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## **STUDIES ON THE DIAGNOSTIC AND TREATMENT IN CANINE MAMMARY TUMORS**

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**Key words:** mammary tumors, diagnostic, treatment, histopathological exam

### **SUMMARY**

Cancer is one of the most spread diseases and that is why, for veterinary and human medicine, this malady represents a problem hard to approach due to its evolution, frequently incontrollable, and its therapy, which results in contradictory results.

Mammary gland tumors are frequent in female dogs and cats, and very sporadic in other domestic animals.

The aim of this study was to evaluate a diagnostic protocol and to apply a therapeutic scheme in bitches with mammary gland tumors in different stages of evolution. These protocols are justified considering that cancer is a gradual process, so a predictable one.

Multi-disciplinary therapy included neoadjuvant cytostatic chemotherapy, surgical exeresis, total or partial depending on the localization of primary tumor and the extension degree, adjuvant cytostatic chemotherapy, immunotherapy and hormonotherapy.

Canine mammary tumors have a gradual evolution, the diagnostic and treatment being individualized depending on stage and patient.

Cancer is one of the most spread diseases and that is why, for veterinary and human medicine, this malady represents a problem hard to approach due to its evolution, frequently incontrollable, and its therapy, which offers contradictory results.

This malady is actually a complex of syndromes having as a common characteristic the uncontrollable proliferation of malignant cells (Crînganu, 2009; Manolescu et al., 1993).

Considering that curative therapy, resulting in the destruction of the tumor, must be applied in early stages, it is highly important that the diagnosis to be established as soon as possible.

The purpose of this study was to highlight the importance of histopathologic exam for an accurate diagnosis in mammary cancer in bitch and to reveal the essential histopathologic aspects in different stages of mammary tumors in bitch, which would allow the establishment of an adequate therapy.

## 1. MATERIALS AND METHODS

Histopathological examination was applied to four different types of mammary tumors, depending on the stage of evolution ( $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ), surgically removed from bitches, in order to apply an appropriate therapeutic protocol.

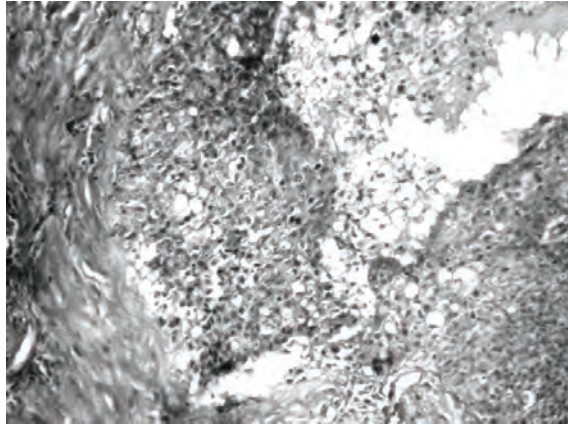
The diagnosis was established upon clinical and paraclinical exams (histopathological, hematological and biochemical exams). Corroborating the data obtained it was established a certain diagnosis regarding the presence and dimensions of the primary tumor, tissular or lymph node regional and eventually at distance invasion, as well as visceral or bone metastases. Thus, it was determined the tumoral type and it was evaluated its extension degree.

Anatomical extension of malignant tumoral process is encoded by TNM system, this representing a specific oncological language worldwide. Therapeutical strategies were based on the association of as much techniques, such as cytostatic chemotherapy before and after surgical intervention, oncological surgical protocols, immunotherapy, hormonotherapy, adjuvant therapies (Crînganu and Crivineanu, 2009). The major purpose of therapy in these diseases is represented by the extending of life duration and the improvement of its quality, because complete healing is attained in relatively few cases.

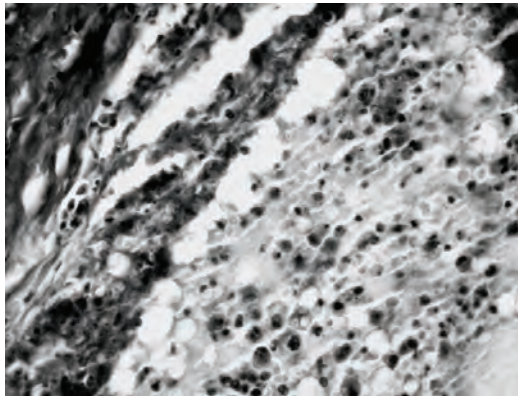
## 2. RESULTS AND DISCUSSION

$T_1$  represents the infiltrative stage of the tumor, clinically visible, but the number of tumor cells and their spread distance is low. In order to form metastases, the tumor crosses two stages, simple epithelial dysplasia and aggravated epithelial dysplasia. Metastases are formed via lymph.

In figures 1 and 2, it is presented the histopathological aspect of a mammary tumor in bitch in the stage of evolution  $T_1N_xM_0$ .  $N_x$  stage denotes unknown lymphadenopathy, while  $M_0$  certifies the lack of metastases in this stage. Histopathologically it could be observed lobular simple carcinoma with cellular necrosis, cytoplasmatic vacuolization and nuclear pyknosis and also the dysplasia and anaplasia of tumoral cells. At high objective (40) there could be noticed large areas of necrosis in tumoral mass, with remaining groups of anaplastic cells which maintained their integrity.



**Fig.1.** Mammary tumor in bitch – stage  $T_1N_xM_0$ . Histologic aspect: lobular simple carcinoma with cellular necrosis, cytoplasmic vacuolization and nuclear pyknosis; it can be observed the dysplasia and anaplasia of tumoral cells (ob. 20; tricromic Masson coloration)

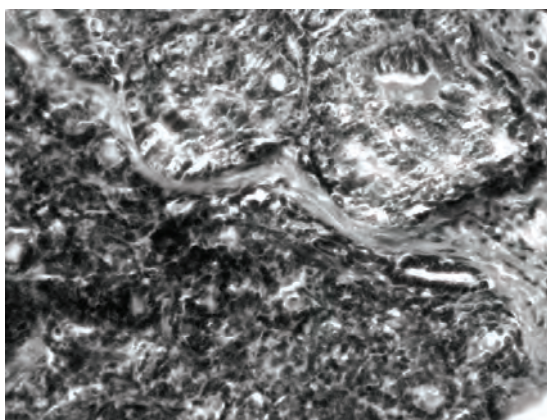


**Fig. 2.** Mammary tumor in bitch – stage  $T_1N_xM_0$ . Histologic aspect: large areas of necrosis in tumoral mass, with remaining groups of anaplastic cells which maintained their integrity (ob. 40; tricromic Masson coloration)

The tumor in  $T_2$  stage realizes vascular connection (neoangiogenesis), it elaborates angiogenetic factors that determines mitosis of endothelial cells in that region and then vascular burgeons appear.  $T_2$  tumor is a red vascular tumor and metastases are formed both via blood and lymph.

In figure 3, it is presented the histopathological aspect of a mammary tumor in bitch in the stage of evolution  $T_2N_1M_x$ .  $N_1$  stage is generally defined as palpable but mobile regional lymphadenopathy, while  $M_x$  denotes unknown metastases. Histopathological exam revealed adenosis areas (benign hyperplasia) with carcinomatous transformation.





**Fig. 3.** Mammary tumor in bitch – stage  $T_2N_1M_x$ . Histologic aspect: adenosis areas (benign hyperplasia) with carcinomatous transformation (ob. 40, trichromic Masson coloration)

In stages  $T_1$  and  $T_2$ , the presumptive diagnosis was established based on anamnesis and clinical signs, while certitude diagnosis was established by histopathologic exam of the tumor, which was surgically removed. Radiologically, in this stage there were not identified pulmonary metastases, aspect that confirmed  $M_0$  or  $M_x$  stage.

Hematological exam revealed changes in blood parameters, as leukopenia, lymphocytopenia, thrombocytopenia, aspects that confirmed the existence of paraneoplastic syndromes in incipient phases.

In mammary tumors in bitches caught in stages  $T_1$  and  $T_2$ , the treatment consisted in:

- neoadjuvant chemotherapy - Holoxan (iphosphamide) associated with antioxidants, diuretics, liver protectors, cardiac tonics, while locally it was used Theranekron, an antiproliferative product obtained from tarantula venom;

- surgical intervention – mammary gland was partially removed; it was also removed inguinal lymph node, even if it was not reactive (Tobias, 2010);

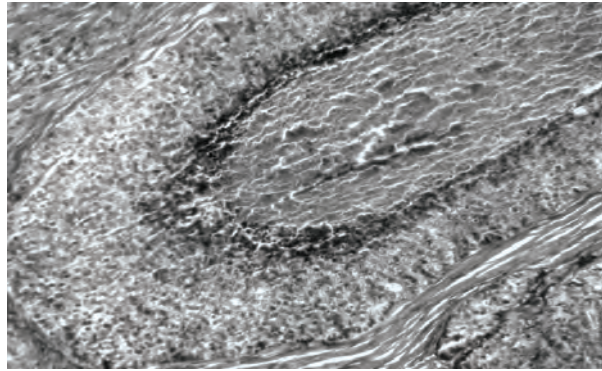
- adjuvant chemotherapy - Holoxan and Gemcitabine in order to prevent pulmonary metastases; also, there were administered liver and cardiac protectors and antioxidants;

- hormonotherapy – there were recommended cortocosteroids (dexamethasone) and antiestrogen substances (Tamoxifen).

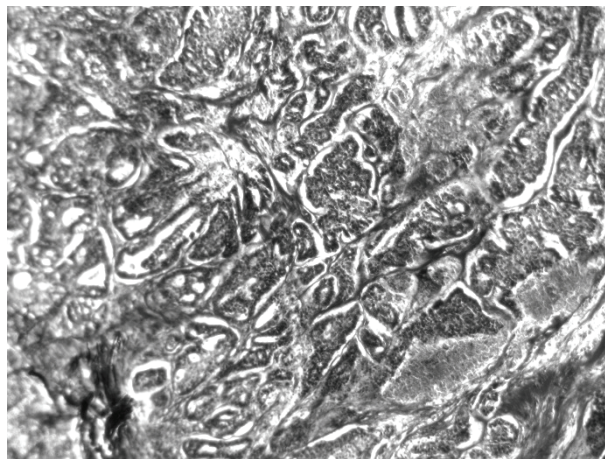
Applying this therapeutic protocol, the bitches had a good physiological state, without paraneoplastic syndromes.

The tumor in **T<sub>3</sub>** stage is bigger than 5 cm, with a regional extend of primary tumor. Metastases are formed via lymph and blood.

In figures 4 and 5, it is presented the histopathological aspect of a mammary tumor in bitch in the stage of evolution **T<sub>3</sub>N<sub>2</sub>M<sub>1</sub>**. Histopathologically it could be noticed the tendency to become cribriform carcinoma – there were observed masses of epithelial proliferation (cordons) that filled the dilated channels; between neoplastic elements, there were small secondary lumens that conferred this aspect. At a bigger objective (40), it could be noticed hyperplasia of epithelial cells, with intratubular and intraacinar papillary proliferation and conjunctive perilobular stroma well represented.



**Fig. 4.** Mammary tumor in bitch – stage **T<sub>3</sub>N<sub>2</sub>M<sub>1</sub>**. Histologic aspect: tendency to become cribriform carcinoma – there are observed masses of epithelial proliferation (cordons) that fill the dilated channels; between neoplastic elements there are small secondary lumens that confer this aspect (Ob. 20, tricromic Masson coloration)

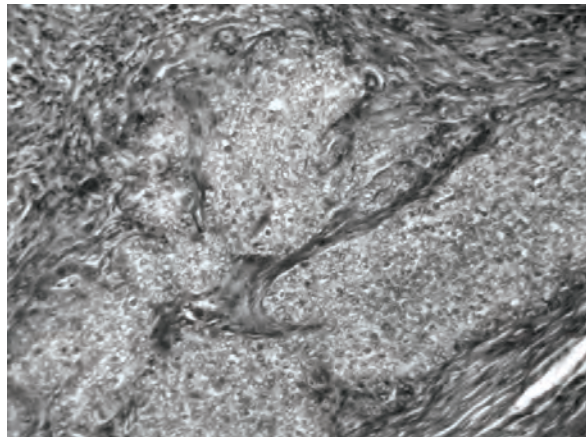


**Fig. 5.** Mammary tumor in bitch – stage **T<sub>3</sub>N<sub>2</sub>M<sub>1</sub>**. Histologic aspect: hyperplasia of epithelial cells, intratubular and intraacinar papillary proliferation; conjunctive perilobular stroma well represented (Ob. 40, tricromic Masson coloration)

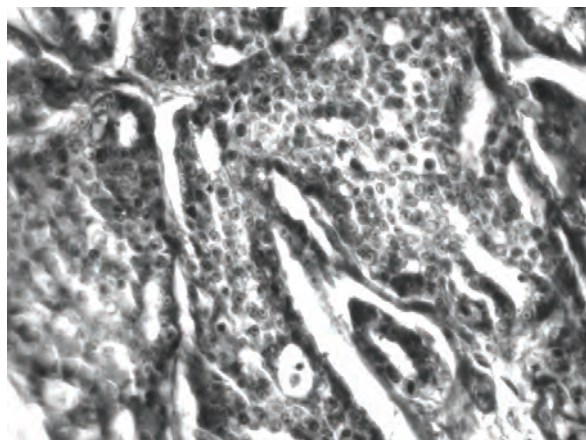
The tumor in **T<sub>4</sub>** stage is characterized by lymph nodes and visceral metastasis. In this stage, tumor's dimensions are not important any more, the tumor reaching under the skin and provoking its ulceration.

In figures 6 and 7, it is presented the histopathological aspect of a mammary tumor in bitch in the stage of evolution **T<sub>4</sub>N<sub>2</sub>M<sub>1</sub>**. **N<sub>1</sub>**, **N<sub>2</sub>** represent increasing stages of regional lymph nodes invading, while **M<sub>1</sub>**, **M<sub>2</sub>** represents different degrees of metastasis. Histopathological exams revealed areas of papillary carcinoma with tendency to spread/solidify, with hyalinization of the tumoral stroma as well as the infiltrative nature and tendency of tumoral lobes to join.

Presumptive diagnosis in stages **T<sub>3</sub>** and **T<sub>4</sub>** was established based on anamnesis and clinical signs, some of them being obvious: cachexia, apathy, respiratory dyspnea. Superficial and in some cases profound lymph nodes were rough and increased in volume. Thoracic radiography revealed pulmonary metastases of different sizes and with different locations. Blood tests revealed also hematological changes, as leukopenia or leukocytosis, lymphocytopenia, thrombocytosis.



**Fig. 6.** Mammary tumor in bitch – stage **T<sub>4</sub>N<sub>2</sub>M<sub>1</sub>**. Histologic aspect: area of papillary carcinoma with tendency to spread/solidify, with hyalinization of tumoral stroma (Ob. 20, tricromic Masson coloration)



**Fig. 7.** Mammary tumor in bitch – stage T<sub>4</sub>N<sub>2</sub>M<sub>1</sub>. Histologic aspect: infiltrative nature and tendency of tumoral lobes to join (Ob. 40, trichromic Masson coloration)

In mammary tumors in bitches caught in stages T<sub>3</sub> and T<sub>4</sub>, the treatment consisted in:

- neoadjuvant chemotherapy with cytostatics of the second line - Pharmorubicin, Holoxan, Carboplatin, Gemcitabine associated with hormonotherapy (Tamoxifen, prednisone, dexamethasone), interferon and therapy of paraneoplastic syndromes;

- surgical exeresis, but only if the animal was clinically and hematologically stable;

- adjuvant chemotherapy mainly with Carboplatin and Gemcitabine, associated with hormonotherapy, immunotherapy and paraneoplastic syndromes therapy.

Paraneoplastic syndromes were represented by cachexia, dehydration, anorexia, vomiting, liver and kidneys disorders, hematological changes (anemia and leukocyte abnormalities) and they were properly treated (metabolism rebalancing, rehydration, antivomiting substances, analgesics, etc.).

### 3. CONCLUSIONS

3.1. Certitude diagnosis in mammary tumors in bitch caught in stages T<sub>1</sub> and T<sub>2</sub> was established by histopathologic exam of the tumor, while the therapy consisted in chemotherapeutic drugs of the first line associated with surgical exeresis, hormonotherapy and immunotherapy.

3.2. Certitude diagnosis in mammary tumors in bitch caught in stages T<sub>3</sub> and T<sub>4</sub> was established by histopathologic exam of the tumor, while the

therapy consisted in chemotherapeutic drugs of the second line followed by large surgical exeresis, adjuvant polychemotherapy, hormonotherapy and immunotherapy.

3.3. Diagnostic protocol of mammary tumors comprises clinical exams, histopathologic exams, radiological exams, ecographic exams, biochemical and hematological exams.

3.4. Mammary tumors in bitch have an evolution in stages, the diagnosis and therapy depending on each stage.

#### **ACKNOWLEDGEMENTS**

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## **CHEMOTHERAPY ASSOCIATED WITH POLYPHENOLIC EXTRACTS: THE EVALUATION OF THERAPEUTIC RESPONSE BY ANALYZING SOME BLOOD PROTEINS**

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**Key words:** polyphenols, chemotherapy, cancer, proteins

### **SUMMARY**

Cancer is one of the most terrible diseases in humans and animals, a complex pathological process, which affects the whole organism and has various clinical aspects. Whereas cancerous disease is systemic, it is necessary to establish an early precise diagnostic in order to allow the treatment in initial phases.

Many studies demonstrated that chemotherapeutic substances induce, due to their toxicity, the decrease of organism's capacity to annihilate free radicals and that is why the treatment includes also antioxidant agents.

The experiments conducted in this study had as aim to evaluate the effect of some polyphenolic extracts on dogs diagnosed with two forms of cancer and treated with cytostatic chemotherapies, by analyzing some blood proteins.

The obtained results demonstrated that the association of polyphenolic extracts with anticancerous therapy led to an increase of therapeutic efficacy accompanied by the decrease of chemotherapeutic substances' toxicity.

Cancer is one of the most terrible diseases in both humans and animals, a complex pathological phenomenon that affects the whole body, having different clinical manifestations from individual to individual, even within the same histological tumor type.

Since cancer is a systemic disease, affecting the entire body, it is necessary to make an early precise diagnosis, which can allow starting the therapy in the initial stages. Therapeutic strategies require an association of many techniques, such as polychemotherapy before and after surgery, surgery protocols, immunotherapy and hormonotherapy, adjuvant therapies, including vegetal polyphenols (Conklin, 2000; Lambert et al., 2005).

Adjuvant and neoadjuvant cytostatic chemotherapy is nowadays the most used treatment for patients with tumors, although the complete destruction of the tumors is recorded in a small number of subjects and the adverse reactions of chemotherapy are very intense (Crînganu, 2009; Crînganu and Crivineanu, 2009). In addition, in patients with cancer it was

noticed that free radicals resulted from oxidative processes can induce oxidative stress, which generates secondary reactions such as modifications of cellular membranes' permeability (Okada et al., 2001).

The purpose of this study was to evaluate the influence of a polyphenolic vegetal extract upon the efficiency of cytostatic chemotherapy in two types of cancer in dog by the analysis of some specific proteins: M protein, C-reactive protein and Bence-Jones protein.

### 1. MATERIALS AND METHODS

The performed experiments had the purpose to evaluate the effect of some polyphenolic extracts on dogs diagnosed with various types of cancer and treated with chemotherapies adequate for each type of tumor.

For the investigations there were selected 12 dogs, from which 10 female dogs with mammary gland tumors (stages I and II) and 2 dogs with B cells lymphoma (Waldenstrom multiple myeloma).

The animals received standard treatments with specific chemotherapeutic drugs associated with the oral administration of a polyphenolic mix obtained from sea buckthorn (*Hippophae rhamnoides*), bilberry (*Vaccinium myrtillus*), Saint John's wort (*Hypericum perforatum*) and hawthorn (*Crataegus monogyna*). Dogs treated only with chemotherapies, without polyphenols, represented control lot (Tables 1, 2).

