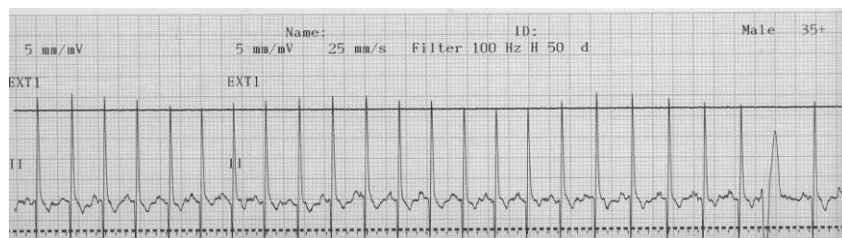


Fig. 1. Ventricular cardiomegaly with sinus tachycardia and ventricular extrasystole

The echocardiography performed in the left parasternal window revealed the increased atrial and ventricular left side diameter of 5 cm while the literature specifies that the maximum size must be of 4.5 cm in this category. A vegetative aspect of the mitral valve has been established at the mitral valve level in the autonomic free terminus (fig. 2 - 3).

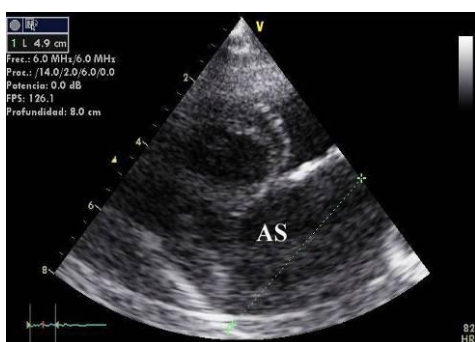


Fig. 2. Left atrial enlarge

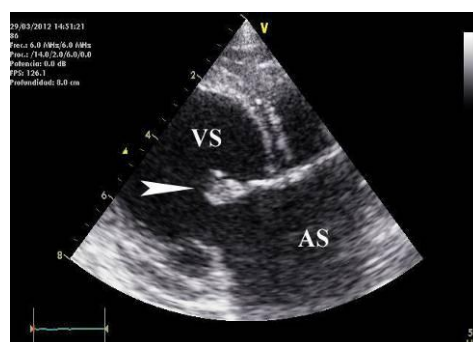


Fig. 3. Increased ventricle and left atrium with a dilatation aspect and mitral degeneration

The Doppler echocardiography, color and pulsed mode reveals the presence of some turbulence in the aortic orifice (emphasized by the presence of yellow, orange and green together with red/blue colours (fig. 4-5).

The diagnosis of degenerative mitral valvulopathy that lead to a 3rd grade heart failure (NYHA) has been established by putting together all the paraclinical investigations. The drug therapy consisted of:

- inhibitors of the angiotensin converting enzyme (ACE): Benazepril, 0.5 mg/kg *bid*;
- diuretics: Furosemide, 2 mg/kg, *bid*, for 5 days;
- Panangin (Mg and K aspartate having an important role in the cardiac cell function improvement: 1 tablet/day, for 2 days) (8).

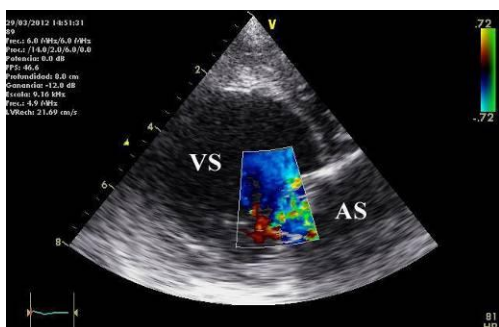


Fig.4. Turbulence in the aortic orifice (color Doppler)

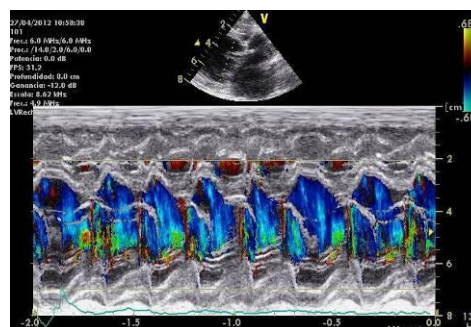


Fig. 5. Combining M mode ECG with color Doppler

After 14 days of treatment there was an improvement of the general condition, but the ascites also appeared. To reduce the blood pressure in the small circulation, we administered Furosemid injections followed by Wrecker abdominal puncture. The initial treatment was completed by Pimobendan administration at a dose of 1.25 mg/kg, *bid*, which caused a radical improvement of the general condition of the animal.

In a comparative study performed on a number of 56 dogs with degenerative disease of the mitral valve, regarding the treatment with Pimobendan and Benazepril, was noticed that use of Pimobendan as monotherapy inflicted an increase in cardiac systolic function, unlike Benazepril which showed no improvements of this function (6).

Another case has been a 16 years old neutered male that showed fatigue, dyspnea on minimal effort, dry cough and impaired systolic murmur grade V/VI in the projection area of the mitral valve during the clinical examination.

The ECG revealed the atrial fibrillation appearance (fig. 6) (3).

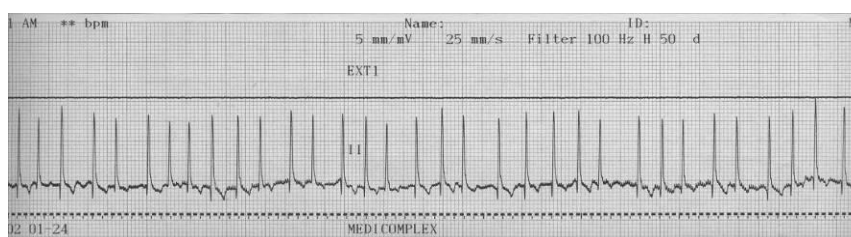


Fig. 6. Atrial fibrillation

The dystrophy and prolapsing of the mitral valve and the transition to the left atrial cavity during the ventricular systole has been established by the echocardiography. The chronic disease has caused an interventricular septal

hypertrophy, namely a hypertrophy of the left ventricle free wall with significant reductions in the ventricular chamber (fig. 7 - 8) (1, 2).