Table 1

Experimental protocol used in case of dogs with mammary tumors

Week	Treatment lot 1		Treatment lot 2	
	Drug	Polyphenolic mix	Drug	Polyphenolic mix
1	Cyclophosphamide - 50 mg/m <sup>2</sup> /day, 4 consecutive days	5 mg/kg daily	Cyclophosphamide - 50 mg/m <sup>2</sup> /day, 4 consecutive days	-
2	Pause	5 mg/kg daily	Pause	-
<b>SURGICAL INTERVENTION (MASTECTOMY)</b>				
3	Pause	5 mg/kg daily	Pause	-
4	Pause	5 mg/kg daily	Pause	-
5	Holoxan (iphosphamide) - 200 mg/m <sup>2</sup> /day, 2 consecutive days	5 mg/kg daily	Holoxan (iphosphamide) - 200 mg/m <sup>2</sup> /day, 2 consecutive days	-
6	Gemcitabine - 200 mg/m <sup>2</sup> one administration	5 mg/kg daily	Gemcitabine - 200 mg/m <sup>2</sup> one administration	-
7	Pause	5 mg/kg daily	Pause	-

8	Pause	5 mg/kg daily	Pause	-
9	Pause	5 mg/kg daily	Pause	-
10	Holoxan (iphosphamide) - 200 mg/m <sup>2</sup> /day, 2 consecutive days	5 mg/kg daily	Holoxan (iphosphamide) - 200 mg/m <sup>2</sup> /day, 2 consecutive days	-
11	Gemcitabine - 200 mg/m <sup>2</sup> one administration	5 mg/kg daily	Gemcitabine - 200 mg/m <sup>2</sup> one administration	-

Table 2

Experimental protocol used in case of dogs with B cells lymphoma (Waldenstrom multiple myeloma)

Week	Treatment case 1		Treatment case 2	
	Drug	Polyphe-nolic mix	Drug	Polyphe-nolic mix
1	Medrol (methylprednisolone) – 1 mg/kg daily Cyclophosphamide - 50 mg/m <sup>2</sup> /day, 4 days Vincristine – 0.7 mg/m <sup>2</sup> one administration	10 mg/kg daily	Medrol (methylprednisolone) – 1 mg/kg daily Cyclophosphamide - 50 mg/m <sup>2</sup> /day, 4 days Vincristine – 0.7 mg/m <sup>2</sup> one administration	-
2	Medrol (methylprednisolone) – 0.7 mg/kg daily Vincristine – 0.7 mg/m <sup>2</sup> one administration	10 mg/kg daily	Medrol (methylprednisolone) – 0.7 mg/kg daily Vincristine – 0.7 mg/m <sup>2</sup> one administration	-
3	Medrol (methylprednisolone) – 0.5 mg/kg daily Vincristine – 0.7 mg/m <sup>2</sup> one administration	10 mg/kg daily	Medrol (methylprednisolone) – 0.5 mg/kg daily Vincristine – 0.7 mg/m <sup>2</sup> one administration	-
4	Medrol (methylprednisolone) – 0.5 mg/kg daily	10 mg/kg daily	Medrol (methylprednisolone) – 0.5 mg/kg daily	-
5	Pause	10 mg/kg daily	Pause	-
6	Pause	10 mg/kg daily	Pause	-
7	Vincristine – 0.7 mg/m <sup>2</sup> one administration Cytosar (cytosine - arabinoside) – 100 mg/m <sup>2</sup> one administration	10 mg/kg daily	Vincristine – 0.7 mg/m <sup>2</sup> one administration Cytosar (cytosine - arabinoside) – 100 mg/m <sup>2</sup> one administration	-
8	Vincristine – 0.7 mg/m <sup>2</sup> one administration	10 mg/kg daily	Vincristine – 0.7 mg/m <sup>2</sup> one administration	-



9	Vincristine – 0.7 mg/m <sup>2</sup> one administration Cytosar (cytosine - arabinoside) – 100 mg/m <sup>2</sup> one administration	10 mg/kg daily	Vincristine – 0.7 mg/m <sup>2</sup> one administration Cytosar (cytosine - arabinoside) – 100 mg/m <sup>2</sup> one administration	-
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*Obtaining polyphenolic extracts.* Dried plants were minced and extracted with ethanol 60% for 3 hours. The obtained extracts were filtered, centrifuged and concentrated on a rotary evaporator. Quantitative evaluation of polyphenols' content was performed by the method with Folin Ciocalteu reagent.

*The evaluation of M protein.* In order to evaluate M protein, it was made the electrophoresis in agarose gel of blood serums sampled from the patients with multiple myeloma.

*The evaluation of C-reactive protein.* C-reactive protein was evaluated using a semi-quantitative agglutination kit.

*The evaluation of Bence-Jones protein.* Bence Jones protein was evaluated from urine using a qualitative method.

## 2. RESULTS AND DISCUSSION

### *The evaluation of M protein*

In specialty literature, M protein is described as a protein that occurs only in B cells lymphoma-type tumors (multiple myeloma, Waldenstrom macroglobulinemia), so the detection of M protein was performed only in the 2 patients with this disease (Crînganu, 2009).

Multiple myeloma is a malignant disease characterized by the proliferation of myelomatous cells in bone marrow, with the local destruction of the bone; these cells synthesize a monoclonal protein (M protein) that can be detected in blood and urine. In multiple myeloma appear localized pseudotumors (plasmacytoma) in bone marrow and in soft tissues.

The results obtained at electrophoretic evaluation of plasmatic proteins in dogs with multiple myeloma treated with cytostatic drugs and chemotherapy associated with polyphenolic mix are presented in Table 3.

Table 3

Electrophoretic profile of plasmatic proteins in dogs with multiple myeloma treated with cytostatics and cytostatics associated with polyphenolic mix

	Dog treated with cytostatics and polyphenolic mix					Dog treated only with cytostatics				
	Albu- mins %	$\alpha$ 1- globulins %	$\alpha$ 2- globulins %	$\beta$ -glo- bulins %	$\gamma$ - globu- lins %	Albu- mins %	$\alpha$ 1- glo- bulins %	$\alpha$ 2- glo- bulins %	$\beta$ - globulins %	$\gamma$ - globulins %
Control (healthy animal)	68.5	4.1	8.8	9.1	9.5	68.5	4.1	8.8	9.1	9.5
day 0 (before treatment)	40.4	6.2	12.1	9.2	32.1	42.3	5.8	11.9	9.5	30.5
week 2	37.3	8.2	12.3	9.5	33.7	35.1	9.9	11.8	9.7	33.5
week 4	35.2	8.7	11.9	9.3	34.9	29.7	9.9	10.6	10.1	39.7
week 8	37.8	9.5	9.6	9.0	34.1	30.2	9.2	10.0	9.9	40.7
week 9	40.0	8.9	9.9	8.1	33.1	29.4	9.5	10.2	9.4	41.5

From table 3 it can be noticed that the association of cytostatic chemotherapy with the polyphenolic mix led to a slight increase of plasmatic albumins accompanied by a decrease of gamma-globulins, compared with the dog treated only with cytostatic therapeutics.

Gamma-globulins concentration's decrease can be attributed to the decrease of M protein concentration in the blood sampled from the patient diagnosed with multiple myeloma treated with therapeutics and polyphenolic mix.

#### *The evaluation of C-reactive protein*

C-reactive protein is an acute phase protein biosynthesised in liver and adipocytes. This protein is also used as a tumor marker and can be detected in solid tumors, in circulating tumor cells, in peripheral blood, in bone marrow, etc. The results obtained in evaluating C-reactive protein in the blood of patients treated with therapeutics and therapeutics associated with polyphenolic mix are shown in Table 4. From the data presented it can be noticed that the values of C-reactive protein in the blood of the animals treated with therapeutics and polyphenolic mix are slightly lower than in patients treated only with therapeutics.

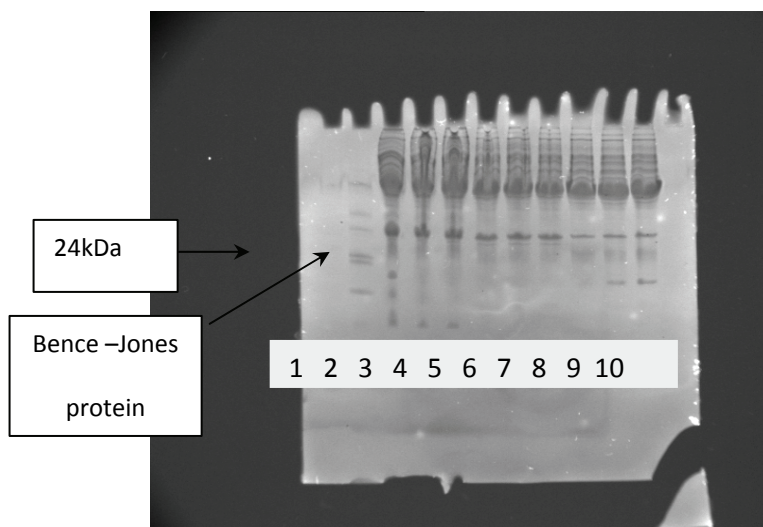
Table 4

Evaluation of C-reactive protein (blood) and Bence-Jones protein (urine) in dogs with mammary tumors and multiple myeloma

	C-reactive protein (mg/dl)	Bence-Jones protein	C-reactive protein (mg/dl)	Bence-Jones protein
	<b>Dog with mammary tumors treated with cytostatics and polyphenolic mix</b>		<b>Dog with mammary tumors treated with cytostatics</b>	
Control (healthy animal)	0.23	-	0.35	-
day 0 (before treatment)	4.28	-	4.34	-
week 2	4.96	-	5.39	-
week 6	6.45	-	6.70	-
week 7	7.58	-	7.98	-
week 11	7.38	-	8.34	-
	<b>Dog with multiple myeloma treated with cytostatics and polyphenolic mix</b>		<b>Dog with multiple myeloma treated with cytostatics</b>	
Control (healthy animal)	0.43	-	0.28	-
day 0 (before treatment)	12.2	+	11.6	+
week 2	12.9	+	13.2	+
week 4	17.4	+	17.9	+
week 8	18.3	+	19.1	+
week 9	17.1	+	20.2	+

#### *The evaluation of Bence-Jones protein*

Bence Jones protein, represented by light chains of immunoglobulins, is present in the blood of patients with multiple myeloma, but also in their urine due to its small dimensions that permit renal filtration; it has a molecular weight between 22-24 kDa. Bence Jones protein was detected in the urine of dogs with multiple myeloma in the whole period of treatment, but it was not detected in the urine samples from patients with mammary tumors (Table 4).



1. molecular mass marker
2. dog with multiple myeloma before treatment (day 0)
3. dog with multiple myeloma treated with cytostatics
4. dog with multiple myeloma treated with cytostatics and polyphenolic mix
5. dog with mammary tumors before treatment (day 0)
6. dog with mammary tumors treated with cytostatics
7. dog with mammary tumors treated with cytostatics and polyphenolic mix
8. dog with mammary tumors treated with cytostatics and polyphenolic mix
9. healthy dog
10. healthy dog

**Fig. 1.** Bence Jones protein. Electrophoresis in polyacrylamide gel in denaturant conditions (PAGE-SDS) for blood serum sampled from dogs with multiple myeloma, dogs with mammary tumors and healthy dogs

Electrophoresis in polyacrylamide gel performed in denatured conditions revealed the presence in high quantities of this protein in the blood of dogs with multiple myeloma before treatment and in smaller amounts in the blood of dogs with multiple myeloma receiving chemotherapy and chemotherapy associated with polyphenolic mix. The smallest spot for Bence-Jones protein was observed in serum sampled from the dog with multiple myeloma treated with chemotherapics and polyphenolic mix (Fig. 1). These results demonstrate the efficacy of cytostatic chemotherapy associated with polyphenolic mix.

### 3.CONCLUSIONS

3.5. The association of polyphenolic mix with cytostatic chemotherapy led to a slight increase of plasmatic albumins and a decrease of gamma-globulins; this aspect can be attributed to the decrease of M protein concentration in case of the patient diagnosed with multiple myeloma.

3.6. In animals diagnosed with multiple myeloma, the association of cytostatic chemotherapy with polyphenols contributes to treatment's efficiency. This observation is sustained by a more pronounced decrease of Bence-Jones protein in the blood sampled from the animal treated with cytostatics associated with polyphenolic mix compared with the dog treated only with cytostatics.

3.7. The association of cytostatics with polyphenolic mix improves proteins' electrophoretic profile both in animals diagnosed with mammary tumors and multiple myeloma.

### ACKNOWLEDGEMENTS

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## **STUDY REGARDING THE RELEVANCE OF THE ULTRASOUND EXAMINATION IN UTERINE DISEASES ASSOCIATED WITH POLYURIA-POLYDIPSIA SYNDROME IN BITCH**

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**Key words:** ultrasonography, uterine, carnivores, polyuria-polydipsia

### **SUMMARY**

The clinical and therapeutic approach polyuria syndrome/polydipsia, of paramount importance is the establishment of primary etiology and pathogenesis complex responsible for its induction. All data included in the diagnostic certainty conclude that the diagnosis of the syndrome of polyuria/polydipsia is made by corroborating elements of clinical and laboratory data (the latter defining the final diagnosis).

From the ultrasound investigated cases with pyometra (n = 21), 85.71% had polyuria-polydipsia syndrome, and from the total number 71.43% were with opened and 28.57% with closed cervix.

Ultrasound diagnosis in cases of pyometra (polyuria-polydipsia syndrome associated) is easily achieved with high degree of accuracy (100% positive) being considered a non-aggressive technique for the patient and the examiner too.

The first intention therapeutically measure is represented by ovary-hysterectomy (especially if the cervix closed pyometra). The young females and the high reproductive value can try conservative therapy, resorting to systemic antibiotic therapy supported with PGF2 $\alpha$  type uterine stimulants, followed by ultrasound examination – for confirming the curative efficiency.

Atypical or unusual urination are concerning and represent one of the most common causes of patient presentation to the veterinarian, as determined by the removal of large quantities of urine, frequent urination, loss of urine apparent, inappropriate urination, or less, due excessive consumption of water.

Formation and excretion polyuria means large amounts of urine (> 50 ml/kg/24h), usually with a low specific gravity. Polydipsia can be defined as increased thirst, resulting in increased volumes of fluid consumption (> 100 ml/kg/24h).

Depending on water content of its diet, a healthy dog drinks an average of 50-60 ml/kg/24h. Normal urinary flow rate varies between 20-40 ml/kg/24h. Exceeding these values lead to polyuria - polydipsia syndrome. Fluid consumption and urine production are controlled by complex

interactions between: plasma osmolarity, volume of fluid in the vascular bed, thirst center, kidney, and pituitary - hypothalamus. The two main mechanisms responsible for maintaining fluid balance are: thirst mechanism and renal concentrating mechanisms.

Any interference with patho-physiological order affecting one of the components involved can lead to polyuria and polydipsia default. In most cases compensate for polyuria polydipsia, polyuria ie is the main issue and results in secondary or compensatory polydipsia. This is true for all diseases that are associated with primary polydipsia or psychogenic polydipsia except where polyuria is secondary and occurs in response to the amount of water in excess.

Antidiuretic hormone (ADH/vasopressin) play crucial role in: control of tubular re-absorption of water, urine production, urine concentration and fluid balance ensuring the body. ADH acts on epithelial cells of distal convoluted tubules and collecting tubules - which increase the hydro-osmotic permeability of these cells.

Primary urine (diluted) of the tube passes into the interstitial (concentrated) in renal medulla. In the presence of ADH's fluid volume decreases the nephron, thereby conserving body fluids. In the absence of ADH's (central diabetes insipidus) or if its action strength tubular structures (nephrogenic diabetes insipidus) - no longer made and water diffusion in the interstitial fluid (renal medulla) - hypotonic glomerular ultrafiltrate passes unchanged in tubules distal tubules and collecting.

## **1. MATERIAL AND METHODS**

The study was performed on 21 bitches, of different breeds, age or body conditions, diagnosed with polyuria-polydipsia, secondary to pyometra.

Were clinical and ultrasonographic examined 21 cases with pyometra (n = 21). From these, in 18 bitches was registered as an important and constant clinical sign, the polyuria-polydipsia syndrome (85.71%).

Clinical examination revealed that in 15 cases (71.43%) were with diagnosed with opened cervix, the other 6 females were diagnosed with closed cervix (in 28.57%).

The ultrasound examinations were performed with usual portable ultrasound (Aquila and Mylab 30 - Esaote Pie Medical machines) with convex probes with different frequencies between 5 and 8 MHz.

In such cases for evaluating the uterus, as a cavitary organ, for ensuring a maximum accuracy of the ultrasound investigations we've followed and

respected strictly the steps for obtaining the most relevant images, without or with minimum artifacts images, in order to enhance the relevance of the obtained ultrasound images.

In most cases we've tried to perform the examinations with filled urinary bladder (actioning as a veritable acoustic window for the uterus).

The conduct of the two different symptoms, so there is polyuria with polydipsia may offset or primary polydipsia (psychogenic). Polyuria with compensatory polydipsia- caused by osmotic diuresis: diabetes mellitus, primary renal glucosuria/Fanconi syndrome, renal failure, polyuria; or interference with the release of ADH and/or response to ADH: chronic renal failure, nephritic syndrome, pyelonephritis, pyometra, Cushing syndrome, chronic liver failure, hypercalcemia, central diabetes insipidus, nephrogenic diabetes insipidus, hyperthyroidism, iatrogenic causes.

Pyometra or cystic endometrial hyperplasia is considered the most important disease in dogs and cats affections. It is considered that the basis of this condition be a progressive endocrine disturbance acting against a particular susceptibility of the uterus to progesterone. Elevated estrogen for short periods followed by long periods of increased levels of progesterone cause cystic endometrial hyperplasia, followed by uterine gland hypersecretion and accumulation of uterine secretions (Carmel and Peterson, 2004; Codreanu and Diaconescu, 2003).

Obvious symptoms and occurs during the luteal phase, usually 4-10 weeks after estrus or after exogenous progestative substances.

The clinical picture is varied as evolutionary (with closed or open cervix). The general registered symptoms were: depression, dehydration, anorexia, polydipsia and polyuria. Sometimes vomiting and rarely diarrhea was registered. Rectal temperature was mostly normal or close to/or hyperthermia.

The local clinical signs of the pyometra were dominated by abdominal distension (enlargement of the abdomen), sometimes pendulous appearance (these cases the abdomen is painful and prevent animal movement). The abdomen may feel a pasty mass occupying much of it.

Shape of the uterus in pyometra is determined by the content of uterine horns. Usually, uterine horns are uniformly dilated. Can be registered situations where uterine horns are unequal or similar to nodular aspect (similar to gestation of 30-35 days), situations avoided by examining in different incidences.

When is missing the vaginal discharge the uterine collection in about a third of cases of pyometra is sterile. The other cases are secondary



infections (with *E. coli*, staphylococci, streptococci, etc.)

Endotoxins from *Escherichia coli* (most commonly associated with pyometra) prevent ADH's recognition in the kidney structure (renal tubules), practically a form of reversible nephrogenic diabetes insipidus (Codreanu *et al.*, 2011).

## 2. RESULTS AND DISCUSSION

The most important objective of our study it was to achieve and to correlate the clinical expression – dominated by polyuria/polydipsia syndrome in cases of pyometra in bitches, in order to confirm the ultrasound diagnosis.

The uterus horns and body can be very difficult to identify and to appreciate in normal females (Codreanu and Diaconescu, 2003).

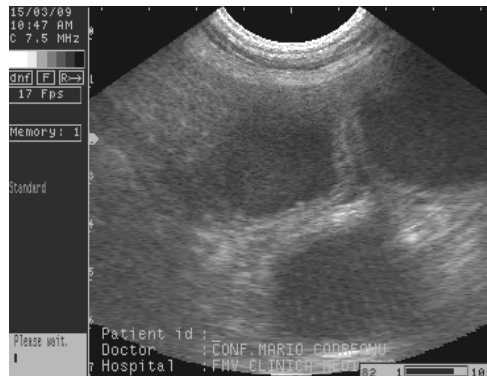
In terms of ultrasound, pyometra can provide a variety of issues rather than depending on the form of development, age, etc. (Codreanu and Diaconescu, 2003; Han *et al.*, 1994; Penninck, 1995).

In case of closed cervix pyometra we have registered the most characteristic aspects: area round/ovoid shaped or oblong, large (3.5-10 cm) containing anechoic (with artifactual distal enhancement) and thin-wall - without parietal thickening (Figure 1 and Figure 2).



**Fig. 1.** The uterus is distended by anechoic content (with distal enhancement), without severe alteration of the uterine wall.

In some cases, because of folding uterine horns, the cavity layout is unique or poly-compartment, with thinned uterine wall (Bîrțoiu and Seiciu, 2006, Crivineanu and Codreanu, 2008).



**Fig. 2.** The uterine horns became ultrasonographic visible, as rounded/ovoid shaped structures, because of the fluid accumulation (with corpuscular elements). The uterine wall is not visible affected.

Uterine contents may sometimes particulate matter, which in light of the abdominal wall movements baling are triggered and generates characteristic appearance of "snow storm".

Sometimes within the uterine cavity, relaxed purulent content, you can see areas of varying sizes echoic, adherent to the uterine wall (polyps or tumors of the uterine wall), or type hypo/anechoic (glandular-cystic endometrial degeneration), quite common in animals (Fig. 3 and Figure 4).

The forms of development with open cervix, the image obtained is totally different. The uterus contains significantly lower quantities of pus, therefore will be lower uterine distension. Instead a parietal hypertrophy appears obvious uterine wall appeared thickened and hyperechoic.

Often, the appearance of the uterus in pyometra the cervix is opening nodular, the dilation is of round/ovoid shape aspect, which can be confused with early pregnancy (Codreanu and Diaconescu, 2003).

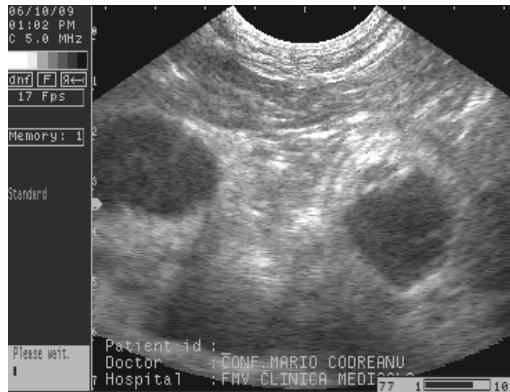
Ovario-hysterectomy is the treatment of choice (Bîrțoiu and Seiciu, 2006, Crivineanu and Codreanu, 2008). If it is aimed at saving the female reproductive capacity should be considered medical management. In this respect, it should start the fluid therapy and antibiotics - using broad-spectrum substances (intravenously).

Correction is done as soon as possible fluid imbalances, electrolyte or acid-base, before the ovario-hysterectomy. Bacterial infection is responsible for the bad condition of the dam and will not settle until it is removed uterine exudates. Typically, oral antibiotics should be continued for 7-10 days after surgery.

Conservative therapy, prostaglandin (PGF $2\alpha$ ) can be used to stimulate uterine contractions and uterine mucus default (Crivineanu and Codreanu, 2008).

Particular caution is advised if the cervix pyometra closed (because of the risk of uterine rupture).

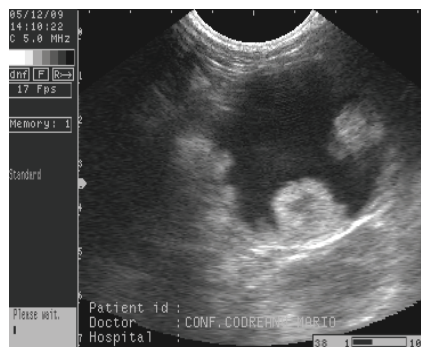
Natural prostaglandins are recommended (0.25 mg / kg, 5 days), synthetic analogues (cloprostenol, fluprostenol) are more intense as the action, but side effects are incompletely studied until now (in carnivores).



**Fig. 3.** The uterine horns contents particular elements (pus), with affection on different levels of the mucosa, but without loosing the parietal architecture/or reduced endometrial involvement.

Animals should be ultrasound reevaluated at 2 weeks after medical therapy for identifying and appreciating the presence or absence of the uterine content (Codreanu and Diaconescu, 2003).

If a leak is still presented bloody or mucous or purulent or if the uterus is enlarged, PGF $2\alpha$  therapy may be repeated using the same protocol, the prognosis becomes reserved.



**Fig. 4.** Chronic case of open cervix pyometra, with endometrial mucosa reaction (hypertrophic changes) – medium/high echogenicity proliferations and hypo/anechoic content (with specific distal enhancement).

### 3. CONCLUSIONS

Synthetic analysis of the results of investigations, allows detachment following conclusions:

3.1. Polyuria and polydipsia is a symptomatic association, rarely manifesting unilaterally, which met and demonstrated with the other syndrome conditions dominated or accompanied by polyuria/polydipsia (diabetes insipidus, diabetes mellitus, Cushing's syndrome, renal failure).

3.2. The clinical and therapeutic approach of the polyuria syndrome/polydipsia, of major importance is the establishment of primary etiopathogenetic complex, responsible for its induction.

3.3. All data included in the diagnostic certainty conclude that the diagnosis of the Polyuria-Polydipsia syndrome is made by corroborating elements of clinical and paraclinical – additional data (the latter defining the final diagnosis).

3.4. From the ultrasound investigated cases with pyometra (n = 21), 18 bitches had polyuria-polydipsia syndrome (85.71%) and from these, 15 (71.43%) were with opened and 6 with closed cervix (28.57%).

3.5. Ultrasound diagnosis in cases of pyometra (polyuria-polydipsia syndrome associated) is easily achieved with high degree of accuracy (100% positive) and non-aggressive for the patient and the examiner, dominated by the distensions of uterine horns by hypoechoic/anechoic content, without or with minimum wall thickening.

3.6. The first intention therapeutically measure is represented by ovary-hysterectomy (especially if the cervix closed pyometra), but in young females or of high reproductive value, can try conservative therapy, resorting to systemic antibiotic therapy supported with PGF<sub>2</sub> $\alpha$  type uterine stimulants, followed for controlling by ultrasound examination – for confirming the curative efficiency.

3.7. In addition and in close connection with the management complexity of all etiopathogenetic and it must be done with maximum care, curative effectiveness is closely linked to precocity, accuracy and consistency of application of measures to be taken.

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## **COLLECTING OF MARE'S OOCYTES BY USING THE METHOD OF ASPIRATION IN SEASON AND OUT OF SEASON FOR IN VITRO FERTILIZATION**

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**Key words:** oocyte. fertilization, collecting, slaughter.

### **SUMMARY**

The most important source of oocytes that can be used for in vitro fertilization technologies is the ovaries of animals slaughtered in slaughterhouses. Because harvest rate is as large oocytes, ovaries from slaughterhouse are transported in special conditions to the laboratories. Thus, the time between slaughtering and oocytes collection and storage temperature are two key factors that influence both oocyte quality and fertilization capacity and development in vitro.

Oocytes derived from ovaries collected from slaughtered animals, are obtained through the follicles aspiration method, because of its advantages related to speed (3 times faster than the stalk of the follicle), cost, ease of excution and quality (Duran hd,2000).Follicle aspiration technique requires harvesting oocytes through a needle attached to a syringe or a vacuum pump (Macak V.et al., 2004).

The method of aspiration to collect oocytes follicular aspiration used needles with diameters between 18 G and 22G, attached to a syringe or a suction system. The aim of this research was to assess and identify the techniques of harvesting oocytes for in vitro fertilization in equines, and morfological evaluation of the mare oocytes and their classification according to structural aspects were also proposed.

### **1. MATERIAL AND METHOD**

Research was conducted between November 2010 - July 2011, a number of 94 ovaries collected from 47 mares aged between 4 and 20 years, and a total of 24 ovaries from 12 mares at the age of 2 years and under 2 years immature of sexually were slaughtered in the slaughterhouses of Vintu

de jos (Alba Iulia) and Cetina (Baia Mare). The animals were brought to the slaughterhouses for slaughter.

### ***1.1 Collecting of mares oocytes from slaughtered animals***

#### ***1.1.1 Collection of ovaries from slaughterhouses:***

Ovaries were collected after 10 minutes of mares slaughter and transported to the laboratory in solution of chloride Na 0.9% and supplemented with 100µm/ml streptomycin and penicilline 100 UI/ml or in a solution of PBS supplemented with antibiotics at a temperature of 30-33 C°, to ensure maintaining the viability of oocytes until processing. The goal was also assured by compliance with a maximum of 3-4 hours between collecting of ovaries and oocytes collection. In the laboratory, before harvesting oocytes, ovaries were washed in sterile saline (without antibiotics) and in order to avoid contamination and to remove surface microorganisms, the procedure was repeated with 70% ethyl alcohol. Drying was done by dabbing with sterile swabs.

#### ***1.1.2 Collecting of mare's oocytes by the method of follicles aspiration***

To achieve collecting method by the aspiration of follicular fluid from ovarian follicles visible on the surface syringe needles were used. The harvesting protocol consisted of the puncture of ovarian follicles visible on the surface with a diameter greater than 1 cm. Aspiration of follicular fluid in the syringe was done carefully with a force constant pressure and not to cause destructions in the oocytes or cumulus cell separation.

After collecting and adding follicular fluid in a Petri plate, it was examined for identification and selection oocytes, and morphological assessment was performed (Fig.1).



**Fig.1-** A- method of follicular fluid aspiration, B- adding follicular fluid in a Petri plate

A Nikon inverted phase microscope provided with a video camera equipment from the Department of Breeding, Veterinary Obstetrics and

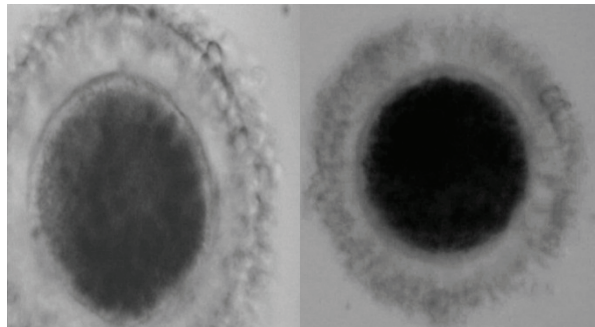
Gynecology, was used for their ranking and selection for in vitro maturation.

Morphological characteristics that were the basis for assessing the integrity and viability of mare oocytes were determined according to:

- Appearance of the zona pellucida: sphericity, integrity;
- Appearance of cumulus ooforus : cumulus cell number, compaction;
- Cytoplasm appearance: texture of cytoplasm, the presence of vacuoles, granularity, opacity, homogeneity;
- Appearance of perivitellin space: the presence of detached cells, thickness, uniformity.

Depending on the identification of morphology using the stereomicroscope and reverse microscope, the mare oocytes collected by this method were classified into two quality classes:

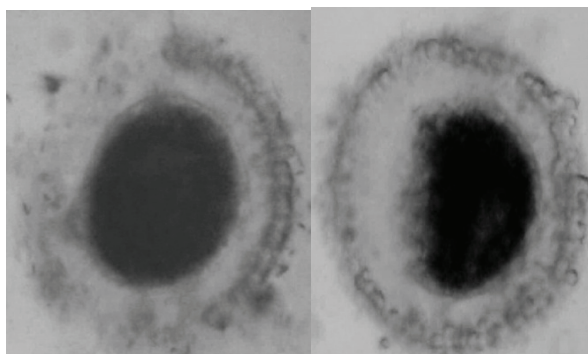
- Cultivable oocytes (Fig.2) which had compact cumulus around the oocyte, multilayered ( at least two to three layers), homogeneous cytoplasm, slightly granular, intact pelucida membrane, without destructions or ruptures and uniform perivitellin space;



**Fig.2-** oocytes suitable for cultivation 20x

- Uncultivable oocytes (Fig.3) who had partial or complete denudation, heterogeneous cytoplasm, distorted and strongly granulated pelucida, broken and uneven perivitellin space.





**Fig.3-** uncollectable oocytes 20x

## **2. RESULTS AND DISCUSSION**

In order to obtain mares oocytes, a number of 94 ovaries from 47 mares slaughtered in season and out of season was used. The method of follicular fluid aspiration was applied, and the collected oocytes were assessed morphologically and classified as two classes of collectable and uncollectable oocytes.

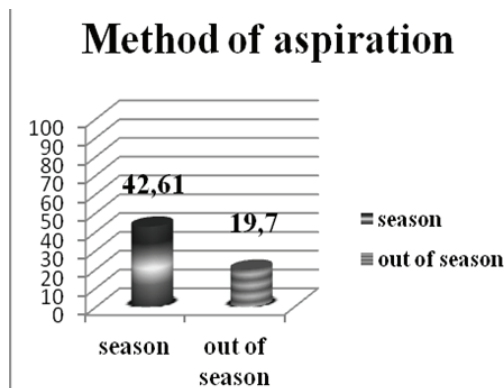
The following goals were considered in choosing techniques and devices to collect oocytes:

- Improving methods of identifying follicles on ovarian cortical surface and inside;
- Full recovery of follicular fluid and oocyte recovery increased efficiency;
- Intervention to be atraumatic to existing oocyte within the follicle;
- The cost price is affordable.

After applying this method of collection on 94 ovaries, 118 oocytes from 353 follicles surface were obtained, with collecting rate 33.42%, averaging 1.25 oocytes/ ovary. Of the 94 ovaries studied, 53 ovaries were harvested in the season and 41 ovaries were harvested out of season. According to on the time of harvesting two groups were made up:

1- Group I: in season, from 53 ovaries a number of 90 oocytes were obtained from 211 follicles, harvesting rate was 42.6% and average 1.69 oocytes/ ovary.

2- Group II: in aut of season, from 41 ovaries a number of 28 oocytes were obtained from 142 follicles, harvesting rate was 19.7% and average 0.70 oocyte / ovary ( chart.1).



**Chart no.1-** presents method of aspiration in season and in out of season

Examination of follicular fluid collected, by stereo microscope in inverted phase, and identification and selection of oocytes based on structural elements (as oocytes, the number of cell layers surrounding the oocyte cumulus, the degree of compaction, cytoplasm appearance and integrity categories: cultivable and uncultivable oocytes.

Of the 118 oocytes ( 90 oocytes in season and 28 oocytes in out of season) collected by follicular aspiration's method and with evaluated morphology, 86 oocytes ( 72.88% ) were classified as cultivated oocytes (chart.2 ), they were divided on the collecting period in two groups:

- Group I: in season, of 90 collected oocytes, a number of 72 oocytes were cultivable ( 80%)

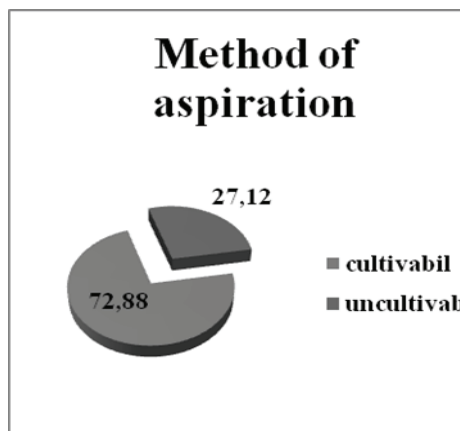
Because they presented two to three layers of cumulus cells, smooth or slightly granular cytoplasm, but no vacuolation, zone pelucide integral and a space perivitelin uniform.

- Group II: in out of season, of 28 collected oocytes , 14 oocytes were uncultivable (50%)

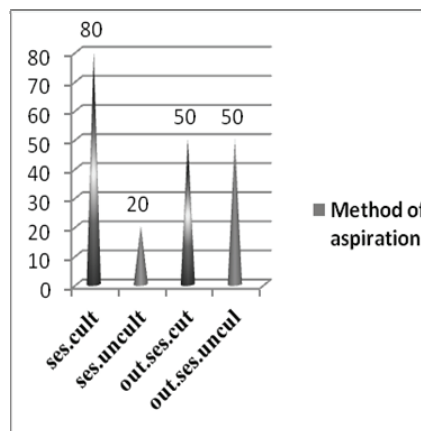
The remaining 32 oocytes ( 27.12% ) were classified as uncultivable and were divided on the collecting period in two groups:

- Group I: in season, of the 90 oocytes collected , 18 oocytes were uncultivable ( 20%).

- Group II: in out of season, of the 28 oocytes collected , 14 oocytes were uncultivable ( 50%) ( chart.3 ).



**Chart.no. 2-** cultivable and uncultivable oocytes



**Chart no.3-** cultivable and uncultivable oocytes in season and out of season

### 3. CONCLUSION AND RECOMMENDATIONS

- 3.1 By using the method of aspiration, a number of 118 oocytes were obtained from 353 follicles, the rate of collecting 33.42%, average 1.25 oocytes/ovary.
- 3.2 A number of 90 oocytes were obtained in season with collecting rate 76.27%.
- 3.3 A number of 28 oocytes were obtained out of season with collecting rate 23.73%.
- 3.4 86 oocytes were classified as cultivable oocytes (72.88%).
- 3.5 32 oocytes were classified as uncultivable oocytes (27.12%).
- 3.6 Highest percentage of recovery has shown for the season.
- 3.7 Most oocytes with different degrees of dedgeneration was identified out of season.

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## THE ZOONOSIS DANGERS- RENAL DISORDERS IN TRICHINELLOSIS

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**Key-Words:**-trichinellosis, renal, glomerulonephropathy, pyelonephritis, hypoalbuminaemia,

### SUMMARY

The renal *disease* in trichinellosis infections in humans manifested through urine-oliguria turbulency or pollakiuria, as well as diagnosis of proteinuria associated with average values of serum albumines, varies according to the clinical type of disease and is directly correlated with the hepatic damage, including a series of glomerulary and tubule cells lesions, the gravity of which depends on the severity of different clinical forms of nephritis and gromenulonephropathy, on the dehydration level, as well as on the renal history of the patient.

Globalization of the food chain causes constant new challenges and risks to health and consumer interests. The main objective of EU food safety policy is to achieve the highest possible degree of protection of human health and consumer interests in relation to food. In this regard, the EU strives to ensure food safety and proper labeling, given the diversity of products, including traditional ones by specific certification bodies (EFSA).

The EU has developed a comprehensive body of legislation on food safety, which is continually monitored and adapted as new developments. Thus, traceability is managed by European legislation and the regulations nr.178/2002 1642/2003 on food safety and the local law no. 150/2004 on food safety and feed quality and standards, such as: 22005:2007, ISO 22000:2005 and ISO / TS 22004:2006 for traceability in the food chain. The EU actively promotes high standards of consumer safety and consumer support organizations to strengthen their role in decision making. Biotechnology researches and development related to food (including genetically modified organisms) is a way to eradicate hunger, which takes into account the basic principle of EU food safety policy by applying an integrated approach, such as "farm to fork" covering all sectors of the food chain, including feed production. Community legal framework on food

safety is common to all Member States, but adapted diversity. EU efforts significant because traditional foods are not removed from the market due to food safety standards and that innovation should not be discouraged and do not have the quality of the Romanian scientific. Each EU country is obliged to ensure that product safety was not compromised in its food chain, and this can be achieved through the implementation and certification of a Food Safety Management System. HACCP is a system of internationally recognized food safety, based on a systematic analysis and preventive production process, which shows that food safety risks are identified, assessed and controlled. HACCP involves risk identification, control and monitoring of critical points where the process could be compromised food quality. The system is based on the Food Code (Codex Alimentations) developed by the UN Food and Agriculture Organization and World Health Organization.

The renal disease in trichinellosis is described most often as glomerulonephritis. Proteinuria associated to a clinical examination pointing towards renal disease including back pain and fever would indicate pyelonephritis rather than trichinellosis, especially if the patient's pathological history hints at a previous reno-urethral disease, and if the urinary sediment proves to be pathological.

All clinical aspects described as possible manifestations in the evolution of various clinical forms of trichinellosis, present since hospitalization, coming as a result of disease evolution, or as complications in the case of severe evolution are each subject to differential diagnosis.

Their interpretation shall mandatorily be made by considering the patient's pathological history, the epidemiological context, associated with specific laboratory data. In the severe disease forms, the kidneys appear enlarged and significantly more fragile. From a microscopic perspective, they correspond to the glomerular-tubular alteration, predominantly epithelial cells lesions[3].

In severe forms, degenerative alterations and sanguine suffusions are found especially in the medulla; the medium-severe forms present spoliation of lipids, an aspect indicative of a hypo functioning of the suprarenal gland.

If at kidney level one finds a series of lesions affecting the elements of the whole nephron, at the level of the suprarenal glands, the organ's spoliation of lipids in different degrees of insufficiency is found at the clinical examination via the typical symptoms (sluggishness, hypotension).

The reversible lesions can be cured by corticosteroids. Hypertoxic forms of disease are an exception to this treatment.

### 1. MATERIAL AND METHODS

Out of the 327 patients suffering of trichinellosis, who were part of the research conducted in the Infectious Diseases Hospital in Brasov' during 1997-2002, 10 people also suffered of a renal disease: nephrolithiasis (7 cases), urinary tract infection (2 cases), chronic pyelonephritis. These patients were excluded from the research..

### 2. RESULTS AND DISCUSSIONS

In our study, only 21 people had urine-oliguria disorders or pollakiuria :

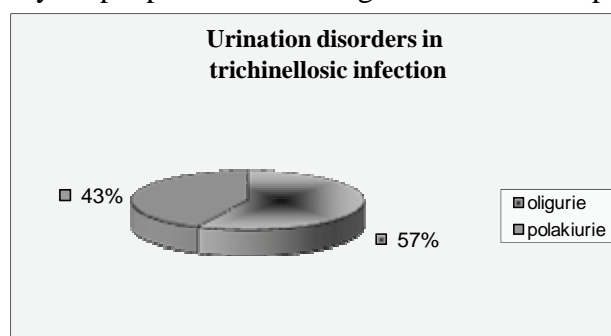


Fig. 1. Oliguria disorders or pollakiuria

In the severe form, a number of five patients presented pollakiuria, contrary to the medium-severe and medium forms, where the same number of patients (two cases) presented this symptom

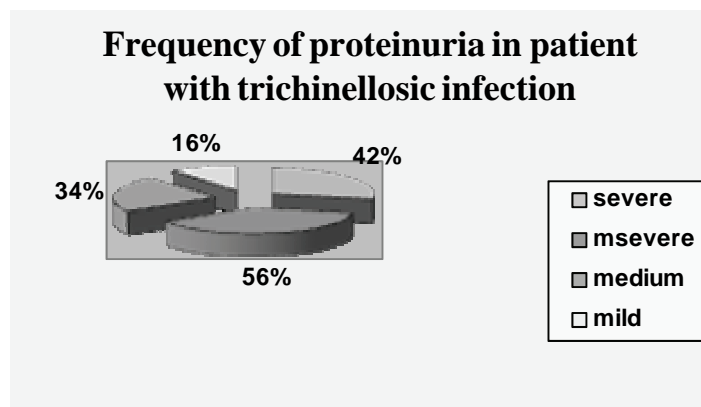
We should mention the fact that, out of the nine patients suffering from pollakiuria, three were pregnant women, in the fourth and fifth months.

From a numeric point of view, the cases that presented oliguria in the medium-severe form were the most frequent (7 cases).

A single patient presented high values of urea (70 mg/dl) and creatinin (1,30 mg/dl), but the later clinical evolution was favourable. The patients suffering of proteinuria, who were included in this research, presented light or medium traces of albumins at the urine examination.

As noticed in Fig. 3, below, the highest percentage of cases to have presented proteins was recorded in the medium-severe form (56%), followed by the severe form (42%). In the mild forms there were no cases of proteins recorded.

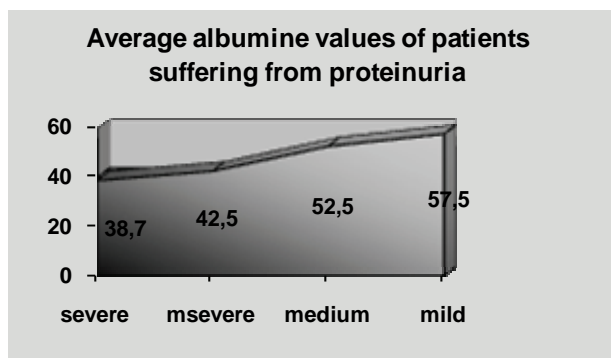
**Fig. 3.** Frequency of proteinuria in patient with trichinellosis infection



We have tried to compare the proteins of the patients infested with trichinellosis with the average values of the serum albumins. We notice, anew, that only in the severe and medium-severe

forms the average value of the serum albumins is of lower percentage, 38,7%, 42,5% respectively; we can state that, in these forms, one of the causes of hypo albumins is represented by their urinary elimination. The urine clinical examination could sometimes reveal the presence of hyaline and granular cylinder, alongside albumins, two of these cases pertaining to the severe form of trichinellosis. In our research, only 4 patients suffering of trichinellosis presented hyaline and granular cylinders, two of them suffering from the severe form of trichinellosis.