Fig. 7. Mitral valve dystrophy

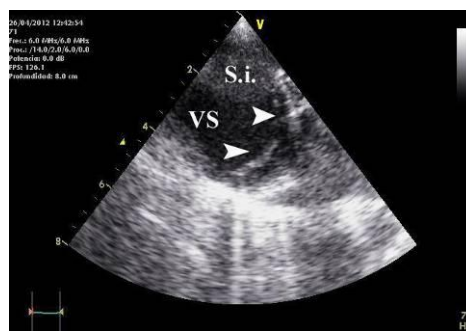


Fig. 8. Longitudinal section of the left ventricle (LV) and interventricular septum (SIV)

Corroborating these symptomatic data with the paraclinical aspects, we can establish the diagnosis of the decompensated 3rd grade heart failure, with degenerative mitral valvulopathy and atrial fibrillation. After the diagnosis has been established, the treatment protocol consisted of:

- cardiotonic (Digoxin 0.01 mg/ kg for 3 days followed by 0.005 mg/kg);
- converting enzyme inhibitors (Enalapril at a dose of 0.25 mg/kg, *bid*);
- diuretics (Furosemide at a dose of 1 mg/kg over 5-7 days).

Although converting enzyme inhibitors (ACE) protect against the loss of potassium, and the furosemide doses had been reduced, the use of potassium and magnesium salts, namely Aspacardin (half a tablet a day) was recommended. The owner has been also given the first emergency recommendations in case of cardiac decompensation and acute pulmonary edema occurrence, meaning the administration of high doses of Furosemide in high doses, about 10 mg every 10-15 minutes until the disappearance of the clinical symptoms.

The next case has been a 19 years old intact crossbreed female. Its medical history revealed a pronounced exhibit dyspnea, especially at medium efforts, fatigue at little effort and a distended abdomen. The examination found that the enlarged abdomen was due to the presence of the ascites fluid.

The echography examination revealed a hepatomegaly with hepatic hilum, respectively the ectasia of the right and the left portal vein (4).

The performed electrocardiogram showed: sinus rhythm, P wave=0.05 s/0.2 mV, PRint.=0.12 sec., QRS=0.07 sec/1.3 mV, QTint.=0.2 sec., right axial deviation, horizontal heart. These parameters allowed us to establish the diagnosis of sinus tachycardia and right bundle branch block (fig. 9).

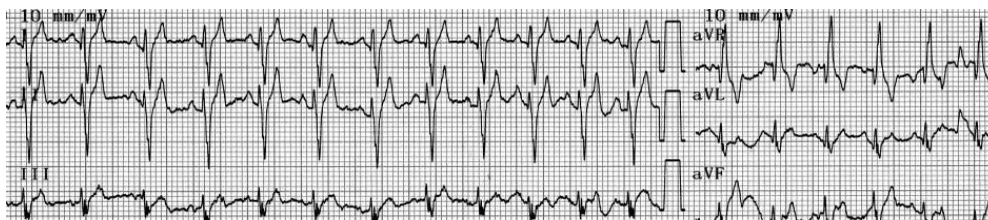


Fig. 9. Sinus tachycardia and right bundle branch block in dogs

The bi-dimensional echocardiography in module B has also proved the mitral valve prolapse which can be seen in the following colour Doppler ultrasound where the appearance of the mitral regurgitation is represented by the presence of yellow-orange-green colour. (fig. 10 -11).



Fig. 10. Mitral valve prolapse in dogs

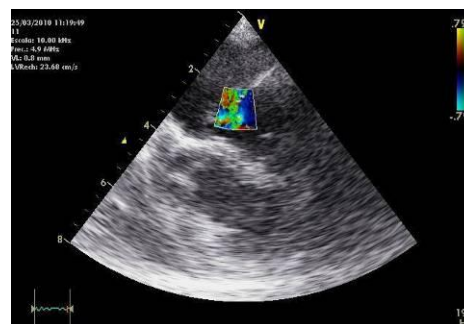


Fig.11. Mitral regurgitation in dogs

By analysing the results, we have established the diagnosis of right dilated cardiomyopathy and decompensated ascites.

The treatment consisted primarily of releasing the ascitic fluid and of drugs administration as follows: Pimobendan at a dose of 0.25 mg/kg, *bid*, Enalapril at a dose of 0.25 mg/kg, for 3-4 days, Aspacardin 1 tablet/day.

In a case of 13 years old spayed Yorkshire female of 5.5 kg, its medical history has shown nocturnal coughing followed by orthopnea and fatigue to common efforts. The clinical examination revealed a slight cyanosis, heart sounds, a strong apex shock and a systolic murmur at the III/VI mitral level. Lung crackles were detected during the auscultation. The electrocardiogram showed a biatrial cardiomegaly with ST segment depression, characteristic to the subendocardial ischemia (fig. 12).

The bi-dimensional echocardiography in B and M mode emphasises the increased right atrium and the hypertrophy of the left ventricle wall. Also, we noticed the right ventricular enlargement, the right atrial enlargement and the thickening of the interventricular septum, while the mitral route aspect examined in M mode highlights the thickening of the endocardial at the mitral level (fig. 13 - 14).



Figure 6 displays a B-mode echocardiographic image. The top portion shows a cross-sectional view of the heart wall, with labels indicating depth (5, 10, 15) and velocity (V). Below this, a larger B-mode image shows the heart wall structure over time, with a scale bar at the bottom right indicating 10 cm.

The patient being known as a "cardiac" and having the symptoms described above exemplified by the tests, was diagnosed with a 3rd grade heart failure with a right heart decompensation. As in the previous cases, the treatment consisted in the administration of the well known "triad": cardiotonics, ACE inhibitors and diuretics.

The next case was a 12 years old German shepherd male. The following symptoms have been revealed from the anamnesis point of view: advanced weakness, moderate dyspnea, dry cough and blurring heart sounds. The electrocardiogram performed has highlighted aspects of sinus tachycardia with sinus rhythm and the ECG microvolt route (fig. 15).