**Fig.4** –Average albumine values of patients - proteinuria



Griffith M. signals cases including nephritis and glomerulonephropathy as renal complications in trichinellosis. We have pointed out the renal complications of the patients included in this research. Observed by



optical microscope, the histological changes at renal level reveal, after SAP (Schiff Periodic Acid) colouring, indicate an augmentation of the glomerular space, following the narrowing of the renal tubes. Hypercellularity is given by the proliferation of the endogenous cells and the leukocyte infiltration [1].

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### 3. CONCLUSIONS

3.1.The higher percentage of cases which presented proteinuria (56%) and hypoalbuminaemia (42,5%) were found in the medium-severe form of trichinosis, followed by the severe form, presenting proteinuria in 42% of the cases, and hypoalbuminaemia in 38,7% of the cases.

3.2. Proteinuria and hypoalbuminaemia constitute a criterion of evaluation in the severe evolution of trichinosis.

3.3. 1,2% of the patients included in this research presented hyaline and granular cylinders alongside albuminuria. 50% of them suffer from the severe clinical form of trichinellosis. No renal complications were noted in the patients included in this research.

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## ETIOLOGY RESEARCH IN A TRANSMISSIBLE VIRAL PROVENTRICULITIS OUTBREAK

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**Key words:** broiler, proventriculitis, PCR

### SUMMARY

Transmissible viral proventriculitis (TVP) is a poorly understood disease of broiler chickens. The major economic problem caused by TVP is its ability to affect overall flock performance in broiler complexes. TVP is considered to be a disease of unknown etiology, several infectious agents, nutritional factors, and toxic factors have been associated as causes.

In this study *avian reovirus* was identified in all age groups, followed in frequency by: ChPV, IBV, CAstV and AvRV viruses, and IBDV, CAV, ANV and CPNV were not identified.

Transmissible viral proventriculitis (TVP) is an infectious disease that develops in broilers, was first reported in the Netherlands, then in the U.S.A. and Australia. In intensive poultry farming is associated with runting stunting syndrome causing significant economic damage.

In 1996, the U.S., Goodwin *et al.*, identified by electron microscopy, positive contrast, a virus, subsequently by other researchers called adenovirus-like. In subsequent years, particularly after 2000, many researchers have studied the disease etiology and according to the results obtained at present it is considered that the disease is caused by a combination of the following viruses: IBV, IBDV, CAstV, ARV, AvRV, CAV, ChPV, ANV and CPNV. Viral association was demonstrated by the fact that TVP reproduction by experimental infection is possible only with the proventriculi triturat with specific injuries and attempts to reproduce only one or two viruses were not possible (6, 8, 9).

In subsequent years, the disease has spread in more countries, including Romania, both imported broilers at the age of one day, and the broiler breeding flocks coming from local farms (1, 2, 3, 5).

Research has been conducted in a broiler farm, raised on litter permanent. In that farm, the disease was suspected clinically at more effective.

## 1. MATERIALS AND METHODS

This study was made in a broiler flock of 6500 chickens, where TVP evolved to confirm the etiology, being made molecular biology tests and serological examinations.

From cadaver with TVP lesions, proventriculi were sampled for examination of molecular biology, respectively, by PCR and RT-PCR techniques. Isolations of viral nucleic acid was made using *QIAamp viral RNA Mini Kit* (Qiagen, Germany) and *High Pure Viral Nucleic Acid Kit* (Roche, Switzerland). Primers used to identify viral agencies are found in Table 1. Revers transcription and amplification were made using *Qiagen OneStep RT-PCR Kit* (Roche, Switzerland).

Table 1  
Primers characterisation used for direct demonstration of viral pathogens causing TVP

Targeted virus	Primer name and sequence (5'-3')	Amplicon size (bp)	Annealing (°C)
ARV	S4-F13:GTG CGTGTGGAGTTTCCCG S4-R1133:TACGCCATCCTAGCTGGA	1120	53
ANV	ANV-ORF1-:AGATACGCTTGCTCGTCTTG ANVORF1R:CCTCTAACCGGCGATATTCT	608	53
CAstV	ANVpolIF:GYTGGGCGCYTCYTTYGAYAC ANVpol1R:CRTTGCCCKRTARTCTTTRT	373	53
IBV	IBV-In1-f:GTGATGACAAGATGAATGAGGA IBV-In2-r:CAGATGAGGTCAATGCTTTATC	402	53
AvRV	NSP4-F30:GTGCGGAAAGATGGAGAAC NSP4-R660:GTTGGGGTACCAGGGATTAA	630	55
IBDV	IBVD-VP2-f:GCCCAGAGTCTACACCAT IBVD-VP2-r:CCCGGATTATGTCTTTGA	743	53
CAV	CAV-VP1-910-f:TGGCACCACCTCAAGCGACT CAV-VP1-1262-:CCGTCCGCAATCAACTCACC	353	55
ChPV	PVF1:TTCTAATAACGATATCACTCAAGTTTC PVR1:TTTGCGCTTGCGGTGAAGTCTGGCTCG	561	55
CPNV	B2F:CGTAGACCTCGTCTTCTGC B2R:GGGCGTAACCATTAGATA	171	55

Serological examination was performed in order to identify specific antibodies against *avian reovirus*, because it had the highest frequency

determined by RT-PCR technique. For this purpose blood samples were taken from chickens, randomized as follows:

- R 1 - at 21 days old (48 blood samples);
- R 2 - at 35 days old (45 blood samples).

Specific antibodies were detected by ELISA, using *FlockChek*<sup>®</sup> *Avian Reovirus Antibody Test Kit*, made by IDEXX Laboratories, Inc.

## 2. RESULTS AND DISCUSSION

The results obtained by molecular tests (PCR, RT-PCR), are shown in Table 2. The cadavers from outbreak were collected from four different age categories to highlight the possible viruses responsible for disease etiology. From the proventriculi samples taken from broiler carcasses, from the PVT flocks, many viruses have been identified. Analyzing data from the table it is observed that *avian reovirus* was identified in all age groups, followed in frequency by: ChPV, IBV, CAstV and AvRV viruses, and IBDV, CAV, ANV and CPNV were not identified.

The results are identical with the results communicated by most research teams dealing with TVP study (7, 8, 9).

*Table 2*

Exam results of molecular biology

Flock	Age	Viruses								
		IBV	ARV	AvRV	IBDV	CAV	ChPV	ANV	CAstV	CPNV
A	14	+	+	-	-	-	+	-	-	-
A	21	-	+	-	-	-	-	-	-	-
A	28	-	+	-	-	-	+	-	-	-
A	35	-	+	-	-	-	+	-	+	-

Serological examination was performed in order to confirm *avian reovirus* infection because the virus was identified by molecular biology tests, samples proventriculi at all age categories.

The results of this examination, performed by ELISA, are shown in Table 3. After reading the responses and process the results, according to the interpretation of the kit software *FlockChek*<sup>®</sup> *Avian Reovirus Antibody Test Kit*, made by IDEXX Laboratories, Inc., were established for each harvest: titer groups, minimum titer, maximum titer and geometric mean. Titres are expressed in optical density (O.D.).

**First harvest**, that the age of 21 days, six groups were identified titers (0-5), the minimum titer of 12 OD and 1022 OD maximum titer.

**The second harvest**, that the age of 35 days, eight groups were identified titers (0-7), the minimum titer of 63 OD and 1453 OD maximum titer.

Analyzing these results, we see that at the age of 21 days (R 1) 29 sera were positive, respectively 60.42% and the age of 35 days 35 sera were positive, 77.77% respectively. These data show that, within 14 days, the proportion of positive sera increased 1.29 times, suggesting horizontal extension of the infection in the flock of chickens examined.

Table 3

Results of serological exam

R 1 / 21 days		R 2 / 35 days	
Group of titer	Number of samples	Group of titer	Number of samples
0	19	0	10
1	5	1	3
2	6	2	5
3	7	3	5
4	6	4	5
5	5	5	6
-	-	6	5
-	-	7	6
<b>Maximum titre</b>	<b>1022 O.D.</b>	<b>Maximum titre</b>	<b>1453 O.D.</b>
<b>Minimum titre</b>	<b>12 O.D.</b>	<b>Minimum titre</b>	<b>63 O.D.</b>
<b>Geometric mean of titres</b>	<b>89</b>	<b>Geometric mean of titres</b>	<b>245</b>

At the age of 21 days, the geometric mean antibody confirmed antireovirus to an actual value of which is 7.42 times higher than the minimum titer considered positive, and after 14 days and 35 days of age, geometric mean was 2.75 times higher than the previous age, geometric mean was 20.41 times higher than the minimum positive titer from the first harvest. Also, at the age of 35 days, maximum serum titer was 1.42 times higher than the maximum titer of serum obtained at the age of 21 days.

Values antireovirus antibody titres, expressed in OD, are similar to values reported by other authors (4, 10), being characteristic of this infection.

The results of this examination confirms the presence of *avian reovirus* infection, and if one takes into account the fact that this virus was identified by RT-PCR in all age groups, could make the claim that *avian reovirus* is the main etiologic agent of TVP or that TVP is a component of malabsorption.

### 3. CONCLUSIONS

- 3.1. By the molecular biology techniques used, more viruses were identified, the most often identified was *avian reovirus*;
- 3.2. Viruses identified in this outbreak are similar to viruses found in other broiler flocks from which samples were taken;
- 3.3. Immunoassay test showed a postinfectious immune response to *avian reovirus* infection and the seroconversion demonstrate its progress;
- 3.4. The presence of *avian reovirus* in proventriculi samples, taken from all age groups, associated with the results provided by serological examination, suggests that this virus occurs as primary agent in the etiology of disease.

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## **ELBOW DYSPLASIA IN DOGS – ETIOPATHOGENESIS DIAGNOSIS AND CONTROL**

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**Key words:** elbow dysplasia, dog

### **SUMMARY**

Elbow dysplasia was originally described as a developmental disease manifested as degenerative joint disease of the elbow with or without an ununited anconeal process. Developmental degenerative joint disease of the elbow has multiple inherited etiologies which may occur singularly or in combination (ununited anconeal process, fragmented medial coronoid process, or osteochondritis of the medial humeral condyle)?

This paper intends to introduce in Romania IEWG protocol, proposing: to provide a standardized evaluation of elbow joints for canine elbow dysplasia, whether due to ununited anconeal process, fragmented coronoid process, osteochondrosis, or any combination there of; and to serve as a data base for control of elbow dysplasia through selective breeding.

As defined by the "International Elbow Working Group - IEWG" elbow dysplasia is a term that defines an abnormal development of this joint and can result in lameness, growth deformities, subluxation and ultimately by progressive degenerative joint damage - arthrosis of the elbow or degenerative arthritis of the elbow (12, 14, 26).

Currently, the literature, under the term of elbow dysplasia, describes three different pathologic entities: fragmented medial coronoid process of the ulna (FMCP) or fragmented coronoid process (FCP), ununited anconeal process (UAP) and osteochondrosis (OC) or osteochondritis dissecans (OCD). Some authors (3, 13) consider that the three pathological entities (to which other researchers add the fourth – elbow incongruity - EI) are causes of the elbow dysplasia. Other consider that the three entities are different diseases (forms) framed in the dysplasia syndrome – arthritis of the elbow, in which osteochondrosis plays the main role (9, 12, 23). This apparent controversy leaves, in fact, to see the incomplete state of knowledge on the etiopathogenesis of the disease.

It is essentially discussed the primary role of osteochondrosis or elbow joint development abnormalities (incongruity), both assumptions are now



acceptate. Arthrotic and arthritic changes are the consequence of the articular cartilage metabolic disturbances, subjected in certain areas to requests for charging excessive body weight, following the faulty development.

The etiopathogenesis of the elbow dysplasia is incompletely elucidated. It is widely accepted the polifactorial etiology with a high genetic predisposition (15). Hazewinkel H.A.W. - 2003 (12) presides for the elbow arthritis causes as having congenital, traumatic and combinations of both origins. In these circumstances, we believe that the dispute on the primary role of the trigger cause (osteochondrosis or anomaly - a joint development mismatch), from which it starts to describe the physiopathological phenomena, it is unnecessary, as both are bone development disorders, with different anatomical locations. Characteristic changes of the elbow dysplasia (OCD, FMCP, UAP, EI) represent pathological reactions to the action of etiological factors at different anatomical levels.

Thus, in the case of osteochondritis dissecans, it is affected the endochondral ossification of the epiphyseal articular cartilage of the humerus of the distal limb, with a possible involvement of the growth metaphyseal cartilage of the medial humeral epicondyle (also responsible for the ununited medial epicondyle of the humerus- unnamed disease among those contributing to the elbow dysplasia) (4).

In the case of the fragmented coronoid process (term also known as fragmented coronoid apophyses), it is affected the osteochondral ossification of its growth apophyical cartilage and/or the closure (premature ossification) of the growth proximal metaphyseal cartilage of the radius, which creates an asymmetry between the length of the radius and the length of the ulna. The rate report of the length of the ulna and the radius is smaller in normal dogs – 0.17-0.19 than in dogs diagnosed with elbow dysplasia – 0.19-0.23. Ulna length grows more than the radius, resulting in an increase in weight distribution and in the forces that press the medial coronoid apophyses of the ulna (9). The following strain of the overload of the medial coronoid apophyses of the ulna, it changes its shape and/or can fracture and fragment.

In the case of ununited anconeal process (term also known as ununited anconeal apophyses of the ulna) the disorder regards one of the secondary ossification centers of the ulna – the disc-shaped growth metaphyseal center of the anconeal process. Premature closure (ossification) of growth distal metaphyseal plate of the ulna (the ulna remains shorter than the radius), common in dystrophic dog breeds, causes increased pressure on the ventral

side of the anconeal process, claiming its separation and/or the caudal subluxation of the elbow. This is termed by some authors as fitting the typical cases of UAP (7), while others, only for the dystrophic breeds classifies it in the classical fracture typology. In the elbow incongruity, there is a misalignment between articular surfaces of the humeral trochlea and those of the radius and ulna (12). Two situations can be encountered: -the ulna shorter than the radius, which coincide with the ununited anconeal process or with exercising undue pressure on the radius by the integral anconeal apophyses (12); -there is a disorder of the development of the articular vertical semilunar notch of the ulna (also known as the trochlear notch of the ulna), which, usually, takes an abnormal oval shape – a situation that coincides with the ununited anconeal process of the ulna and/or fragmented medial coronoid process of the ulna (23).

In this second case, to an animal affected by elbow dysplasia, one can notice a disparity (mismatch) in the joint edge continuity represented by the ulna trochlear notch and the radial head (5). As most researchers consider, this apparent step (the articular line of the radial head is positioned 2 to 5 mm below the articular line of the trochlear notch of the ulna) seems to be due to an asynchronous length growth of the radius comparing to the ulna length – the delayed development of the radius. The assumption made is objectionable because the radiographic changes considered in the premature closure of the growth center of the radius, respectively the tightening of the articular space between the anconeal process and the humerus, are not similar to those seen in the elbow dysplasia, in which case it is notable the increase of the joint space. Wind A. P. – 1986 (23) offers another explanation consisting in: the underdevelopment of the trochlear notch of the ulna (the concavity is lower than the convexity of the humeral trochlea) tries to be offset by a faster growth of the ulna than the humeral trochlea. This tendency results in an increase in the ulna length greater than that of the radius (creating the joint step) but insufficient for the development of the trochlear notch of the ulna to ensure a congruent articulation with the humeral trochlea, making the ulna to move towards caudal and increase the joint space.

In support of this approach of the etiopathogenesis of the elbow, there come the following findings:

- the presence of some developmental abnormalities (mismatch between parts of the elbow bone components) similar in different forms of elbow dysplasia;

-in the terms of the same breed, there is possible to encounter dogs affected by different forms of the elbow dysplasia;

-relatively high frequency of some combined damages, the association of the fragmented medial coronoid process of the ulna with the osteochondritis dissecans of medial condyle of the humerus and/or the association of the ununited anconeal process with the fragmented medial coronoid process;

-different forms of dysplasia met on the elbows of the same animal.

It is possible that the development disorders (enchondral ossification) start in one anatomical location and then expand in the neighborhood, and can not be ruled out any multiple initial damage with varying degrees of severity for each anatomical location. We suppose that it is very likely to exist a sequence to follow the order of closure (ossification) of the seven growth metaphyseal centers of the bones competing at the elbow joint and the epiphyseal articular cartilages of the humerus, ulna and radius.

Given the existence of a genetic predisposition for the elbow dysplasia, the intervention of the environmental factors (nutritional, hormonal, traumatic, etc.) during the growth period triggers a series of pathological changes characteristic for the dysplasia syndrome (OCD, FCP, UAP) and later on, with age, there will occur characteristic changes for the progressively degenerative arthritis (6, 19, 22). Among the environmental factors that predispose to dysplasia, the overfeeding and the excessive intake of dietary calcium are constantly taken into account (11, 16, 17).

Elbow dysplasia affects fast-growing dog breeds, middle and giant sized. High frequencies of elbow dysplasia have been reported in breeds as: Bernese Mountain Dog, Rottweiler, German Shepherd, Labrador, Golden Retriever, Basset. Other dogs are affected, with lower frequencies: Newfoundland, Saint Bernard, Doberman Pinscher, English Setter, Gordon Setter, Mastiff, Sharpei, Chow-Chow, Terrier, Dog (8, 9, 14, 26). Males are more frequently affected than females, indicating a possible genetic involvement of a sex-linkage gene (6).

Symptoms of elbow dysplasia in dogs are dominated by uni or bilateral lameness – small rigid steps and an obvious limitation of the flexion and extension movements of the elbow while walking. Bilateral impairment (both elbow) is found in 20 to 35% of the cases. In these cases, specifying the affected limb and the gait analysis is more difficult. Elbow dysplasia begins as an acute arthritis, dominated by the inflammatory signs, and then evolves to a progressive degenerative joint configurations. Lameness occurs in dogs aged between 5 and 12 months (9). The OCD affects dogs around the age of 6 months and UAP and FMCP is found around the age of 12

months. Up to 18 months, the causes of the lameness are represented by the pathological entities signaled by acute inflammatory symptoms (OCD, UAP, FMCP). After that age, lameness is caused by the arthrotic modifications (degenerative progressive arthritis). Overall, the lameness caused by the elbow dysplasia begins between 3 and 12 months of life, but there are reports on late appearances, until the age of 5 to 7 years. Rapid locomotion and jumpings aggravate the intensity of the lameness.

During the progression, the lameness may become intermittent (periodic), alternating the periods of remission with the relapse. Periods of relapse occur after intense supported effort. Clinical examination reveals living pain when handling the forearm of the affected limb and deep palpation reveals joint swelling, crepitation and the "click-clac" feel caused by the cartilage loose flap (OCD).

Hazewinkel H.A.W. - 2003 (12) notes that in 50% of cases affected by elbow dysplasia, the affected limb can be observed turning outward (supination) and in slight abduction, when the animal is inspected in standing position. The movement examination signals varying degrees of lameness, obviously raising the head when the support is made on the affected limb. Swelling of the elbow is more evident in the UAP than in FMCP or OCD. Lateral decubitus direct examination records the decreased joint mobility, pain and crepitation when the examiner's thumb is placed on the anconeus muscle. If the case of UAP the crepitations and the pain are well highlighted during the joint hyperextension. In FMCP and/or OCD, the crepitation and the pain can be caused by a prolonged hyperextension and, sometimes, by turning outward the forearm under the block of the scapular-humeral joint.

Clinical expression of symptoms is closely correlated with the breed of the affected animal. Thus, the fragmented medial coronoid process of the ulna (comparable injury) is clinically manifested by an intensity much higher in Retriever breed dogs than in Rottweiler breed (12, 22).

The diagnosis is suspected based on clinical signs and confirmed by imaging -radiographic examination, computed tomography and/or magnetic. IEWG with the agreement of the "World Small Animal Veterinary Association - WSAVA" (25) proposed a minimum conduct required for the radiographic examinations, which includes a standard set of three images (no. crt. 1-3) for animals affected by elbow dysplasia, but more often two incidents (no. crt. 4 and 5) are necessary to specify the correct diagnosis of the morbid entity (UAP, FMCP, OCD, IA):

1. -medio-lateral incidence with the elbow in slight flexion (110-120 °) and the X ray beam centered on the medial epicondyle – this permits the assessment of the congruency of the radio-ulnar articular line and, in some cases, the medial coronoid process may be seen as an superimposed image on the proximal extremity of the radius (cranial of the radius' physis);

2. -medial-lateral incidence with the elbow fully flexed (approximately 45°) - offers the highest degree of exposure of the anconeal process, allowing the view of the dorsal localized bone spurs;

3. -cranio-caudal incidence with the forearm rotated inward about 15 ° - offers the best image to view the osteochondritis dissecans lesions located on the medial condyle of the humerus, but, although, it can capture the image of the proximal edge of the medial coronoid process of the ulna, highlighting its fragmentation is difficult;

4. -medio-lateral incidence with the elbow in full extension and the forearm rotated 15 ° outward (supination) –it can highlight the cranial edge of the medial coronoid process of the ulna (viewing is possible in 90% of cases, compared to other medio-lateral incidences that permit the capture of the coronoid process in only 50% of cases);

5. -cranio-caudal incidence with continued right forearm – it allows the view of the osteochondritis dissecans lesions visualization of the medial humeral condyle and, also, the bone spurs located medial to the medial humeral epicondyle and/or medial coronoid process of the ulna.

Radiography of both elbows is indispensable. The main radiographic changes observed are: radio-ulnar joint line incongruity, increased humero-radio-ulnar intra-articular space, loss of the contour or the shape of the abnormal medial coronoid apophyses of the ulna, loss of the radiographic contour or ununited anconeal process; periarticular osteophytosis located around the medial coronoid process of the ulna, cranial to the radius head, dorsal to the anconeal process and medial to the medial epicondyle of the humerus; uneven flattening of the bone layers; subchondral sclerosis, especially on the articular vertical semilunar notch of the ulna (trochlear notch); loose cartilage flap (intra-articular bone spurs) or ossified cartilage flap located on the medial humeral condyle (8).

Osteophytosis represents a response to physiological conditions of the intraarticular environment, consisting of abnormal development of the edges of the joint cartilage and of the nearby bone. Osteophytosis is recorded bilaterally (both elbows) in about 30% of cases with UAP and in 50% of cases of FMCP (12). Depending on their location, bone spurs can be classified into periarticular, periosteal and endosteal.

The presence of osteophytes is the significance of unstable joints, but also of a reparative revival of the cartilage, the periosteum or the affected synovial tissue. Periarticular locations can be identified around the medial coronoid process of the ulna, cranial to the radius head, dorsal to the anconeal process and medial to the medial epicondyle of the humerus, at about 21 days from formation (8).

Periosteal or endosteal located osteophytes indicate a response reaction of the endosteum and the periosteum to trauma, infection, irritation, inflammation or tumors. In the elbow dysplasia, they appear localised around the edge of the trochlear notch of the ulna, the points out attachments to the origins of tendons, ligaments and joint capsule. They reveal the chronicisation of the condition.

The presence of extraarticular (soft tissue mass near the joint) of bone fragments of various sizes, was notable on the X-ray of individuals over 27 breeds. Extraarticular positioning was reported in the following locations: lateral to the medial humeral condyle, medial to the ulna and disto-caudal to the medial humeral epicondyle. The nature of these bone fragments and their clinical significance is unknown (18).

Bone sclerosis of the trochlear notch and the distal edge of the coronoid apophyses of the ulna appears on the radiographic image as a sum of images of the thickened trabecular bone structure with the periosteal and endosteal bone spurs from this location. Sclerosis does not occur if blood flow toward the coronoid process is diminished, recording as an osteochondral defect, lack of ossification of fibrous tissue connecting the coronoid apophyses to the annular ligament.

The bone spurs come from the detachment of cells from cartilage (detachment caused by trauma or osteochondrosis) that develops and subsequently ossify from synovial cell metaplasia into cartilage cells and bone later on. Their presence requires careful examination and differentiation from humeral sesamoid bones present on the side of the joint and from the center of ossification of the lateral collateral ligament.

IEWG clasifies the radiographic changes that occur in the examination designed to identify dogs with elbow dysplasia in four degrees of damage (24, 26).

**0 degree** – healthy animal with no signs of arthrosis

**1<sup>st</sup> degree** -minor arthritis, characterized by one or more of the following details: osteophytes smaller than 2 mm located proximal to the dorsal edge of the anconeal process of the ulna; osteophytes smaller than 2 mm located cranio-proximal to the radial head, or near the dorsal edge of

the medial coronoid process of the ulna, or lateral to the caudal edge of the humeral trochlea; subchondral sclerosis in the caudo-distal area of the trochlear notch of the ulna.

**2<sup>nd</sup> degree**- moderate arthrosis, characterized by one or more of the following details: bone spurs in size between 2 and 5 mm located proximal to the dorsal edge of the anconeal process of the ulna; bone spurs in size between 2 and 5 mm located cranio-proximal to the radial head, or near the dorsal edge of the medial coronoid process of the ulna, or lateral to the caudal edge of humeral trochlea.

**3<sup>rd</sup> degree** - Severe arthrosis, characterized by one or more of the following details: osteophytes larger than 5 mm located proximal to the dorsal edge of the anconeal process of the ulna; osteophytes larger than 5 mm located cranio-proximal to the radial head, or near the dorsal edge of the medial coronoid process of the ulna, or lateral to the caudal edge of humeral trochlea; medial, in the distal portion of the medial humeral condyle; medial to the medial coronoid process of the ulna.

This method of examination is used for the surveillance and the prevention of the extension of the elbow dysplasia in various dog populations, being accepted by the Orthopedic Foundation for Animals (OFA - USA), by the canine federations in Australia, New Zealand and some dog clubs from european countries (Kennel Club Sweden, Norway Kennel Club, Kennel Club Netherlands, England, Switzerland, Germany, France) (1, 2, 8, 10, 21). The program promoted by IEWG is supported by the International Kennel Federation - FCI which, since 1999, issues international certificates for the elbow and hip dysplasia control (13).

The expansion of radiographic control of the elbow dysplasia programs in populations are based on a relatively high correlation between the coefficient of heritability of the disease and the radiographic cataloging based on the IEWG protocol proposal (1, 2, 20, 22). The existence of a positive correlation between the degree of the dysplasia and the percentage of the affected dogs as a result of various forms of cross breeds has been reported for Rottweiler, Bernese Mountain Dog, Labrador and German Shepherd (1, 2, 20). Applying for an elbow dysplasia transmission control program in accordance with the IEWG protocol at about 70% of the dogs of Rottweiler and Labrador breeds led in Sweden (1, 2) to a reduction of the ED prevalence from 59% in 1985 to 28% in 2002 for the Rottweiler breed, and from 36% in 1987 to 9% in 2002 to the Labrador breed.

Other attempts of establishing some programs to estimate the genetic value of the elbow dysplasia transmission based on the elbow angles

measurements have not resulted in obvious advantages in the control of the disease.

Currently, in countries that have accepted the IEWG radiographic control program of the dog population to help prevent the dissemination of the elbow dysplasia, there is the concurrence of the majority of the dog breeders for the exclusion from breeding of specimens categorized as 2<sup>nd</sup> and 3<sup>rd</sup> degree of dysplasia, and some associations do not support even 1<sup>st</sup> degree of dysplasia.

According to the protocol, the elbow radiography of an animal that is assessed for the elbow dysplasia in the purpose of obtaining the reproductive rights, there is usually made at the age of 1 year and only the dogs of large breeds (giant breeds) between the age of 1 and 2 years (23). Animals coming from the elbow dysplasia susceptible breeds must be evaluated early, at the age of the first vaccination (15-20weeks) and the exams (both clinical and radiographic) is repeated at the age of 6-7 months and then at the age of one year. Early detection of one of the entities which contribute to the elbow dysplasia syndrome allows the development of the arthrosis supervision and the setting out of a treatment protocol based on dynamic osteotomy of the ulna. In contrast, Ohlerth S. et al. - 2000 (16) shows that the data obtained by the early X-ray investigation (236 days) of Labrador breed puppies is insufficient to allow a safe diagnosis.

The future of the population dissemination control of the elbow dysplasia is probably represented by DNA tests programs that will become reliable after the completion of the genome map in this species and the exact location of genes responsible for the development of the cartilage and the bone.

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## **MONITORING METABOLIC PROFILE PARAMETERS DURING CATTLE HEAT STRESS AND TECHNOLOGICAL**

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**Keywords:** stress, technopathies, greenhouse effect gases, umoral level, metabolic profile tests, metaphulaxy, nutritional-metabolic surveillance

### **SUMMARY**

Stress as a biological phenomenon is not identified as a pathological status. The limits in which an organism can adapt to stress factors are bound by parameters which are set by resources inside the body that should allow life preservation. Still, limits are dictated by genetic programming through the extreme situations which the body has encountered throughout its evolution and yet has not stopped surviving and multiplying. In intensive breeding, production override and other stress factors such as global warming led to the apparition of real adapting diseases expressed by nutritional and metabolic disorders which are difficult to diagnose and treat.

The current tendency in zootechnics is to reduce the number of caretakers and that of the technical personnel in farms which leads to a decrease of attention on the actual animal.

Other causes of adapting disease refer to the use of food selections which lend to automatic feeding technologies (technology trailer), and ruminants do not ruminate properly any more. In addition, from technopathies, medically induced diseases have emerged, which need to be studied, prevented and controlled.

Causes of adapting diseases linked to global climate changes, food crisis and water quality should be mentioned. Regarding adapting diseases, stress is a very important and variable factor, difficult to detect and treat.

Clinical manifestations of nutritional and metabolic diseases are often nonspecific, with extremely complex pathogenic mechanisms, difficult to deal with in terms of food diversity, breeds improvement and the appearance of genetic lines with maximum production efficiency.

In order to approach these diseases, broad knowledge of epidemiology, clinical, laboratory and technological studies are required to rethink information on intensive breeding of animals, completely different from those of the last century when multi-deficiency aspects were dominant.

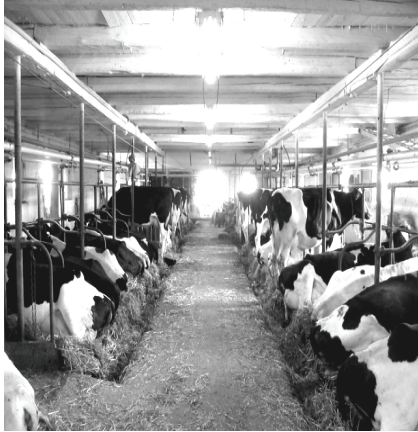


#### Alimentary override

Once prophylactic measures were applied, diseases determined by germs were reduced and the efforts to increase milk production led on one hand to obtaining record milk productions and on the other to the appearance of nutritional and metabolic disorders which resulted in significant economic loss even if some of these illnesses are subclinical and very difficult to diagnose.

Through a rigorous selection lines and highly productive individuals were created but the work to obtain specialized individuals was not a priority in order fructify unconventional fodder supplies: this led to the creation of new individuals with metabolic disorders, directed towards the organs production effector, but very susceptible to nutritional and metabolic disease.

All this is obviously a result of significant reduction of the operating and nutritional spectrum in which animals can maintain homeostasis: for it to be profitable, an exploited livestock should be healthy throughout the production cycle.



### Technological Stress

Sensitivity to various technological or food factors has led to the stress-sensitive individuals who react badly in terms of weight gain and meat quality.

Overcoming homeostasis borders led to onset of asymptomatic expressing adapting diseases (stress, fertility reduction, decreased milk production and milk fat, etc.), which raises larger diagnostic group issues, including prevention and control.

Nutritional disorders caused by the lack in one or more nutrients are common manifestations, which may be indispensable for the proper functioning of the body, or affect the quality of animal production.

Poor nutrition has immunosuppressive effects at the cellular or umoral level, affecting even the immuneprophylactic measures.

Metabolic disorders may be a consequence of genetic factors, but the environment is important too for living animals and the soil that is obtained based on forage, which is interdependent with its mode of fertilization.



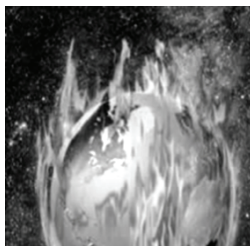
### Caloric Stress

"We live in a warmer world." Biological Diversity, which is a defined notion of expression of the full diversity of life on Earth is currently experiencing one of the most complex phenomena: global warming.

Ecosystems evolution for thousands of years is a direct outcome of the quasi equilibrium between different components and between species and abiotic factors and may be strongly affected by the direct impact of climate changes.

It may be indirectly affected by the relationship between species to define new terms of reference of the ecosystem in training, particularly related to the direct correspondence between species and abiotic factors (temperature, humidity, water mode, pH, O<sub>2</sub> concentration, the concentration of other gases solvit, soil structure, etc.).

Increasing of the concentration in greenhouse effect gases in the atmosphere, especially carbon dioxide is the main cause for warming during the last 50 years of the 20th century. Different climate scenarios of global climate models have predicted an average global temperature rise until the end of the XXI century (2090-2099) than during 1980-1990 between 1.8°C-4.0°C, depending on the scenario for emission of greenhouse gases.



With global warming, heat stress appeared (both summer and winter), so true adapting diseases should be monitored and treated. The metabolic profile refers to evaluation of nu-triational and

meta-bolic integrity in randomly chosen animals, by perfor-ming hematological and biochemical tests [10].

Although these determinations can be helpful, both to highlight the nutritional imbalances or previous infections and for prevention of metabolic disorders by adopting a certain medical conduct.

After Ghergariu S. (1985), metabolic health is based on the body's physiological steady state, which allows it to develop and provide optimal yields without any detectable changes in the hematological and biochemical parameters which considered normal regarding the species, age, physiological status and season.

In nutritional pathology, diagnosis based on clinical manifestations is late; it is possible only when nutritional disorders have become well defined. At this stage fighting illness is difficult and complex and the measures are etiotope and symptomatic, often followed by positive results. become incurable.

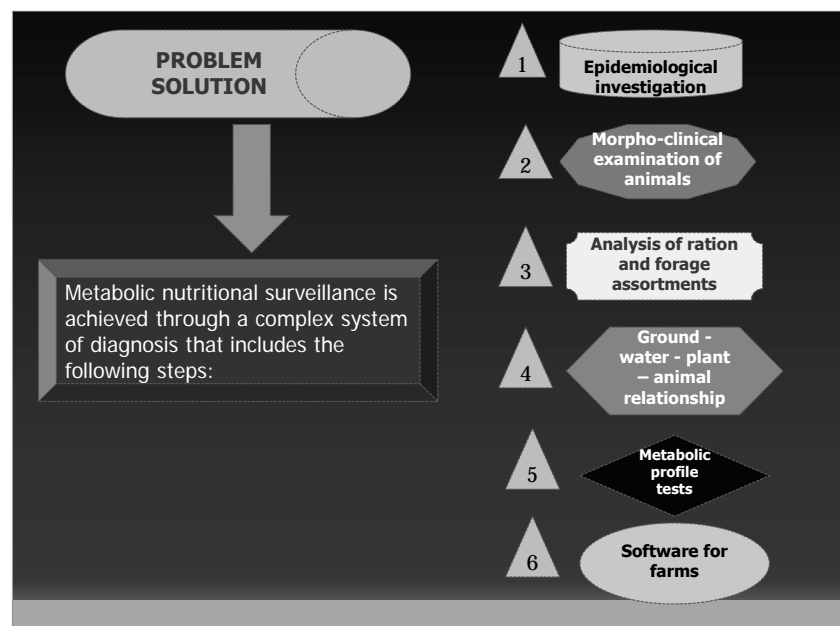
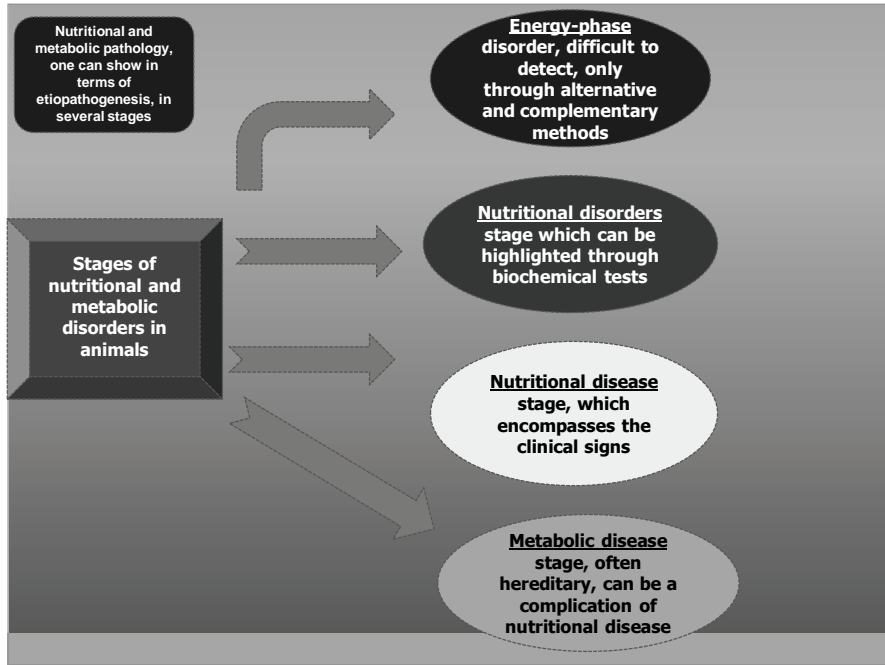
The metabolic profile is required when:

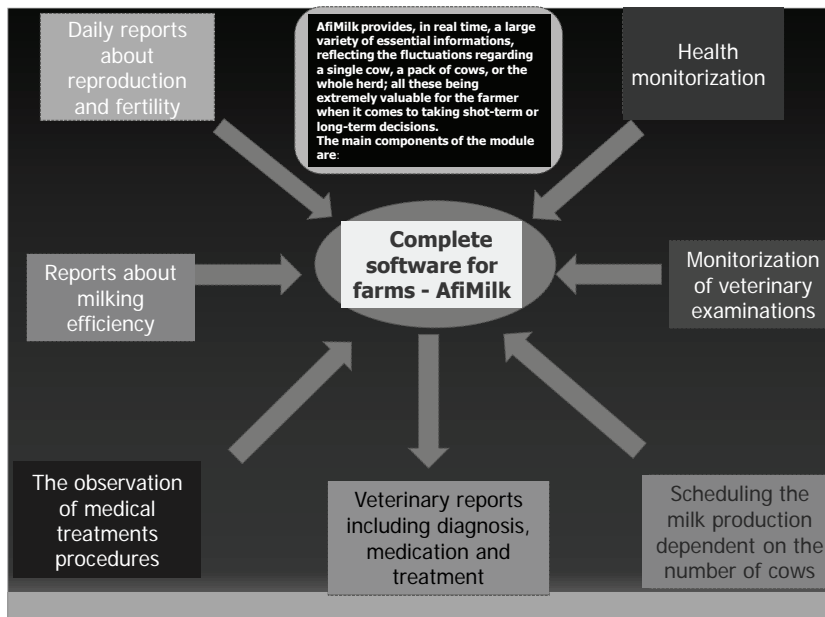
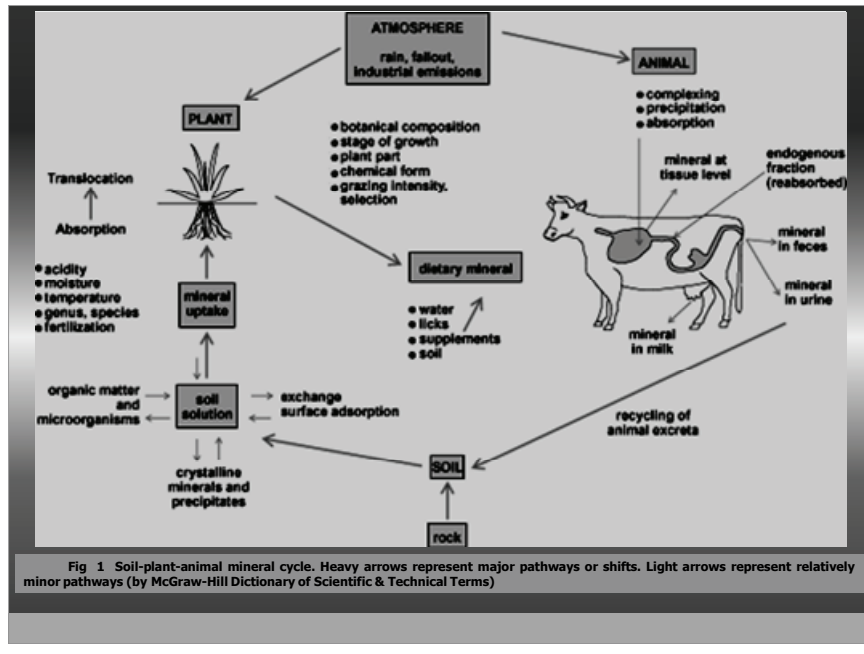
- Self diagnosis is replaced by group diagnosis;
- When nutritional disease is suspected;
- When there is a decrease in milk production and milk fat;
- When reproductive disorders multiply;
- When complex diseases with difficult etiology occur;
- For routine preventive exams.

### **Stages of nutritional and metabolic disorders in animals**

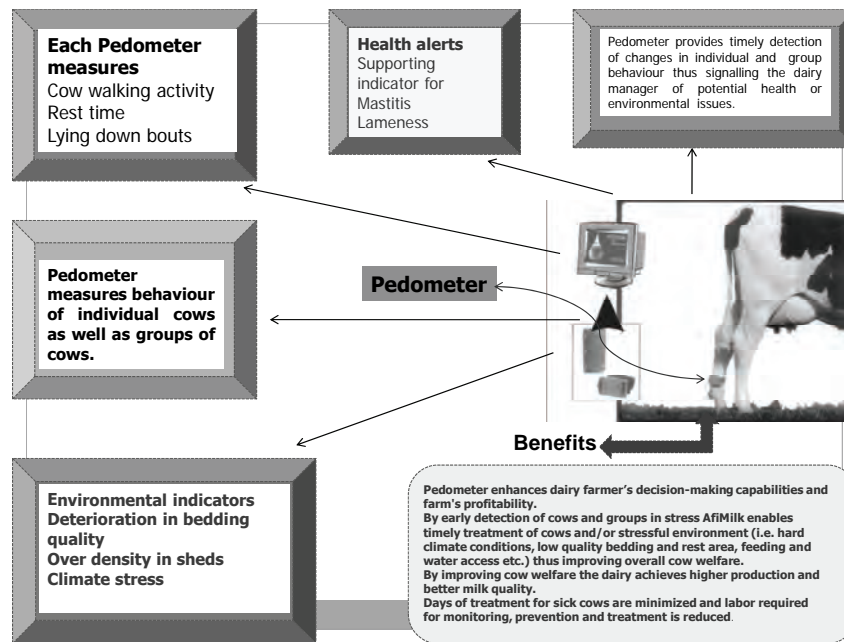
Nutritional and metabolic pathology, one can show in terms of etiopathogenesis, in several stages:

- Energy-phase disorder, difficult to detect, only through alternative and complementary methods;
- nutritional disorders stage, which can be highlighted THROUGH biochemical tests;
- nutritional disease stage, which encompasses the clinical SIGNS;
- metabolic disease stage, often hereditary, can be a complication of nutritional disease.