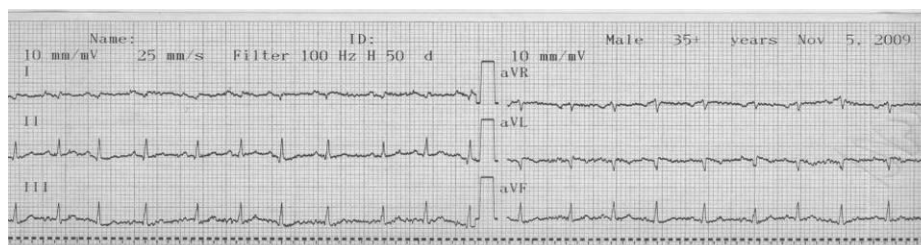


Fig. 15. Sinus tachycardia and the ECG microvolt route in dogs

This aspect required a two-dimensional echocardiography investigation in B module that revealed the thickening of the pericardial sack, the presence of the fluid not only in the pericardial sack, but also at the pleural level (fig. 16).



Fig. 16. Pleuropericardic fluid in dog

The diagnosis of exudative pericarditis was established based on the results of the investigations. Because the animal's general condition did not require an urgent intervention (patient did not have orthopnea and cyanosis) the pleural and pericardial puncture was not necessary. Since we suspected a pericarditis secondary to a dilated heart disease, the treatment was required. By administering Digoxin associated with Pimobendan, Benazepril, Furosemide and Panangin, after about a week there was a clinical improvement manifested by the reduced amount of fluid, which confirmed the diagnosis.

### Conclusions

Clinically, the subjects in the study presented fatigue to common effort, dyspnea accompanied by orthopnea, dry cough, especially at night, without fever, ascites.

A systolic murmured of V/VI insufficiency could be heard during the auscultation.

Sinus tachycardia, atrial fibrillation, biatrial cardiomegaly accompanied by ST segment depression, characteristic to subendocardial ischemia, have been highlighted by the EKG examinations.

Enlarged left heart, dystrophy with prolapsing the mitral valve, reducing the thickness of the interventricular septum (IVS), the presence of fluid in the pericardial and pleural sack have been highlighted by the ECG examinations.

Doppler ECG has revealed the presence of turbulence in the aortic orifice secondary to mitral valve prolapse.

Digoxin, Pimobendan, Benazepril, Furosemide and Panangin administration led to improvement in the general condition.

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## ANGIOCARDIOGRAPHY WITH CONTRAST MEDIUM IN SWINE

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### Summary

Pigs are a widely used species in various medical experiments. Our study aims to investigate a segment of the cardiovascular system in this species. For the accomplishment of this study we performed several thorax radiographs highlighting the heart and the main vessels originating from this level. All individuals used in this study were subjected to neuroleptanalgesia. The aim was to obtain quality images focusing on various anatomical and radiological aspects such as: the amount of contrast medium injected in the vein, the position of piglets and radiation exposure, the duration of radiation exposure and the progression of contrast medium with the blood in the cardiovascular system.

**Key words:** angiography, swine, contrast medium, cardiovascular.

The main reason we chose the swine as the study species, is the correlation between humans and pigs. It is known that there are anatomic studies of pigs, but there are also comparative studies between different animals and humans. Most researchers focused on the anatomic correlations between humans and pigs. The statements made above show that not only veterinarians have undertaken studies of pigs, but also human physicians.

### Materials and methods

The biological material used for the angiographic investigation consisted of 5 male piglets, aged between 30 and 65 days, with a weight range between 20 and 35 kg. All pigs were obtained from a private farm from Zalau County and were Mangalitza breed.

The angiocardiology was performed at the Radiology Laboratory of the Cluj-Napoca Faculty of Veterinary Medicine, using the TEMCO GRx-01 radiological device (K&S Röntgenwerk Bochum GmbH & Co, Germany). Automatic processing, type JP-33 was used to develop the radiological film. For highlighting the cardiovascular system, we used Iomeron 350 (Bracco U.K. Ltd), solution for injection, as an X-ray contrast medium.

Iomeron 350 contrast medium is used, according to the concentration, for different types of examinations: peripheral arteriography, venography, urography, myelography (for skull and/or spinal column visualisation, including the intervertebral disks). The solution is also intended for the clinical investigation of internal organs - gall bladder, different glands, fistulas, but also articulations and



male/female genital organs (11). The main therapeutic indications are for aortography, angiocardiology and left ventriculography (12).

The clinical examination was conducted according to an observation sheet. The piglets were monitored throughout the period of investigation, recording physiological constants: pulse, respiration, temperature. For optimal accommodation, the subjects were housed in individual pens, with two days of rest before the beginning of the experiment.

The angiocardiology with contrast medium technique requires the induction of neuroleptanalgesia for the proper conduct of investigations. This method reduces the risk of obtaining erroneous results due to body movements performed by subjects during radiation exposure.

Neuroleptanalgesia was induced to each pig by an intramuscular injection of 2%Xylazine, 2-4 mg/kg body weight and 10%Ketamine, 10-20 mg/kg body weight.

For the injection of the contrast medium we prepared the jugular region, more specifically the external jugular vein. After that, through the circulation it reached the heart and thus the major blood vessels. The amount of contrast agent injected was 25 ml Iomeron 350, for each subject.

Before the injection of the contrast medium, we prepared the area by trimming, shaving and disinfection with alcohol the entire jugular region.

To reveal the external jugular vein we first performed a local anaesthesia with lidocaine and then we incised the skin and muscle to highlight the external jugular vein. Afterwards we introduced a cannula and attached a syringe in order to inject the contrast medium into this vein (Fig.1).

Radiological exposures in pigs were made in dorso-ventral and latero-lateral decubitus. Radiological exposures were executed serially as follows: during the injection and then at 1 and 5 minutes after the administration of contrast medium.



Fig. 1. The injection of the contrast medium into the jugular vein

### Results and discussions

In terms of injecting the Iomeron 350 contrast medium into the external jugular vein, we can say that the results were identical for all five subjects undergoing radiological investigation.

By injecting the Iomeron 350 radiological contrast medium into the external jugular vein, we obtained a radiological image that determined a uniform opacity of the jugular vein path, which continues with the cranial vena cava.

Latero-lateral decubitus of the subject and latero-lateral radiological exposure yielded images that allow us to visualize the path of the jugular vein, thus observing its relationship with the medial part of the shoulder joint, but also with the scapulo-humeral articulation (Fig.2). Radiological exposure was taken during the injection of contrast medium.