## CONCLUSIONS

1. Animal health is based on their welfare, a minimum of biological confort necessary for expressing natural behaviour, suitable for changes which occur in the environment. Vital space is necessary as well as daily surveillance of all the animals and constant monitoring of microclimate factors, ventilation, fodder and water consumption and prophylactic measures.

2. The necessity to replace old technology with new one. Industrial techologies will be examined trough progress in the domain of animal behaviour knowledge, ethical rules regarding animal interraction and national organizations which respect international considerations regarding animal welfare and protection and which respect the physiologic requests of animals and allow fructifing of the productive biological potential of animals in the best conditions.

3. Revising breeding technologies is imposed also by food security reasons, old technologies developing consumer mistrust for some aliments and the appearance of illness such as food poisoning, flu viruses, prionic illness, trichinosis, dioxin poisoning, etc.

4. Artificial breeding conditions does not imply ignoring the physiologic needs of animals, on the contrary, it allows us to know exactly how to avoid useless suffering and economical loss.

5. Mandatory computerization of farms along breeding flows and obtaining alimentary products, including monitoring physiological and metabolic parameters.

### Acknowledgments

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This work was cofinanced from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project POSDRU / 89 / 1.5 / S / 63258. "Postdoctoral school for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by ecosangenesys"

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## **THE ROLE AND THE PLACE OF NUTRITIONAL-METABOLIC PATHOLOGY IN RUMINANTS, IN THE CONTEXT OF WORLD FOOD CRISIS**

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**Keywords:** homeostasis, nutrients, biomass, pollution, nutritional imbalances, metabolism

### **SUMMARY**

Farm animal husbandry assures the welfare of farmers and population by obtaining superior quality products of animal origin. Achieving this goal is becoming more and more difficult, as the rapid demographic increase aggravates the world food crisis, which is more obvious with the global climate changes. Ordinarily, the doubling of the population would mean the doubling of zootechnic productions, but actually, it would take more than that, because more than half of the world's population is currently suffering from the insufficiency of food products of animal origin. In this context, the fodder assortments obtained in the corrupted environment (ecopathology) will suffer alterations which will act upon the quality of the fodder, obtained in the food chain: soil-water-fodder-animal-man. First of all, animals will have to suffer from deficit of fodder, especially from low quality fodder, which will lead, first of all, to true deficiency diseases. The last link of the food chain is man, who will be the beneficiary of this nutrition; therefore, adequate measures need to be taken to ensure food safety. The researches done now and in the future will verify the traceability of the food chain in the concept "from the farm to the fork".

### **1. INTRODUCTION**

Feeding represents the most important technological link in the management of high productive milk cows and it conditions the level, the quality, the economicity, the health of the animals and the efficiency of the exploitation. The specific of feeding high productive milk cows is given by the remarkable intensity of their metabolism and the individual productive level. Therefore, the specific digestion in ruminants and, none the less, the metabolism of nutrients from the fodder (azotated compounds, lipids, vitamins, mineral substances etc.) must be known. The selection of animals

for bigger productions leads to unstable homeostasis, even to the most insignificant stress from the environment. Exceeding the boundaries of homeostasis leads to nutritional and metabolic disorders, that are asymptomatic (not accomplishing the growth randament, the decrease of milk production and diminution of the fat percentage in milk etc), which raises problems in group diagnosing, including prevention and combating. Animal selection for certain superior productive abilities makes the animals vulnerable to certain nutritional and metabolic diseases, especially the most productive animals, at the peak of their productivity. The determinant factors of nutritional and metabolic diseases can operate independently from the organism, so that the imbalance between different components of the fodder ratio can condition inadequate proportions between classes of nutrients: the excess of one of them can determine, besides its direct harmful effect, the secondary deficit of other nutrients, or the deficiency in one or more nutrients, by interfering with their absorption [10, 11, 12, 14].

The nutritional imbalances can be caused both by altering the proper composition of the fodder plants, and by errors in formulating the rations, the undifferentiated nutrition by species, race, age, physiological state, the sudden change of fodder ratio.

There are frequent manifestations of nutritional deficit, determined by the lack of one or more nutrients, essential for the well-function of the organism or that affect the quality of the animal production. Deficient nutrition could have even cellular or humoral immunosuppressive effects, affecting the efficiency of immunoprophylactic actions in animals [10].

The concept „*nutritional-metabolic surveillance*” was imposed as a necessity after extending of the modern farming and intensive exploiting, with the benefic ensemble, but with all the evil, also. In this condition, the nosogenetic nutritional-metabolic factors can become prevalent because the excessive exploitation, uncompensated by ensuring the proper nutrition, can lead to extremely costly imbalances, both by decreasing the production and fertility, and by increasing the morbidity and even the mortality in animals. Because of these motives, the specialists need to focus upon the better knowledge of the biological and physiological needs and production particularities of each species, as well as the optimal correlation with the new technologies in farming and intensive exploitation with the environment health [17].

It can also be the issue of animal malnutrition, even in the conditions of ensuring proper quality and quantity of fodder, because of deficiencies

along the food chain, respectively soil-water-plants-animals-man [10, 11, 12].

The same malnutrition can be determined by unsuitable conditions of the environment, respectively in the case of global warming when the animals refuse the food and the water, if polluted, or if the soil that is dried, deserted, poor in minerals, is fertilized, treated with pesticides or other chemical compounds without judgment.

## **2. PROBLEM FORMULATION**

The problem of malnutrition in animals was consistent during the last century, without talking of a world food crisis, but only of an uneven repartition of fodder on the geographic areas. Many countries have been thru this stages, including during the intensive farming, but once with the technological development and the scientific progress in animal farming, there is an exact knowledge of the animals' physiological needs, that meaning exceptional economical results.

Today we are facing some globalization issues in which the striking differences between different areas are no longer existent, and, moreover, there are new elements linked to the incapacity of ensuring fodder and food resources, as a consequence of geo-climatic changes or of the effects of long term polluting of the soil, of the water, of the plants and of the animals [1, 6, 15].

It is the issue of analyzing the resources and the great ecologic challenges of contemporary world.

### **2.1. The demographyc increase and food resources**

The human resource is the only one on Earth that, besides regeneration, also has the chance of becoming, it being the only resource that operates with the spirit and the knowledge (science), that being the reason for which man is the „manager” in putting good use of other resources.

The capacity of sustaining the world's population targets ensuring the food, the energy, the water and the living space, including the conditions of physical and psychical comfort.

From the exhaustive analysis of the report resources-population-environment, the fact that this is today into a total imbalance, that the natural resources are quantitative and qualitative reduces, the population is numerically increasing and the exigency for its comfort as well, the environment is more and more degraded and the food resources are fewer

care be ascertained. As well, the requirements are needed for the animals that offer proteins needed by the men.

### **2.2. The soil**

The soil is a natural strategic unique resource and its degrading affects its main quality, fertility. Regaining the fertility of the soil requires expenses and long periods of time, sometimes even centuries and millennium. The soil is the product of synthesis of other components of the environment. In it, there are found, either direct or indirect, all the components of the geo-system, and any change of one of components will influence the physical, chemical and biological characteristics of the soil [7, 8, 9].

Soil erosion represents a determinant factor in the loss of the fertilizing capacity of soil and it is caused by a series of factors, of which: deforestations, destructions of vegetation in fires, excessive grazing, applying improper agro-technical measures, covering with industrial and household waste products, industrial pollution etc.

The fodder harvested of these soils can be low in nutritional and mineral elements, especially in selenium [11, 12].

### **2.3. The water**

Water is the element indispensable to living beings. Although the planet is occupied with three quarters water, paradoxally, today is the issue of drinking water crisis [7].

The industrialization, the implementing of technology, the economic development, the excessive chimisation of agriculture, the urbanization etc., have been greatly affected the hydrographic structures and the aquatic habitats.

The quality of water is constantly degrading, especially because of the pollutants discharged into it: agricultural fertilizers, pesticides, detergents, heavy metals, cyanides, radioactive substances, industrial chemicals that are dangerous thru themselves, but are also dangerous when drank by the living beings. For the animals, the quantities of water used for living and for production are enormous and the poor quality of water can determine severe illnesses both in animals as in humans [7, 8, 9].

### **2.4. The plants**

In the last half of the past century, the agricultural productions of the world, especially the cereal ones have increased by 3 times (The Green Revolution), increase also registered in the less developed countries that have imported top technology. Subsequent (after 1980), the financial efforts

gave no more results, the varieties of culture plants have poorly developed in the new soil and climate conditions, becoming sensible to diseases and pests, requiring huge expenses, and also economic losses because the animal populations which were heavily chemified, intoxicated the human populations.

The limiting factors of agricultural production increase refer to the influence of the climate changes, the pollution, the soil erosion, the fertilizers and the use of fossil fuels [7, 8, 11].

Furthermore, the plants incorporate everything the soil, the water and the air offer, the quality of agricultural products significantly influencing the health and productivity of animals. Actual studies reveal the direct relation between the quality of the fodder assortment and the health of the consumers.

### **2.5. The animals**

Proteins are essential for physical and psychical development of the man. The more essential aminoacids are contained by the proteins, the more nutritive value they have: the basic food of animal origin is richer in proteins than the one of vegetal origin [7, 11, 12].

Although the world production of meat has significantly increased, the need for proteins is not ensured for the entire population, especially for the people living in poor countries.

Once with the demographic explosion, it is necessary to ensure the proper feeding of the population. The physiological limits of many species of animals of economic interest have been reached, they have been stimulated by force to deposit more biomass; by artificial feeding and productive force, meat with qualitative disturbance has been obtained, that is dangerous for men health [2, 5].

The new technologies foresee the exclusion of some pollutants from the environment of animals, the prohibition of hormone and antibiotic use in therapy or as growth bio-stimulants, the restricting of using industrial residues in food and applying the environmental protective measures in regard to organic and biologic pollution by zoo-technical residues.

### **2.6. The Man**

The dynamic of population in 2050 and the consume rate of cereals and animal products recognizes the international standards for food safety; the European concept “from the farm to the fork” and “from farm to plate” [3, 4], which have strict rules imposed by FAO, must be taken into account by the year 2050.

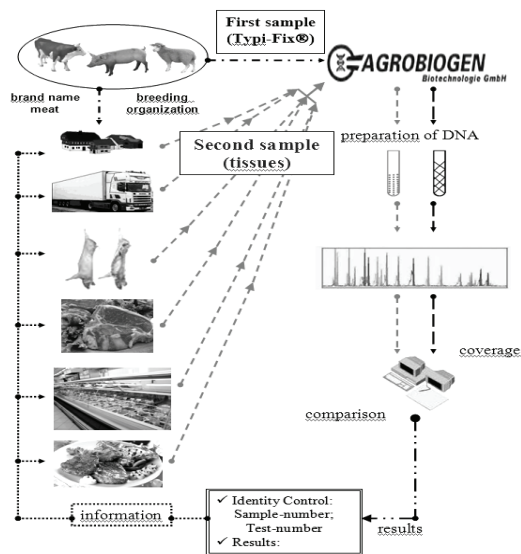


There are more branches interested with the identification of animals and their paternity, besides breeders and veterinarians. In the E.U., the current identification of animals is based on their traceability with the use of passports, earrings or procedures. A great disadvantage of this tackle is the susceptibility to fraud as the control instruments are missing. Currently, the technologies based on the DNA fingerprinting are not only 100% precise as regard to the traceability of the animals and the animal products, but also represent a powerful instrument for the animals' health and wellbeing improvement.

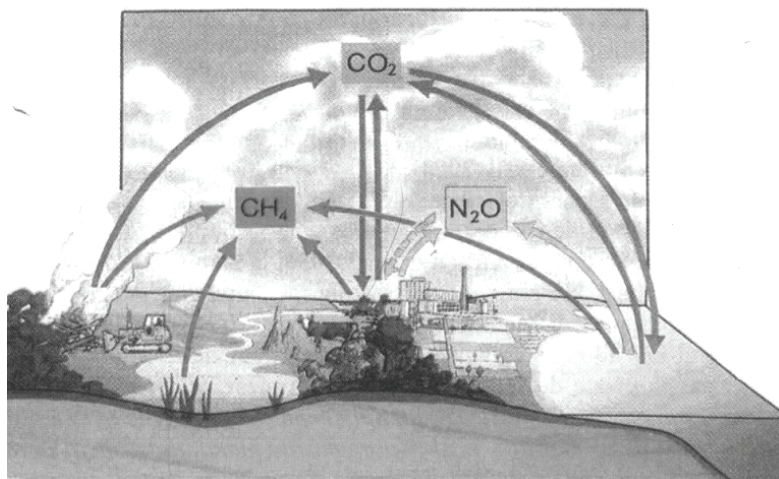
## 2. PROBLEM SOLUTION

Starting from the principle of Le Châtelier (any intervention upon a system that operates as a perturbing factor, obliges the system to self-adjust from within, so that, in time, the system balances and comes back to its initial state, or to the state most close to the climax), it can be said that once with the increase of the surface of the Planetary Ocean, and keeping in mind that the depth of the new waters is relatively small, the evaporation will intensify, also sustained by the high temperatures of air: therefore the atmospheric humidity will increase and the water vapors will accelerate the greenhouse effect. Thus, in high humidity conditions, with high temperatures and surplus of carbon dioxide in air, luxuriant vegetation will develop all over the planet, which will cause a true Green Revolution and will ensure food for consumers at all levels. Gradually, the luxuriant vegetation will absorb, by photosynthesis, the surplus of CO<sub>2</sub> from the atmosphere, which will cause graduate decrease of temperature: in these conditions, the warming of the lower atmosphere will be reduced, process that will constitute negative feed-back, existing the possibility that the cooling of the climate would become inevitable, that being a component of a cycle of changes [7].

From the decomposing of the immense quantity of biobase will result more CO<sub>2</sub> and CH<sub>4</sub>, which will recharge the terrestrial atmosphere and the climate will again become appropriate as parameters to the one before the global warming process.



**Fig. 1** The leading lab correlation with traceability of meat products through the new method of Prof. G. Brem (By A.T. Bogdam at all, published 2009 in Bulletin UASVM, nr. 66 (1-2) /2009 - Veterinary Medicine, Print ISSN 1843-5270; Electronic ISSN 1843-5386 pg. 427



**Fig. 2.** Surse Global change research in the Netherlands, 2001

The scenario presented above could take place over decades, centuries or millenniums, but one thing is certain: every beginning has an end, which becomes a new start: from here results that everything has a cyclic

evolution, that nothing is perchance and that every aggressive intervention onto a dynamically equilibrated system is followed by repercussions with feed-back effects. Some researchers have expressed other hypothesis that droughty years alternate with rainy years, that warm years alternate with cooler years and that afterwards, there is a comeback to normality.

Anyhow, the prognoses are not precise, but it is known that our planet can endure and will endure many thermal and other types of stresses, with all the consequences that follow.

As referring to the animals, it can be said that they will adapt to the new pedoclimatic conditions and will abide the nutritional and metabolic changes created in the ecopathologic conditions that will generate major changes, including appearance of new diseases or the disappearance of other. It is important that these ones to be controlled by diagnosing, monitoring and profitability actions, including technologies and biotechnologies, in a clean environment which would ensure ecosanogenesis.

#### 4. CONCLUSIONS

1. The ruminants, because of their specific digestion, are considered to be the species of the future, because they are capable to transform the poorest fodder resources into the best quality proteins.

2. The prognoses for assuring the food for the world's population are:

- optimistic (increase of food production can support 30-40 milliard of people);

- realistic (there can only be a doubling of the actual agricultural productions, conditioned by the proper ecologist exploiting of the lands and by the stabilizing of the increase of the population);

- pessimistic (the actual food production will remain constant, or it will slightly decrease because of the pollution, but the population will increase, situation which will generate a great food drama, hard to estimate, with social, political, ecological and economical implications).

3. The welfare of humankind depends on the welfare of animals. The selection of animals for certain superior productive capacities makes them vulnerable to certain nutritional and metabolic diseases, especially the best productive animals, at the maximum production ages.

4. The determinant factors of nutritional and metabolism diseases can act independently from the organism, so that the imbalance between different components of the fodder ratio can condition an improper ratio

between classes of nutrients, the excess of one can determine, among the harmful effects, the secondary deficit in other nutrients, or the lack of one or more nutrients interferes with the absorption of other nutrients.

5. The nutritional imbalances can be due to both altering of the proper composition of fodder plants, as well as to the misjudged ratios, the undifferentiated alimentation per species, race, age, physiological state, sudden change of fodder ratio.

6. Achieving the goals regarding the productivity and the quality of animal products obliges to certain corrections in the mentality of the farmers. It is imposed especially the elimination of the old mechanical routines in the favor of metaphylaxis concept (a sort of prophylaxis), for the achieving of ecosanogenesis.

7. Large productions can be obtained only with the productive health of the animals. It is imposed the periodic analysis of the fodder assortments, of the composition of the soil, the determination of the quality of water and environmental studies.

### **Acknowledgments**

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## **CORRELATIONS BETWEEN OVARIAN ACTIVITY IN EWES AND SUMMER WEATHER CONDITION IN S-E ROMANIA**

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**Keywords:** Ewe, ovarian activity, weather conditions, ovulation

### **SUMMARY**

Due to climatic conditions of S-E Romania, sheep manifest seasonal estrus, mainly in autumn and secondary in spring. By reference to literature we can say that due to summer conditions ewes are in physiological anestrus. The purpose of this research is to observe spontaneous ovarian activity in Țigăie breed along summer in southern Moldova, by morpho-functional.gynecological examination of gonads. In experiments it was found that in 46.6%, ovarian activity was recorded by identifying preovulatory follicles on the ovaries. In 13.3% of cases we identified corpus luteum during the active phase or proliferation and organization phase, this indicates the presence of ovulation. Ovary size in summer was on average 1.8 cm with a range between 1.1 and 2.5 cm. In June, cycles accompanied by ovulation reached the highest level (20%) and the values of the minimum temperature (16°C) and average temperature (21.6°C) for that period were the lowest. Also and precipitation level (2.8 mm/m<sup>2</sup>), and cloudiness (3.6) reached the highest values. However, estrous phase manifestation is reduced due to environmental conditions in summer (extraseason). But in approximately half of the number of the ewes are present unapparent sexual cycles, and some even ovulatory.

In terms of frequency and types of sexual cycles, sheeps are poliestric females (polycyclic), showing estrus cycles several times a year. Sheep manifests seasonality markedly, seasonality is dependent on climatic conditions, food, maintenance, race, degree of improvement.

Fotoperiodism is an inductor of melatonin synthesis, stimulating the function of reproduction. The heat appearance is influenced by light-dark ratio during the day (1,4). Reproductive functionality is particularly true in the autumn season, and the sheep who have not remained pregnant during this period sexual cycle may occur in spring. Under favorable conditions of feeding and microclimate some breeds can become polycyclic, meaning that heats are manifested during the entire course of the year. A good quality forage administrated during breeding season for 2-3 weeks makes the percentage of twins gestation to increase from 6-8% to 40% (2,5).

Some authors state that ovarian activity during the summer continues, but ovulation is not accompanied by heat (2, 3), which is the objective of this study.

## **1. MATERIALS AND METHODS**

Ovarian activity in Țigaie sheep breed, was observed on a total of 30 nonpregnant sheeps, at optimal age for breeding activity. Sheep were divided into three experimental groups, benefiting from the optimum conditions for maintenance and nutrition, located in a sheep farm in southern Moldova (Galați-Brăila area).

For monitoring gonadal activity, the sheep were slaughtered in three different stages, in the summer months (june, july, august).

Following slaughter the ovaries were examined from morpho-functional and obstetrical point of view: size, follicle growth, ovulation and corpus luteum formation. Data were centralized and expressed as average. The data obtained were combined with local weather activity during summer (June to August 2011). Meteorological data were taken from the National Institute of Meteorology and Hydrology (INMH), the local station in Galați.

## **2. RESULTS AND DISCUSSION**

After the examination of sheeps gonads from those three experimental groups, following data were obtained (Table 1):

In June, the average size of the ovaries from 10 sheep slaughtered was 1.8 cm (1.7 for the left ovary and 1.9 for the right ovary). The number of ovarian follicles found was three, and the proportion of ovaries with follicular activity was 30%. A number of 2 corpus luteum were identified, and the percentage of the ovaries with CL was 20%. In July, the average size of the ovaries was 1.75 cm. 40% of ovaries presented follicular activity and CL`s were found in 20% of ovaries. In August the ovaries were on average 1.8 cm, with a rate of 30% of follicular activity and CL`s have not been seen on any ovary. Ovarian follicular activity was considered present when on the surface of the ovaries were identified evolutionary follicles greater or equal to 5 mm. From this size is considered that cavitary follicles turn in preovulatory follicles, and could possibly occur ovulation and oestrous phase (fig. no.1)

Luteal activity analysis was focused on identifying the cyclic corpus luteum in proliferation and efflorescence phase and not those in involution. Monitoring the corpus luteum formation in a preset time, shows us exactly if the ovarian follicular activity was ovulatory (fig. 2).

*Table 1*

Average of ovarian constants in Țigaie ewes in summer 2011

Average month/season	Sheep no.	Ovary size (cm)		Ovarian follicles > 5 mm (no./%)		Cyclical CL's (nr/%)	
		Left ov.	Right ov.	Left ov.	Right ov.	Left ov.	Right ov.
<b>June</b>	10	1,7	1,9	1 (10%)	2 (20%)	1 (10%)	1 (10%)
<b>July</b>	10	1,8	1,7	2 (20%)	2 (20%)	0	2 (20%)
<b>august</b>	10	1,7	1,9	2 (20%)	1 (10%)	0	0
<b>Season</b>	<b>30</b>	<b>1,7</b>	<b>1,9</b>	<b>5 (16,6%)</b>	<b>5 (16,6%)</b>	<b>1 (3,3%)</b>	<b>3 (10%)</b>

Analyzing the data table 2, we observe that ovarian activity during the summer season was present at 16 from 30 ewes examined. The number of sheep that showed follicular activity was 10 and only in 4 cases was identified corpus luteum, consecutive to a ovulations.

*Table 2*

Activity of ovarian physiological formations in Țigaie ewes in summer 2011

month/season	Ewes no.	Ewes with ovarian activity		Ewes with follicles		Ewes with CL	
		nr	%	nr	%	nr	%
June	10	5	50	3	30	2	20
July	10	6	60	4	40	2	20
august	10	3	30	3	30	0	0
<b>season</b>	<b>30</b>	<b>14</b>	<b>46,6</b>	<b>10</b>	<b>33,3</b>	<b>4</b>	<b>13,3</b>

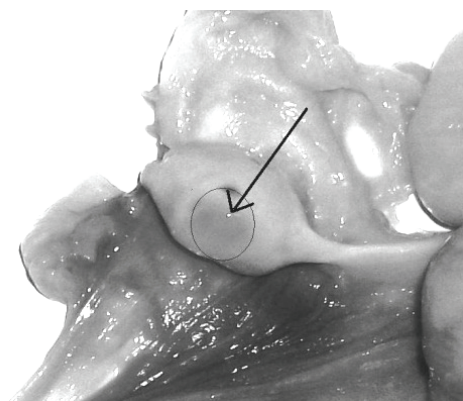
By summing the follicular and luteal activity (table no. 3), the sexual cycle activity can be seen in summer months. At 14 sheep we identified sexual cycle activity during summer, based on anatomic and physiologic changes in ovaries. Of these, 71.4 percent had follicles on ovaries and 28.6% presented corpus luteum on the surface of the ovaries.



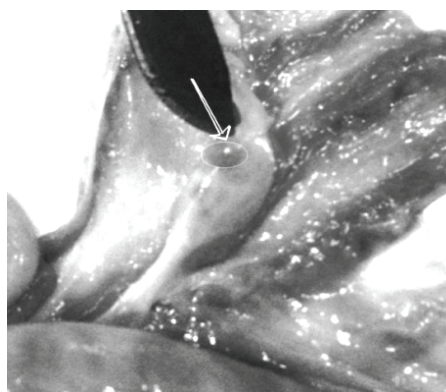
Table 3

## Summer cyclicity in Țigaie breed

month/season	No.	Ewes with ovarian follicles		Ewes with CL	
		No.	%	No.	%
June	5	3	60	2	40
July	6	4	66,6	2	33,3
august	3	3	100	0	0
<b>season</b>	<b>14</b>	<b>10</b>	<b>71,4</b>	<b>4</b>	<b>28,6</b>



**Fig. no. 1** Sheep right ovary with preovulatory follicle



**Fig. no. 2** Right ovary with corpus luteum in active phase

Meteorological data, during the study, were taken from the National Institute of Meteorology and Hydrology (INMH) Galați, for the entire Galați-Brăila area.

Qualitative assessment of meteorological activity followed the three indicators: temperature, precipitation and cloudiness.

Regarding the temperature, was monitored maximum, minimum and average temperature values, expressed in degrees Celsius. The level of precipitation is the average volume expressed in mm/m<sup>2</sup>. Cloudiness is an indicator that shows the degree of coverage of the sky with clouds, and is closely related to light intensity. Is measured in eighths of the sky and is expressed by a code from 0 to 9.

The average temperature in summer was 23.7°C, with limits between 16°C (June) and 30.8°C (July).

The level of precipitation was an average of 1.3 mm/m<sup>2</sup>, with limits from 0.3 to 2.8 mm / m<sup>2</sup>.

Cloudiness value reached a average of 2.7, and limits between 1.7 and 3.6.

Table. 4

Weather conditions during 2011 summer in Galati-Braila area

Month	T <sup>0</sup> C max	T <sup>0</sup> C min	T <sup>0</sup> C medie	Precipitation (mm/m <sup>2</sup> )	Cloudiness cod 0 - 9
June	27.3	16	21.6	2.8	3.6
July	30.8	18.3	24.5	0.3	2.9
August	29.3	17.2	23.2	0.9	1.7
Average summer season 2011	<b>29.1</b>	<b>17.1</b>	<b>23.1</b>	<b>1.3</b>	<b>2.7</b>

Climate and fotoperiodism have inductive effect on melatonin synthesis, especially in sheep, stimulating breeding function. Incentive is decreasing day length and temperature.

After we analized the data related to cyclic ovarian activity in Țigaie breed, in the south of Moldova (Galați - Brăila), coupled with meteorological and hydrological data, we see that 46.6% of the cases examined had oestrous cyclical activity at an average temperature of 23.1°C, cloudiness 2.7 and precipitation 1.3 mm/m<sup>2</sup>.

We note that under these conditions ovulatory estrous cycles occurred, identified by the presence in four cases of corpus luteum.

We note that, in June when cycles accompanied by ovulation reached the highest level (20%), the values of the minimum temperature (16°C) and average temperature (21.6°C) for that period were the lowest. Also and precipitation level (2.8 mm/m<sup>2</sup>), and cloudiness (3.6) reached the highest values in June 2011 (Table 2 and 4).

So, oestrus activity takes place in Țigaie breed in weather conditions from southern Moldova, at a value of 46.6% but ovulatory cycles are found in 13.3%.

### 3. CONCLUSIONS

3.1 In Țigaie sheep breed, increased in summer weather-climate in the south of Moldova, the activity of the sexual cycle occurs in 46.6% from the ewes capable for breeding.

3.2 Estrous phase manifestation is reduced due to environmental conditions in summer (extraseason), but in approximately half of the number of the ewes are present unapparent sexual cycles, and some even ovulatory.

3.3 Ovulation rate in Țigaie breed, in southern Moldova in summer is 13.3%.

3.4 In June, cycles accompanied by ovulation reached the highest level (20%) and the values of the minimum temperature (16°C) and average temperature (21.6°C) for that period were the lowest also and precipitation level (2.8 mm/m<sup>2</sup>), and cloudiness (3.6) reached the highest values.

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## **CORRELATIONS BETWEEN SOME SPERMATIC INDICATORS AND SUMMER CLIMATIC CONDITIONS IN ȚIGAIE BREED RAMS**

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**Keywords:** ram, ejaculate, concentration, climate

### **SUMMARY**

The rams sexual activity is influenced and controlled by a series of external and internal factors. Knowledge of seasonal dynamics, plays an important role in the production and sperm quality. Environmental factors represented by temperature, light and precipitation affect spermatogenesis, libido and sexual reflexes. Research goal is to assess sexual activity in rams of the Țigaie breed, in extraseason, by age and environmental factors (climate), from southern Moldova (Galați-Brăila area). Sperm indices monitored during the summer season were represented by volume and concentration, reported to the summer climate conditions from the south of Moldova (temperature, precipitation, degree of cloudiness). Ejaculates average volume recorded in the extraseason was 0.76 ml, with minor variations by category of age. Sexual activity and function are reduced at an average temperature of 23.1°C, cloudiness 2,7 and precipitation 1.3 mm/m<sup>2</sup>. Under these conditions, through spermatogenesis occurred a summer average concentration of 2.04 billion/ml, with a volume of 0.76 ml.

Weather and climatic factors influence the production of semen by variations in temperature and light (6). Research supports the finding that, unlike ewes, rams exhibit libido and can perform mating all year round, but the number and quality of ejaculates obtained from rams varies by month and season, autumn being the greatest (2, 7).

There is a reduction in semen quality in rams due to high temperature in summer months. The high temperature associated with long light during the day can reduce mating desire to its disappearance. Sperm quality is influenced by season (1, 5). The effect of external environmental temperature on fertility varies according to species, race, age, height. Temperature influence can be removed by selective growth. Also, meteorological factors and other factors influence the sexual behavior of breeding males and sperm quality, such as: humidity, atmospheric pressure, UV emanations (4, 7).

## 1. MATERIALS AND METHODS

Spermatogenesis function and some sperm indicators in Țigaie breed males, was carried out on breeders grouped into five age categories. They had optimal conditions for maintenance and nutrition in a sheep farm in southern Moldova (the Galati – Braila area). For monitoring gonadal activity were collected ejaculates four times a month in three summer months (June, July and August). Sperm collecting was made by using artificial vagina.

Semen volume was measured directly in the collector glass. Sperm concentration was performed using semen analyzer SPERMAQE. The data obtained were combined with local weather activity during summer (June to August 2011). Meteorological data were taken from the National Institute of Meteorology and Hydrology (INMH), the local station in Galați.

Qualitative assessment of meteorological activity followed three indicators: temperature, precipitation and cloudiness.

Regarding the temperature, was monitored maximum, minimum and average temperature values, expressed in degrees Celsius. The level of precipitation is the average volume expressed in mm/m<sup>2</sup>. Cloudiness is an indicator that shows the degree of coverage of the sky with clouds, and is closely related to light intensity. Is measured in eighths of the sky and is expressed by a code from 0 to 9.

## 2. RESULTS AND DISCUSSION

Average volume of ejaculate obtained from the rams by age category is shown in Table 1.

Ejaculate volume was assessed by direct measurement in collector glass that is graduated. Durring this occasion we found thar it varies between collections, from period to period, and is influenced by age, temperature and atmospheric humidity. In 1,5 years rams, volume ranges between 0.5 ml and 1.2 ml, during those 4 monthly collections. The summer average was 0.7 ml. In 2,5 years rams, average volume of ejaculate was 0.8 ml, with range between 0.6 ml in August and 1.0 ml in June.

Maximum annual average volume is recorded in rams aged 3.5 years, with range between 0.6 ml in August and 1.2 ml in July. As rams get older, annual volume average gradually decreases, and the average of monthly oscillations has small deviations. In 4,5 years rams, average of volume was 0.8 ml, and in 5,5 years old rams it was 0.7 ml.

Analysis of these data reveals that in Tîgaie breed rams, from southern Moldova during summer, the ejaculate volume average was 0.76 ml.

*Table 1.*

Average values of semen volume in Tîgaie rams, by age groups, during the summer

Month	Average volume of semen (ml) in rams				
	1,5 years	2,5 years	3,5 years	4,5 years	5,5 years
VI	0,9	1,0	0,9	0,9	0,9
VII	0,6	0,8	1,2	0,9	0,7
VIII	0,7	0,6	0,6	0,6	0,5
Average	<b>0,7</b>	<b>0,8</b>	<b>0,9</b>	<b>0,8</b>	<b>0,7</b>
Season average	<b>0,76</b>				

After assessing the semen obtained from rams in this study, we found that in the months of the extraseason semen was found to be rare (R) and medium (M). In Table 2 are shown average concentrations of sperms in semen, by age, in summer season.

*Table 2*

Average of sperms concentration in ram semen, by age in summer season

Month	Sperms concentration (billions/ml)				
	1,5 years	2,5 years	3,5 years	4,5 years	5,5 years
VI	2,1	2,3	2,3	1,8	2,1
VII	2,1	2,0	2,5	2,2	1,9
VIII	1,9	2,0	1,8	1,7	2,0
x	<b>2,03</b>	<b>2,1</b>	<b>2,2</b>	<b>1,9</b>	<b>2,0</b>
Season average	<b>2,04</b>				

From Table 2 we note that, sperms concentration varies from subject to subject during summer season. The average in summer season was 2.04 billions/ml. There were no large differences between the monthly average values.

Average concentration of semen in the summer season is lower than the one from the natural breeding season, when normally meet 3-4 billion/sperms/ml.

The average temperature in summer was 23.7°C, with limits between 16°C (June) and 30.8°C (July). The level of precipitation was an average of 1.3 mm/m<sup>2</sup>, with limits from 0.3 to 2.8 mm / m<sup>2</sup>. Cloudiness value reached a average of 2.7, and limits between 1.7 and 3.6.

Table 3

Weather conditions during 2011 summer in Galati-Braila area

Month	T <sup>0</sup> C max	T <sup>0</sup> C min	T <sup>0</sup> C medie	Precipitation (mm/m <sup>2</sup> )	Cloudiness cod 0 - 9
June	27.3	16	21.6	2.8	3.6
July	30.8	18.3	24.5	0.3	2.9
August	29.3	17.2	23.2	0.9	1.7
<b>Average summer season 2011</b>	<b>29.1</b>	<b>17.1</b>	<b>23.1</b>	<b>1.3</b>	<b>2.7</b>

Analyzing the monthly average values according to breeders age we can say that in all age groups the lowest concentration was recorded in August when the number of sperms/ml of ejaculate decreased to 1.88 billion/ml.

These statements are explained by the fact that the average temperature in this month has been elevated (23.2°C) and nebulosity marked the lowest value (1.7) (Table 3).

Although sperms concentration in the ejaculate depends on the specimen we note that the season has influence on this index. Long and sunny summer days not only reduce sexual reflexes but ejaculates concentration, too.

Largest volume of ejaculates was obtained in June (with an average of 0.95 ml), when summer temperature values were among the lowest (average temperature of 21.6°C, maximum 27.3°C, and minimum 16°C). Maximum values of ejaculates in June, coincide with the highest values of precipitations (2.8 mm/m<sup>2</sup>) and cloudiness (3.6). Therefore, we appreciate that the ejaculates volume in summer season is negatively influenced by increased temperature and positive by high level of precipitation and increased cloudiness.

Also, the ejaculates concentration in June was 2.14 billions/ml. It has not undergone too much from summer average of 2.04 billions/ml. Weather conditions have a more striking influence over spermatogenesis and ejaculates concentration than over their volume. Climate and has fotoperiodism induce melatonin synthesis especially in ram, stimulating the function of reproduction. Incentive is the decreasing of day length and

temperature. The analysis of data related to Țigaie breed rams, from the south of Moldova (Galați - Brăila), in conjunction with meteorological and hydrological data, reveals that activity and sexual function are reduced at an average temperature of 23.1°C, cloudiness 2,7 and precipitation 1.3 mm/m<sup>2</sup>.

We note that under these conditions, through spermatogenesis occurred a summer average concentration of 2.04 billion/ml, with a volume of 0.76 ml.

### 3. CONCLUSIONS

3.1 Țigaie breed rams from southern Moldova had a volume of 0.76 ml average to ejaculate, during summer. Volume limits ranged between 0.7 ml (at the 1.5 years old and 5.5 years old rams) and 0.9 ml (in 3.5 years old rams);

3.2 In rams of the Țigaie breed medium volume of the ejaculate is not major influenced by age (although the minimum volume limits were recorded for the categories of 1.5 and 5.5 years old rams);

3.3 Semen concentration average in summer season was 2.04 billions/ml. There were no large differences between the monthly average values;

3.4 Ejaculates concentration in June was 2.14 billions/ml. It has not undergone too much from summer average of 2.04 billions/ml. Weather conditions have a more striking influence over spermatogenesis and ejaculates concentration than over their volume;

3.5 Sexual activity and function are reduced at an average temperature of 23.1°C, cloudiness 2,7 and precipitation 1.3 mm/m<sup>2</sup>. Under these conditions, through spermatogenesis occurred a summer average concentration of 2.04 billion/ml, with a volume of 0.76 ml.

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## **PERIOSTEAL BONE HEALING CAPACITY UNDER THE LC-DCP**

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**Key Words:** LC-DCP, periosteum, fractures, healing, dog.

### **SUMMARY**

The aim of this study was to determine the influence of LC-DCP (Low Contact Dynamic Compression Plate) on the fracture healing in two different circumstances. The study was carried on clinical cases from the Surgery Department of the Faculty of Veterinary Medicine Timisoara. The healing evolution was followed by X-ray performed every 30 days. The suprapariosteal fixation of the plate accelerates the fracture healing.

The purpose of our study was to:

- assess the influence of LC-DCP type plates over the healing process of the diaphysar fractures;
- assess the influence of periosteum manipulation in relation to the fractures fixation with plates of LC-DCP type

### **1. MATERIAL AND METHODS**

The study was conducted on 16 canine patients, divided in two groups, on which, under general anesthesia (Acepromasine - Ketamine - Propofol - Isoflurane), the stabilization of the diaphyseal fractures of the long bones was achieved (Fig. 1) using an osteosynthesis plate of LC-DCP type (Low Contact - Dynamic Compressive Plate) (Fig. 2) and cortical screws - cortex screw, internal hex head with 2.7 and 3.5 diameter, self-tapping and lengths between 10 and 24 mm, depending on the individual bone conformation.

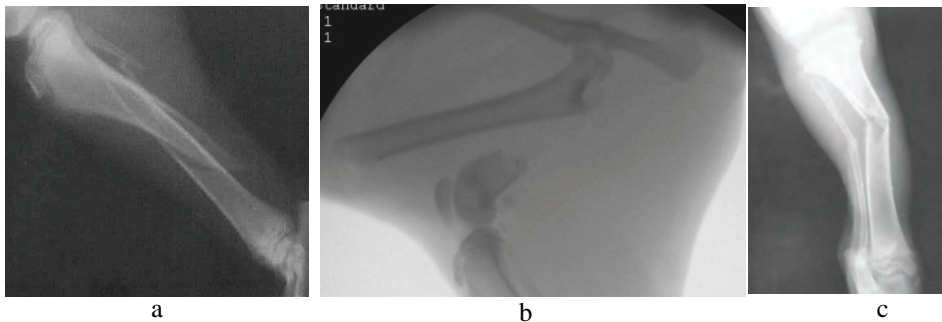
The surgical approaches of the bones (femur, tibia and humerus) were classical (5, 6, 12).

In the group 1 the periosteum was preserved in contact with the bone surface. In the group II, the periosteum was removed from the bone surface on which the LC-DCP was applied.

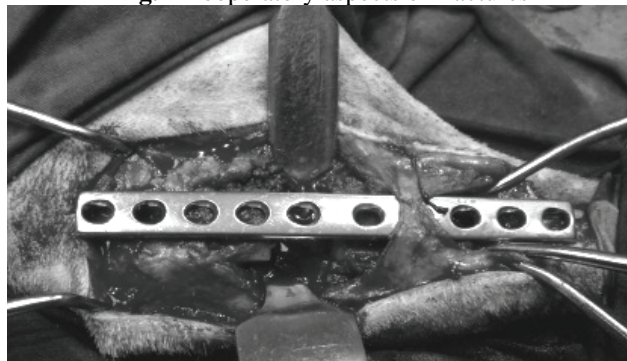
Fracture reduction was accomplished by pull on, angulated and putting head to head the bone fragments.

To stabilize the fracture and fix the plate, this was shaped on the bone surface. Muscle layer was sutured in continuous thread with resorbable material<sup>1</sup>, the skin was sutured in single interrupted sutures with nonabsorbable wires<sup>2</sup>.

X-ray examination was performed every 30 days for over 12 weeks.



**Fig.1** Preoperative aspects of fractures



**Fig. 2** Intraoperative aspects - LC-DCP fixation

*Table.1*

Clinical Characteristics of cases fixed with LC-DCP

Group	Subject	Age (years)	Weight (kg)	Fracture type
I	1	2	10	Simple, oblique, diaphyseal, middle 1/3 , femur
	2	4	17	Simple, transversal, diaphyseal distal 1/3 , tibia
	3	6	14	Simple, oblique, diaphyseal, middle 1/3 , femur, tibia
	4	3	18	Simple, transversal, diaphyseal, middle 1/3 , tibia
	5	5	15	Simple, oblique, diaphyseal, distal1/3, femur
	6	2	10	Simple, transversal, diaphyseal, middle 1/3, humerus
	7	7	21	Simple, oblique, diaphyseal, distal1/3, femur

<sup>1</sup> 3/0, 2/0 or 1/0 PDS II, PDS plus, or Vicryl - Ethicon, Johnson & Johnson Ltd.

<sup>2</sup> 2/0 or 1/0 Mersilk - Ethicon, Johnson & Johnson Ltd.

	8	4	23	Simple, oblique, diaphyseal, middle 1/3, tibia
II	1	5	11	Simple, oblique, diaphyseal, proximal 1/3, tibia
	2	2	13	Simple, transversal, diaphyseal, middle 1/3, tibia
	3	2 <sup>1/2</sup>	15	Simple, transversal, diaphyseal, middle 1/3, tibia
	4	2	17	Simple, oblique, diaphyseal, distal 1/3, femur
	5	6	24	Simple, transversal, diaphyseal, proximal 1/3, femur
	6	8	16	Simple, transversal, diaphyseal, proximal 1/3, tibia
	7	3	19	Simple, oblique, diaphyseal, middle 1/3, tibia
	8	1	14	Simple, transversal, diaphyseal, distal 1/3, humerus

## 2. RESULTS AND DISCUSSION

During hospitalization, general alteration of clinical status was not observed in any patient.