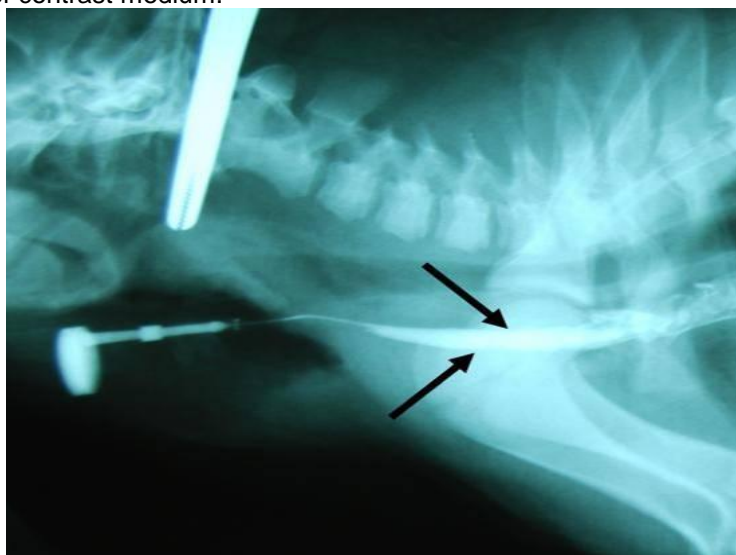


Fig. 2. External jugular vein trajectory (black arrows)

From the external jugular vein, Iomeron 350 contrast medium propagated up to the cranial vena cava. This resulted in the opacification of the right atrium, knowing that at this level the opening of the cranial vena cava is situated. Due to right atrium and right ventricle communication, through the atrioventricular orifice, we noticed the passage of the contrast medium into the right ventricular chamber, a process that took place through the tricuspid valve during atrial systole and ventricular diastole (Fig.3).

The distribution of contrast medium produced a uniform opacification of the right part of the heart, thus the radiographic images obtained from all subjects

revealed that the lomeron 350 substance penetrated both right atrium and right ventricle cavities (Fig.3).

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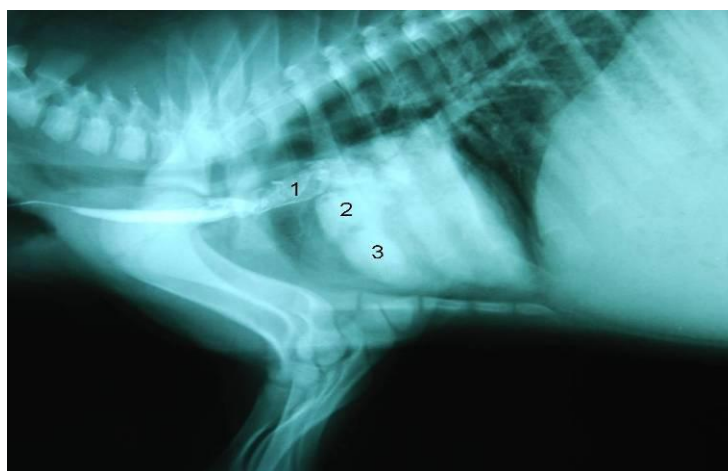


Fig. 3. 1.Cranial vena cava; 2.Right atrium; 3.Left ventricle

Due to the right ventricular systole, the contrast medium was distributed in the pulmonary arterial trunk. In this way we obtained a uniform opacification of the pulmonary arteries, determining the opacification of entire bronchial tree (Fig.4).

Angiographic images performed during the injection of lomeron 350 in subjects positioned in the latéro-lateral decubitus and latero-lateral exposure revealed a good view of the topography of the heart in pigs. Radiographic images show its position in the cardiac mediastinum, between the intercostal spaces 3 and 6, dorsal to the sternum. The apex of the heart has a ventral-caudal orientation in the direction of the diaphragm muscle. In swine, the heart has a globular aspect, with the longitudinal axis tilted to sternum (Fig.3).

Both latero-lateral decubitus of subjects with the latero-lateral exposure and ventro-dorsal decubitus of subjects with dorso-ventral exposure helped to obtain a good image in terms of heart topography in this species (Fig.5).

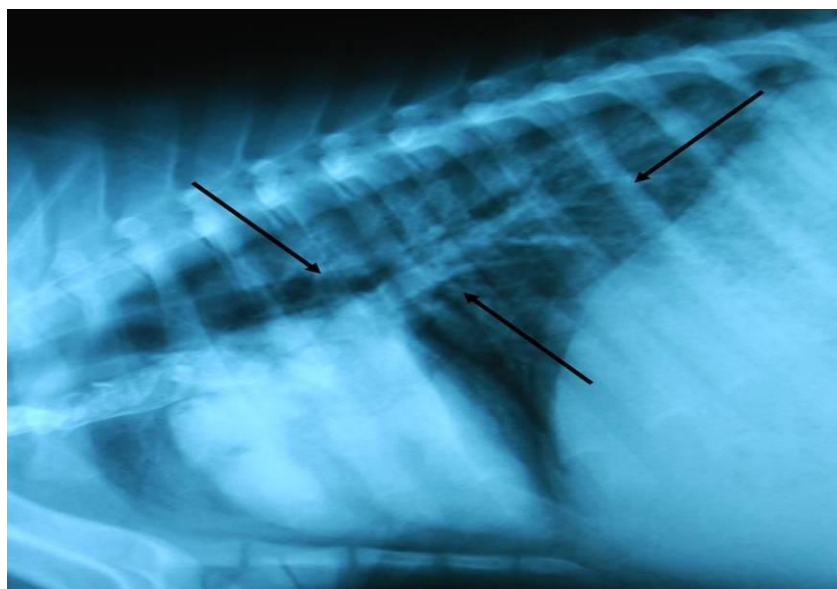


Fig. 4. Vascular opacification of bronchial tree

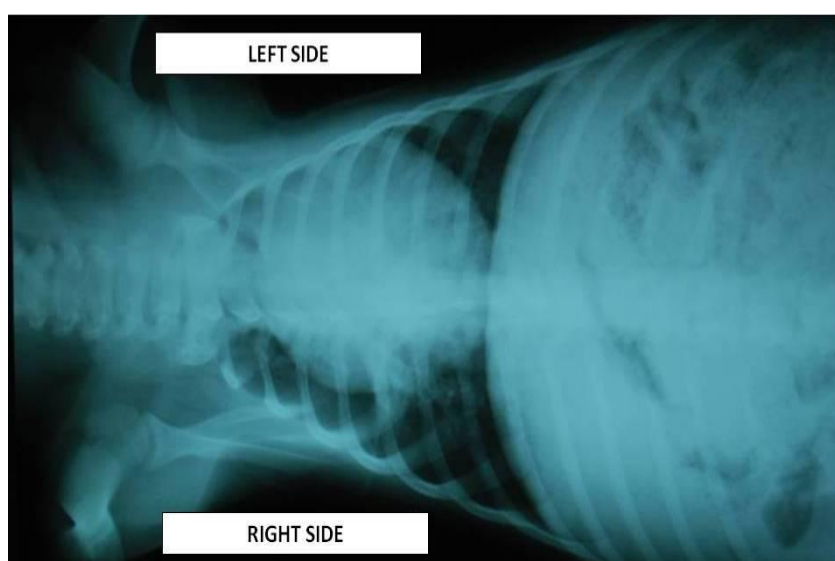


Fig. 5. Topography of swine heart: ventro-dorsal radiological exposure

Regarding the return of contrast medium from the lungs to the heart, we must affirm that it has occurred in a small percentage in all subjects investigated.

This statement is supported by the obtained radiographic images, where a weak aorta opacification can be observed. This process did not involve the brachio-cephalic trunk, knowing that this trunk detaches directly from the aortic arch (Fig.6).

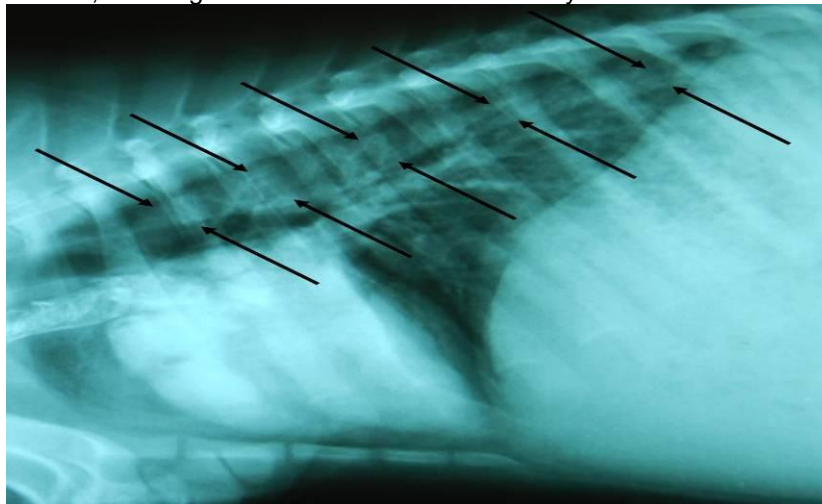


Fig. 6. Weak opacification of the aorta

Radiographic images obtained at 5 minutes after injection of the contrast medium indicates that the Iomeron 350 substance is no longer present in the heart, in any of the subjects (Fig.7). This finding is due to high blood flow at this level.



Fig. 7. Latero-lateral radiological exposure 5 minutes post injection of Iomeron 350

Non-ionic contrast agents used to view the cardiovascular system in pigs, offer arguments to consider this species as an experimental model in comparative medicine as imaging technique performed in this study did not result in loss of any subject.

The review of the literature shows that not only veterinarians have undertaken studies in pigs, but also human physicians (2, 7, 8, 9, 10). Regarding the topography and shape of the heart, we mention that both latero-lateral radiological exposure with lateral-lateral decubitus of the subjects and ventro-dorsal exposure of subjects in dorsal-ventral decubitus provided quality images. Based on these results we can say that the heart's shape and topography in the investigated subjects corresponded to the description of other authors (1, 2, 3, 4, 5, 6, 7, 10).

The arteriography image obtained confirms the origin, path and distribution of the pulmonary arterial trunk, highlighted by other authors through various dissection techniques in pigs (4, 9).

### **Conclusions**

The angiocardiology with contrast medium technique proved to be a useful imaging technique in pigs.

Due to angiocardiological images we were able to visualize the heart and main vessels with their origin, path and distribution.

The amount of the contrast agent used in this study yielded heart opacification in all of the investigated individuals.

The contrast medium used for the opacification of the arteries heart did not change the state of health of the subjects included in our study.

The best results regarding heart and arteries opacification were obtained by performing radiologic exposure during the injection and one minute after it.

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## RESEARCHES REGARDING CONTROLLED REPRODUCTION IN NILE TILAPIA

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### Summary

Currently, the domestication of indigenous fish species is promoted and the hormonal stimulation is intended to multiply the initial number of natural reproductions per year and the amount of eggs deposited at each spawning. *Oreochromis niloticus* was introduced in Romania in 2008 through a project in The Research and Knowledge Center on Fish Growth Nucet from Dâmbovița. Various ways have been tried to attain its acclimatization and a rise in both reproductive capacity and adaptability of this species to the Romanian climate.

In this study, between 01.07.2012 and 01.07.2013, based on the methods described bellow, we investigated and determined the possibility of controlling the reproduction of the Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1757). Eco-technological conditions have been provided on the farm SC KAVIAR HOUSE BUCHAREST BRANCH TULCEA SRL and we attempted breeding technologies of Nile Tilapia adapted to aquaculture in our country.

We used 36 fish including nine males and 27 females divided into three pools of 300 liter capacity each, immediately after each fish has been weighed and measured in length. We created three groups, of eight, 12 and 16 fish in each pool, having the male-female ratio of one:three. The average weight and length of reproducing fish was about the same, i.e. 80-110 grams and 13 to 15cm in length. Males and females can be easily distinguished by the shape and color of the body. Water temperature ranged during the experiment from 26°C to 28°C.

In the first experiment, with six females, 53 larvae/female were obtained, the fertility percentage was 83%. In the second experiment, with nine females, 49 larvae/female were obtained, the fertility percentage was 77.7%. In the third experiment, with 12 female, 38 larvae/female were obtained, the fertility percentage was 58%. Males are very territorial, and the increase of their density may lead to increased fighting and the decrease of their reproduction activity. Smaller number of larvae per female achieved within higher density experiments can be explained by the intensification of cannibalism, especially because females do not have a synchronous spawning and fish that carry no eggs or larvae in the mouth will eat up the larvae or eggs dropped in the tank by other females.

**Key words:** reproduction, Nile, Tilapia, biometry, *Oreochromis niloticus*



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Based on the methods described bellow, we investigated and determined the possibility of controlling the reproduction of the Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1757). Eco-technological conditions have been provided on the farm SC KAVIAR HOUSE BUCHAREST TULCEA BRANCH.

### **Materials and methods**

Researches have been conducted between 01.07.2012 and 01.07.2013 in SC KAVIAR HOUSE BUCHAREST TULCEA BRANCH in the project "Building and location of ponds for raising fish annex building construction and purchase of specialized transport means in Tulcea, Horia town."

We used three tanks with a capacity of 300 liters, a 1m<sup>3</sup> capacity water tank, three current water supply systems, three systems for water circulation, three automatic feeders and 30 kg feed especially for tilapia (1, 7).

36 fish including 9 males and 27 females were divided into three pools of 300 liter capacity each, immediately after each fish has been weighed and measured in length. We created three groups, of eight, 12 and 16 fish in each pool, having the male-female ratio of one:three (2, 5).



Fig. 1. Morphological aspects of both Tilapia male and female breeders (orig.)

The average weight and length of reproducing fish was about the same, i.e. 80-110 grams and 13 to 15cm in length. Males and females can be easily distinguished by the shape and color of the body. Water temperature ranged during the experiment from 26°C to 28°C.

Breeding fish were placed in fiberglass tanks of 300 liters capacity water immediately after being weighed and measured in length each. After breeding, juveniles were moved into a 1m<sup>3</sup> water tank. Water supply could be achieved by gravity from a reservoir (Horia accumulation) and the supply system used was the one with continuous flow.

Table 1

**Biometry results in females**

Identification number	Weight (grams)	Length (cm)
1	83	13
2	87	13.2
3	93	14
4	96	13
5	100	14
6	110	13
7	89	14.1
8	103	14
9	101	15
10	99	14.8
11	98	14.7
12	92	15
13	88	14.6
14	105	15
15	84	13.8
16	82.9	13.9
17	82	13
18	95	13.6
19	106	15
20	85	13.1
21	98	13.2
22	108	15
23	109	15
24	110	15
25	107	14
26	97	14.2
27	83	13

Table 2

**Biometry results in males**

Identification number	Weight (grams)	Length (cm)
1	85	13
2	112	15
3	109	14.2
4	88	13.8
5	87.3	13.6
6	92	14
7	96	14.9
8	101	15
9	100.2	14.2

During spawning males are much easier to identify, they have red lip and red areas on the ventral side of the head and on the pectoral fins. Females are not difficult to recognize, though they do not have a specific color and can be easily seen by the increased abdomen.



Fig.2. Male (left) and female (right) of tilapia during mating period (orig.)

Other external differences between the sexes are based on the fact that males have two holes: one is the anus, while the other is the urogenital opening. Females have three holes: anus, the second is the genital opening and the third is

the urinary tract. Sexing fish is a simple, but quite stressful procedure. Therefore it is recommended that this procedure be performed only once per year. After sexing, fish are left in the tank, without other interventions for four days, and the next meal in the program will be over another four hours.

For the controlled breeding experiments conducted at KAVIAR HOUSE were selected males releasing sperm at a mild abdominal pressure. In females, the selection criteria were represented mostly by increased volume and soft abdomen including sexing with methylene blue for certitude. (3)

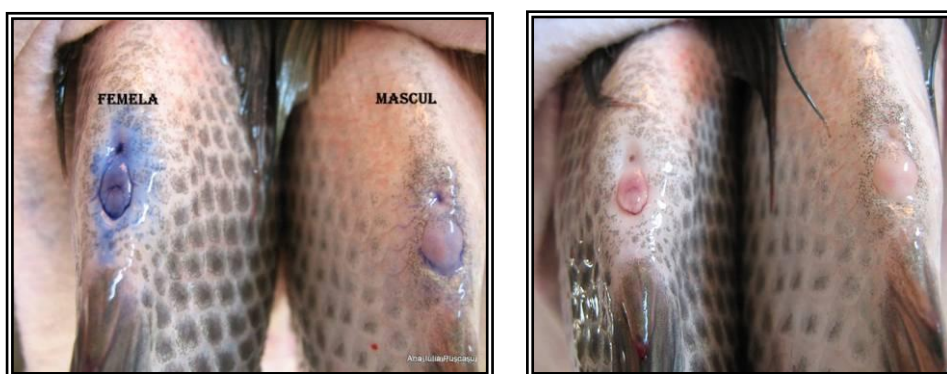


Fig.3. Sexing Tilapia fish tilapia with methylene blue (left) and with no special help (right) (orig.)

The Nile tilapia female repeatedly releases a string of about 20-50 eggs and then the male goes right over the eggs with opened genital papilla which will release a milky cloud of sperm. The female returns immediately and takes the fertilized eggs in her mouth. Studies have shown that eggs fertilization occurs inside the female's mouth. (5, 6, 7)

Incubation of fertilized eggs occurs within the female's mouth. The larvae remain in female's mouth until the yolk sac is absorbed completely and juveniles start to feed themselves. When larvae were discharged from the mouth of females they had already 11 days of life, the yolk sac was absorbed. They were moved in special incubators where they were fed every day, at every four hours with a special diet for tilapia represented by *Aller Aqua* feed (*Aller Tilapia*) with a diameter of one mm and 33% crude protein.



Fig.3. Incubation of fertilized eggs within the female's mouth (orig.)

### Results and discussions

Interval between the onset and the end of the experiment reflects a period of one year, during which we have overseen one spawning of Tilapia.

The water temperature varied within 26-28°C in all three experiments. The incubation temperature for the best survival rate varied between 25-32°C. Decreasing water temperature below 22°C in the subtropics may produce a delay or decrease the number of eggs deposited by the female, as it was reported in Nile tilapia in Vietnam and Egypt.

The physico-chemical properties of water indicate some differences between the three experimental variants. As it can be seen, the differences for chemical parameters to experimental data values were high, but the values did not exceed the normal range for reproduction Nile tilapia.

Although values of the minimum oxygen dissolved in water, in all three variants have been under four mg/l, they did not affect the reproductive process.

Table 4

**Physico-chemical parameters of water during the experiment (day 10)**

No.	Parameter	Unit	Critical/ideal Values	Exp. 1	Exp. 2	Exp. 3
1.	pH	pH units	4-11	8.0 – 8.2	7.8- 8.2	7.8-8.4
2.	Alkalinity	ml HCl /l		2.0-3.2	2.2.-2.9	1.5 -2.5
3.	Total hardness	(grade D)		3.80- 5.96	3.92-5.82	2.35- 4.14
4.	Dissolved oxygen	Mg O <sub>2</sub> /l	<2 / > 6	3.5-14.1	3.8-6.2	3.1-5.7
5.	Ammoniac	Mg/l	>15/<3	1.29 – 2.91	1.43 - 2.67	1.09-2.99
6.	Nitrites	Mcg/l	>5 /<1 mcg/l	0.0381- 21.02	0.019- 134.64	0.026- 0.174
7.	Nitrate	Mcg/l	>500/ <20 mg/l	0.06-193.1	0.02- 128.31	0.02- 0263

At the end of the experiment we had to determine the number of breeding females and the number of larvae per female in each experiment.

Table 5

**Final results of the experiment on breeding females and the larvae obtained from them**

No.	Experiment	Female no.	Larvae in total	Fecundity percent
1.	I	6	53 larve/femelă	83%
2.	II	9	49 larve/femelă	77.7%
3.	III	12	38 larve/femelă	58%

As it can be seen, the best results were recorded in experiment I, eight breeding fish in the pool. The decrease in the number of breeding females is inversely proportional to the increasing of the number of youngsters in the tank. This phenomenon can be explained by the fact that males are very territorial and the increase of their density may lead to increased fighting and the decrease in reproduction. Smaller number of larvae per female achieved wherein the breeding density was higher can be explained by cannibalism intensification, especially because females do not have a synchronous spawning and fish eggs or larvae dropped by other females in the tank will be eaten by other fish which have no eggs in the mouth (4).

The development time of the fertilized tilapia egg is represented by a period ranging from a minimum of three days to maximum six days, depending on a number of factors, including the water temperature, salinity and water flow rate.

### Conclusions

Controlled reproduction without hormonal stimulation or without water heating clearly demonstrated that Nile tilapia can be cultivated as any native species of fish, as long as the water temperature during winter in the culture system does not drop below 18°C.

The report between females and males can range from one:five in ponds, but the ratio of one:three in the pools seems to be optimal.

Increasing the density of breeding fish decreases spawning activity because of the aggressiveness and fighting among males that will lead to the decrease of the natural reproductive behavior, decreased fertilization and lack of incubation.

Also, by increasing the density we obtained the phenomenon of cannibalism among fish breeders and juveniles.

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## THE IMPACT OF THE ANTIINFLAMATORY TREATMENT ON DOGS WITH INTERVERTEBRAL DISC DISEASE BASED ON OLBY RECOVERY SCORE

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### Summary

The present study is a description of the evolution of motor neurological deficits in dogs diagnosed with intervertebral disc disease, which were treated with anti-inflammatory substances for 3 days. The evolutions of motor neurological deficits have been registered using Olby recovery score for 7 days.

Analysis of Olby recovery score has showed clinical improvements since the third day of monitoring for all patients from study. Also, the patients that were treated with non steroid anti-inflammatory drug have shown increases of 50% recovery score after completing treatment than patients that were treated with steroidal drug.

**Key words:** intervertebral disc disease, anti-inflammatory drugs, Olby recovery score, dogs.

Intervertebral disc disease (IVD) herniation is a common cause of neurologic dysfunction in dogs and approximately 2% of the canine population suffers from this disease. For the last 60 years, IVD herniation has been the focus of significant research aiming to describe and understand this debilitating condition and to improve therapeutics options for clinical patients (2, 5).

Thoracolumbar disc herniation can cause varying degrees of back pain and neurologic deficits that range from mild paraparesis to paraplegia with or without loss of deep pain perception. Upper motor neuron (UMN) signs are associated with most IVD extrusion but lower motor neuron (LMN) signs are possible and indicate a lower lumbar lesion (10).

The treatment of IVD herniation is depending on the neurologic status of the animal and the owner's ability to proceed with the recommended therapeutic plan, because this condition may be managed conservatively or with surgical decompression of the spinal cord (9).

Generally, conservative management of IVD herniation typically consists of restricts confined rest, anti-inflammatory drugs, muscle relaxants, analgesics and physical therapy (8, 11, 13).

Though, the most important part of conservative management for any animal suffering from disk herniation is restrict confinement for 4-6 weeks to prevent continued nuclear extrusion or protrusion and reduce the risk of self-trauma



as a result of in coordination (11,13) a retrospective evaluation revealed that the duration of cage rest did not affect the success of medical therapy (8).

Drug therapy for spinal cord injury, including acute disk herniation is controversial. Steroids are not the mainstay of treatment for the disease and do nothing to alleviate the compression on the neural tissue. Non-steroidal anti-inflammatory drugs can be used for pain control instead of steroids.

This therapy is becoming more popular in referral institution to help prevent the potential side effects of steroid use and the potential detrimental effects of steroids on the healing spinal cord (9).

There are currently 3 validated scoring systems (6, 7, 12) to assess the neurologic status of patients experiencing spinal cord injury. In particular, the 5-point grading system (6) is useful for predicting the outcome following intervertebral disc disease and 14-point motor score is a system that was developed as mean of scoring and monitoring pelvic limb function as a result of thoracolumbar injury.

Although, the Olby recovery score is used for monitoring thoracolumbar trauma of small animals (12) it can be used to monitor the motor recovery of neurological disorders of patients with other spinal pathology.

### Material and methods

The study was conducted in a heterogeneous group of 10 dogs that have been diagnosed with acute intervertebral disc disease. Following diagnosis, dogs were divided into two equal groups. The first group was treated with steroidal anti-inflammatory drug and the second group was treated with non-steroid anti-inflammatory drug. Both groups have received the anti-inflammatory medication for 3 days and then they were monitored during 7 days. The evolution of motor neurological deficits was registered using Olby recovery score.

### Results and discussions

During 7 days of monitoring clinical, recovery score increased for each patient so that at last assessment all patients were registered ambulatory but with varying degrees of neurological deficits. (Table 1, Figure 1)

Table 1

**Olby recovery score of patients with intervertebral disc disease**

	Patient number	I	II	III	IV	V	VI	VII
<b>Group I</b>	<b>1</b>	8	9	10	10	11	12	13
	<b>2</b>	7	7	8	8	8	8	8
	<b>3</b>	7	7	7	7	8	8	8
	<b>4</b>	8	8	9	10	11	11	12
	<b>5</b>	5	5	6	6	6	7	7

<b>Group II</b>	<b>6</b>	8	8	9	9	10	11	13
	<b>7</b>	8	8	9	10	12	12	13
	<b>8</b>	9	9	10	10	11	12	13
	<b>9</b>	10	10	11	11	11	12	12
	<b>10</b>	9	9	9	10	10	11	11

For the 10 dogs that were included in the study, 2 dogs have showed only with one recovery point and 3 dogs have presented only with two recovery points. Others patients have displayed more than 2 recovery points.

Patients of group II have showed superior increasing of recovery score with a total of 18 recovery points and an average of 3.6 than patients of first group that have presented 13 recovery points and an average of 2,6.

The increasing of recovery score by the second day of monitoring was recorded only in one patient of first group; the patients of group II did not register any change. On the third day of treatment were recorded equal increases of recovery score for both groups of dogs (4 patients in each group).

After 3 days of treatment with anti-inflammatory drugs, recovery score has recorded significant increases for patients of group II that have gained 14 recovery points (50%) than patients from first group that have gained 8 recovery points.

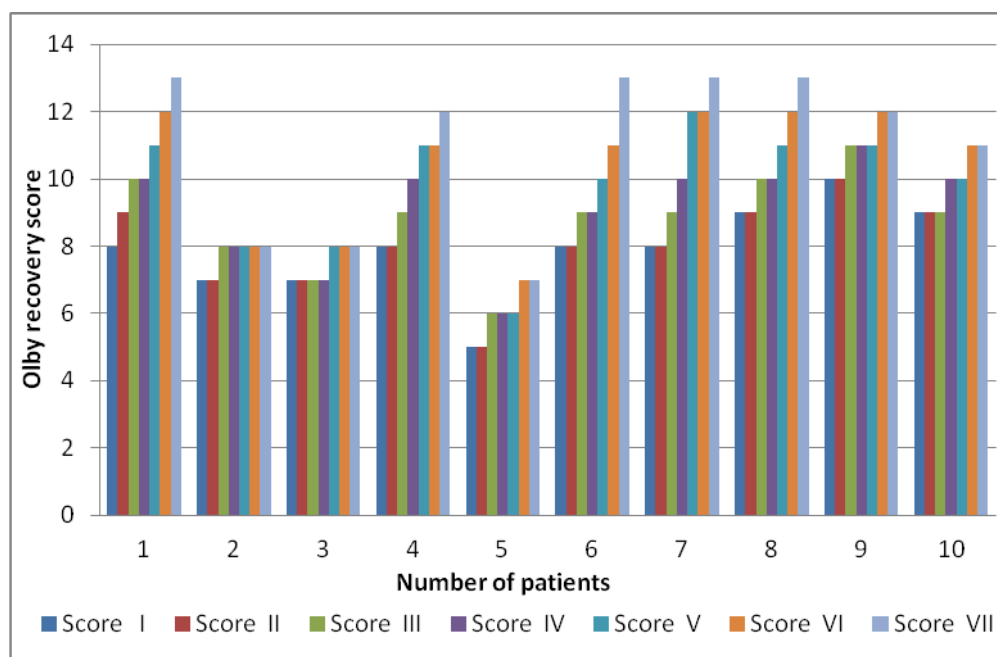


Fig. 1. The graphical representation of the Olby recovery score of patients with disc herniation during the 7 days of monitoring

In group I were recorded two patients that have presented less than 2 recovery points while in group II there was no patient less than 2 recovery points.

Analysis of patient outcomes from this study may highlight importance of monitoring motor function of patients with neurological deficits and during treatment with anti-inflammatory drugs by means of a recovery score.

Even if the monitoring period was reduced, the clinical outcomes were significant to highlight the motor recovery of patients in the 2 groups of dogs.

Though, on the third day of treatment both groups of patients have recorded equal results, after the 4th day of monitoring the recovery score was significantly improved to the patients that were treated with non-steroidal anti-inflammatory drug. The fact that on these patients were recorded increases of the recovery score by 50% more than the patients that were treated with steroidal drugs, it highlights the limited effect of the steroids on patients with disk herniation.

Practical, medication with dexamethasone is forbidden on dogs with disk herniation (1, 9), because steroid administration has not really been proven beneficial in the treatment of these disease and it can potentially be harmful according to studies (1, 3, 4). Instead, no steroidal anti-inflammatory drugs can be used for pain control instead of steroids and this therapy is to help prevent the potential side effects of steroid use and the potential detrimental effects of steroids on the healing spinal cord.

Although therapy for spinal cord injury, including acute disk herniation is still controversial, the results of this study highlight once again the importance of choosing treatment with anti-inflammatory drugs, even if the number of dogs was reduced.

### **Conclusions**

Analysis of Olby recovery score at patients with intervertebral disk disease showed clinical improvements starting with the third day of monitoring for both groups of patients.

The patients that were treated with non steroid anti-inflammatory drug have shown increases of 50% recovery score after completing treatment than patients that were treated with steroidal drug.

The clinical effects of steroids are limited on dogs with disc disease compared to the effects of non steroid anti-inflammatory drugs.

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