The healing of the operative wounds were achieved primarily without complications during 7 to 10 days after the operation, for 14 individuals. In two cases the occurrence of seroma was noted.

Functional recovering of the limb (Table 2) occurred between 14 and 25 days (18.5 days on average) in group I and between 14 and 30 days (22 days on average) in group II.

X-ray examination where made every 30 days (Table 3). The X-ray examination carried out 30 days after the operation, indicates the presence of callus with reduced radiodensity especially in the fracture lines areas for patient 4 from group I. At patient 6, in group II, mobilization of the first two proximal screws was observed as well as the persistence of a fracture line (Fig. 3).

X-ray examination performed at 60 days after surgery, shows a "very good" healing for 75% of the individuals in group I and 50% for those from group II.

At 90 days, on the x-ray images was observed a uniform radiodensity all over the bone surface on individuals in group I and a lower radiodensity than of the normal bone tissue in 25% of cases of group II (Fig. 4).

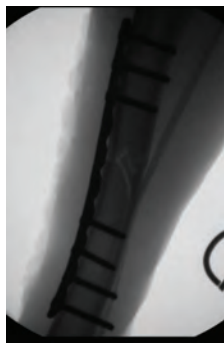
Table 2

Group	Time	1 Week (subjects)	2 Weeks (subjects)	4 weeks (subjects)	8 weeks (subjects)	12 weeks (subjects)
	Lameness					
I	0	-	3	7	8	8
	1	-	3	1	-	-
	2	5	2	-	-	-
	3	3	-	-	-	-
	4	-	-	-	-	-

II	0	-	1	6	8	8
	1	1	4	2	-	-
	2	3	3	-	-	-
	3	4	-	-	-	-
	4	-	-	-	-	-

Table 3

Group	Time Healing	30 days	60 days	90 days
		Very good	4	6
I	Good	2	1	2
	Satisfactory	2	1	-
	Poor	-	-	-
	Very good	3	4	5
II	Good	2	2	1
	Satisfactory	3	2	2
	Poor	-	-	-



a

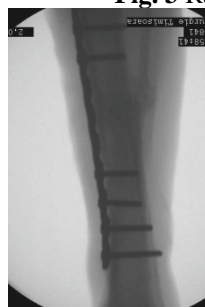
Patient 4 group I, cranio-caudal view



b

Patient 3 group II, cranio-caudal view

**Fig. 3** Radioscopy evaluation at 30 days



Patient 4 group I, cranio-caudal view



Patient 3 group II, cranio-caudal view

**Fig. 4** Radioscopy evaluation at 90 days

Shaping the LC-DCP type plates is easier than the DCP type plates, a finding reported also by other authors (1, 2, 7, 8, 9). Even more, in group I the use of LC-DCP type plates, that constructively reduce the contact with the periosteum at minimum required for an adhesion that ensure fixation and inter-fragmental compression led to shortening by about seven days of the time required for primary bone healing (compared with DCP type plates placed supraperiosteal) (10, 11).

### 3. CONCLUSIONS

3.1. Plates of LC-DCP type promotes periosteum perfusion by limited contact with the bone surface.

3.2. Strengthening of the fractures fixed with LC-DCP type plates occurs in about 58 days in cases with supraperiosteal fixed plate (group I) and over 90 days for 25% of the patients that had plates applied directly to the bone cortical (deperiosted - group II).

3.3. Primary healing of fractural defects stabilized with plates of LC-DCP type, occurs rapidly under coaptation in perfect, fast and dynamic compressive fixation conditions of the bone ends with periosteal integrity preserved intraoperatively.

### ACKNOWLEDGEMENTS

This work was supported by CNCSIS – UEFISCSU, project number PNII – IDEI 130/2008

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## OBSERVATIONSON NEMATODE INFESTATION IN TURKEYS AND GUINEA-FOWLS IN HOUSEHOLD SYSTEM

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**Key words:** turkey, guinea fowl, nematodes, *Heterakis*, *Syngamus*.

### SUMMARY

The authors followed the evolution of parasitic nematode infestations in turkeys and guinea fowls in household system in Ialomița and Giurgiu counties and some morphological and histopathological features in infestation with *Heterakis* sp. The turkeys were identified with *Syngamus trachea* infestations (63.6%), *Heterakis* sp. (40.9%), *Ascaridia dissimilis* (26%) and the guinea fowls with *Heterakis gallinae* (51.4%) *Ascaridia* sp. (33.8%), *Capillaria* sp. (32.3%); *Syngamus trachea* (25.0%).

Current food consumption trends focused upon poultry farms where meat is coming from rearing and feeding conditions close to the biological requirements of birds in recent years make all private breeders more concerned with raising turkeys and guinea fowls in their farms for meat and eggs production.

It is known that the guinea fowl egg has a protein content of 13.5% and the vitamin content is 2-3 times higher. Guinea fowl meat has higher nutritional value than beef or chicken because it is richer in protein. Contains the lowest amount of fat, respectively 2.7% in the pulp and 1.1% in the pectoral muscles (Barbat I., 2001).

Traditional growth conditions impose poultry exposure to contamination by eating the intermediate hosts or interlacing (earthworms, insects, insect larvae) and by eating food or water contaminated with eggs or larvae. This creates a specific profile of nematode infestation, dominated by syngamosis and followed by heterakiosis and ascaridiosis.

The study compared results obtained from turkeys followed in other geographical areas of the country-Cluj, Bistrita-Nasaud and Arges where infestation with *Capillaria* spp., *Trichostrongylus tenuis* and *Ascaridia/Heterakis* dominated (Magadaș C., 2005).

In the case of guinea fowl were taken as benchmarks studies from African countries (Nigeria, Zimbabwe), where the growth of these birds is much more extensive and climatic conditions are favorable. According to these studies *Ascaridia* infestations occurred in 34% of birds with clinical expression in young individuals accompanied by severe symptoms and significant mortality (Ayeni J.S. et al.).

## 1. MATERIALS AND METHODS

The study was conducted between March 2009 - June 2011, in Giurgiu county – Stanesti village and Ialomita county - Moldoveni village, being examined birds from five householders in the village.

The total number was 106 guinea fowls of which 86 individuals aged 10-18 months and 20 individuals aged 3 weeks and 130 turkeys of which 96 individuals aged 5-6 months and 34 individuals aged 3-4 weeks.

Coproparasitological examination was performed through qualitative methods- Willis amended by Lungu and quantitative - McMaster. Monthly fecal examination was intended to capture the influence of climatic factors on eggs or intermediate hosts.

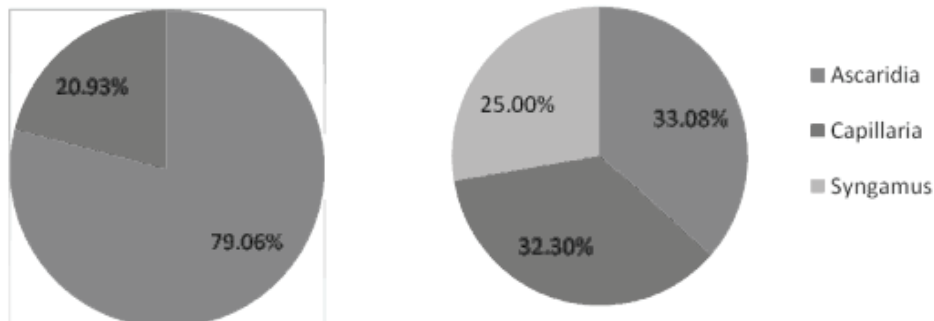
In heavy infestations or clinical, birds were slaughtered and morphopathological examination was performed upon the digestive tract, followed by histopathological examination after sampling the specific lesions, fixed in neutral formalin saline and further processed for inclusion in paraffin. Paraffin blocks were sectioned at 6 microns, stained preparations were obtained by the trichromatic method of Mallory, examined and microphotographed.

## 2. REZULTATE ȘI DISCUȚII

Following coproscopic examination in guinea fowls, infestations were identified in 88 individuals (79.07%) and 18 guinea fowls (20.93%) were negative.

Among these, infestations with *Ascaridia sp.* were identified in 23 guinea fowl (33.8%), with *Heterakis gallinae* in 35 individuals (51.4%), with *Capillaria sp.* 32.3% in 22 guinea fowl, with *Syngamus trachea* 17 (25.0%) (fig. 1). Poor infestations with *Eimeria numidae* have been identified in 33 individuals.



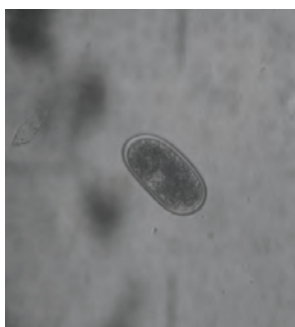


**Fig. 1** – The incidence of nematode infestation in guinea fowls

The identification of nematodes types was based on the specific morphology of eggs, aiming to differentiate comparative *Ascaridia/Heterakis* (aspect of the lateral) and *Capillaria/Syngamus* (plug appearance, structure mass germination) (fig. 2, 3, 4).

The degree of infestation had values of 50-250 OPG for *Ascaridia*, 100-350 OPG for *Heterakis*, *Capillaria* 50-350 and 50-250 for OPG OPG for *Syngamus trachea*.

Out of 68 guinea fowls diagnosed with parasitic infestations 18 individuals were identified with a single species infestations (26.4%); with 2 species in 40 individuals (58.8%); with 3 species in 8 individuals (11.7%), with 4 species in 2 individuals (2.94%).



**Fig. 2** - *Heterakis* sp. - egg



**Fig. 3** - *Syngamus trachea* - egg



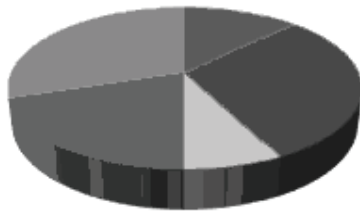
**Fig. 4** - *Ascaridia* sp. - egg

88% of all turkeys had parasitic infestations and only 12% were negative. For these infestations with nematodes were identified as *Syngamus trachea* 63.6%, *Heterakis* sp. 40.9%, 26% *Ascaridia dissimilis*. With one species of nematode infestations were identified 68.1% individuals and 31.8% with two species.

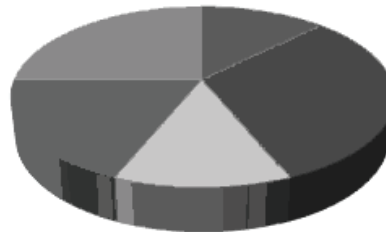
Aiming at progress against parasitic infestations in turkeys from 5 households where parasitological examinations were performed, the results indicated *Tracheea syngamus* infestation prevalence (45-60%), but also a high incidence of phages lice and ectoparasites.

In **household number 1** from the total number of 14 individuals, 2 individuals were clean and 12 individuals were infested as it follows: *Ascaridia dissimilis* - 25%; *Syngamus tracheea* - 60%; *Heterakis* sp.- 15%; lice phages - 40%; ticks - 60% (fig. 5).

In **household number 2** from the total of 21 individuals, 2 individuals were clean and 19 were identified with parasitic infestations as it follows: *Ascaridia dissimilis* - 20%; *Syngamus tracheea* - 50%; *Heterakis* sp. - 20%; phages lice- 30%; ticks - 40% (fig. 6).



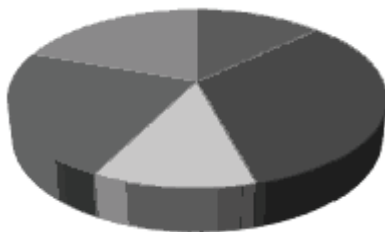
**Fig. 5** – The incidence of parasitic infestation in household 1



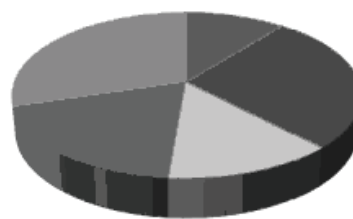
**Fig. 6** – The incidence of parasitic infestation in household 2

In **household number 3** from the total of 35 individuals, 3 individuals were clean and 32 individuals had parasitic infestations as it follows: *Ascaridia dissimilis* - 25%; *Syngamus tracheea* - 50%; *Heterakis* sp. - 18%; phages lice - 35%; ticks - 30% (fig. 7).

In **household number 4** from the total household of 30 individuals, 3 individuals were clean and 27 were identified with parasitic infestations as it follows: *Ascaridia dissimilis* - 20%; *Syngamus tracheea* - 50%; *Heterakis* sp. - 25%; phages lice - 35%, ticks - 55% (fig. 8).

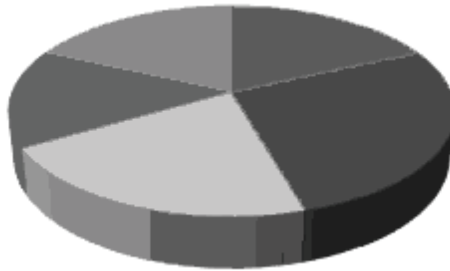


**Fig. 7** – The incidence of parasitic infestation in household 3



**Fig. 8** – The incidence of parasitic infestations in household 4

In **household number 5** from the total of 30 individuals, 4 individuals and 26 individuals had parasitic infestations as it follows: *Ascaridia dissimilis* - 30%; *Syngamus trachea* - 45%; *Heterakis sp.* - 35%; phages lice - 25%; ticks - 30% (fig. 9).

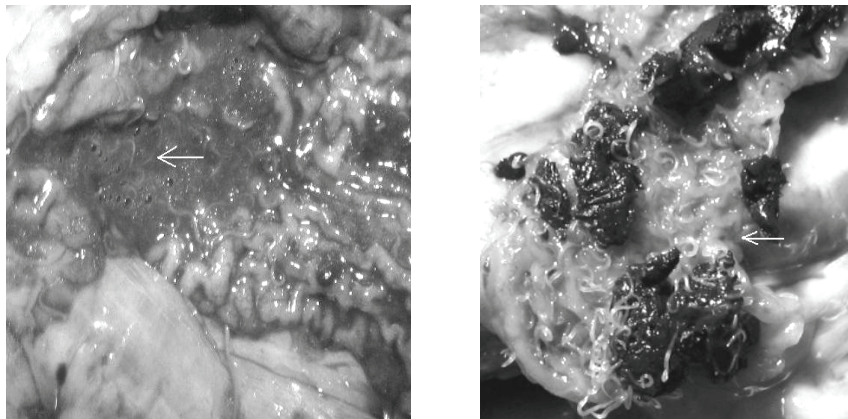


**Fig. 9** – The incidence of parasitic infestation in household 5

Necropsy examination performed in a total of three adult guinea infested with nematode species, slaughtered at the end of the experiment revealed the presence of *Heterakis gallinae* massive infestations after cutting the intestinal caecums.

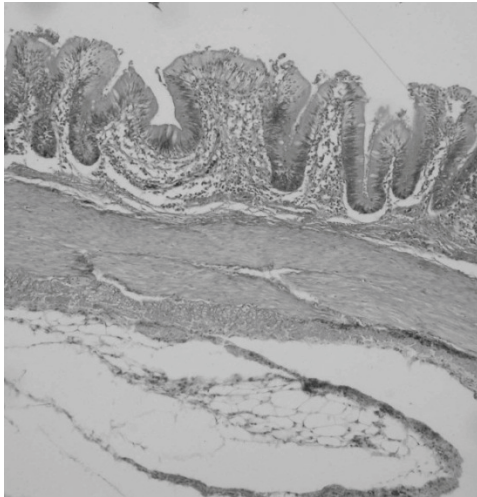
Infestation was accompanied by typhoid catarrhal, areas of necrosis and ulceration at epithelial level caused by the presence and action of pathogenic products of *Heterakis gallinae* specimens (fig. 10).

*Heterakis gallinae* specimens taken from the intestinal lumen of the caecums were subjected to lactofenol clarification process to highlight specific morphological features in order to identify the species.

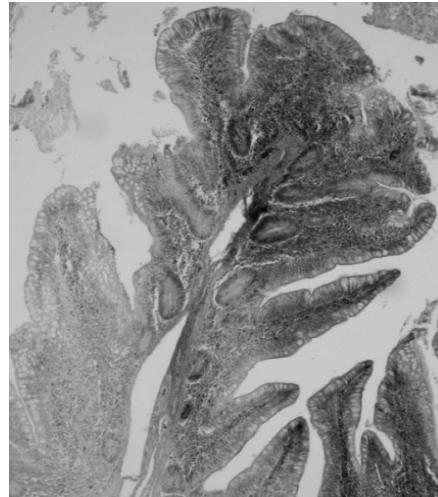


**Fig. 10** - Massive infestation with *Heterakis* in the cecal lumen

In histological sections made from fragments of the guinea fowl intestinal caecums infested with *Heterakis gallinae* and killed at the end of the experiment have revealed the following injuries: typhoid catarrh, hemorrhagic typhoid, fatal necrotic epithelial denudation accompanied by limphohistiocytic infiltration in the chorionic lining (fig. 11, 12).

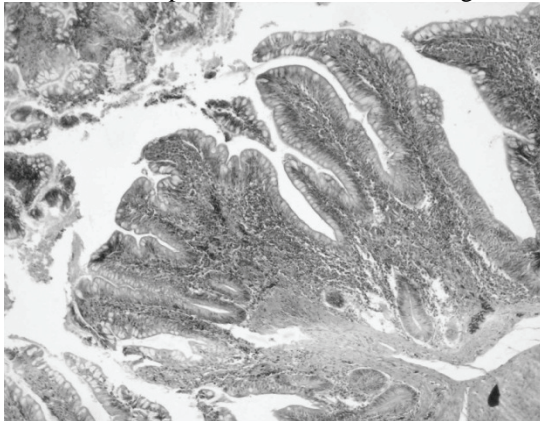


**Fig. 11** - Typhoid intestinal bleeding with epithelial denudation; Col. Mallory trichromatic, Ob 10x

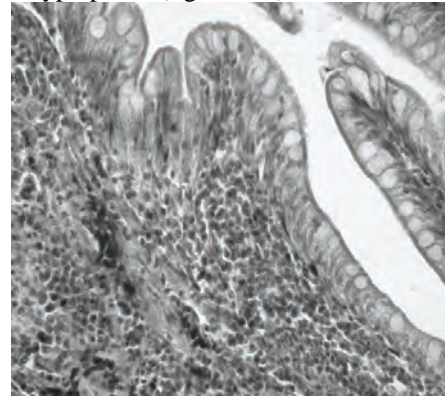


**Fig. 12** - Typhoid ulcers and lymphohistiocytic infiltration in the intestinal lining; Col. Mallory, Ob 20x

The intestinal epithelium is found mucous goblet cell hyperplasia (fig. 13, 14).



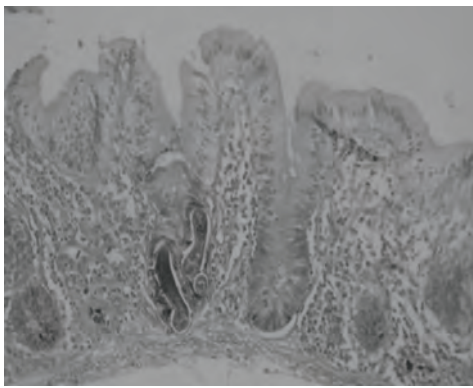
**Fig. 13** - Hypertrophy and hyperplasia of mucous goblet cells; Col. Mallory trichromatic; Ob 20x



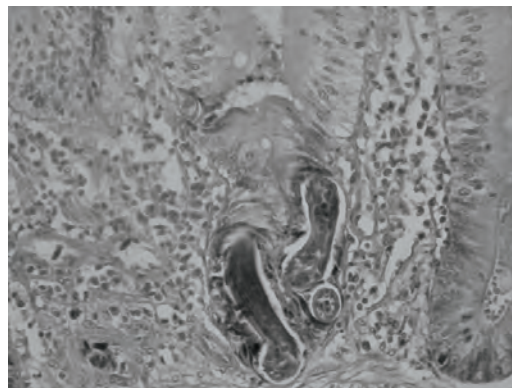
**Fig. 14** - Hypertrophy and hyperplasia of mucous goblet cells; Col. Mallory trichromatic; Ob 40x

Bleeding and limphohistiocytic infiltration in chorionic intestinal villi are accompanied by the presence of 3 *Heterakis larvae* found in sagittal and transverse sections in enteroparietal migration.

It is known that *Heterakis gallinae* larvae evolutionary cycle has an enteroparietal migration that lasts for 10-14 days, during which evolves through two successive processes and become L<sub>4</sub> moult, leaving the intestinal wall back in the lumen where they develop into L<sub>5</sub> and stage adult. Intestinal villi at the mucosal epithelium is found mucosal hypertrophy and hyperplasia of goblet (fig. 15, 16).



**Fig. 15** - Larvae of *Heterakis* in chorionic lining of ceca; Col. Mallory trichromatic; Ob 20x



**Fig. 16** - Larvae of *Heterakis* in chorionic lining of ceca; Col. Mallory trichromatic; Ob 40x

There is an limphohistiocytic infiltration in the chorionic lining as a response of the host to the presence of larvae in the cecal wall and to the pathogenicity of adult parasites located in the lumen of the digestive segment affected.

### 3. CONCLUSIONS

3.1. Parasitic nematode infestations identified in turkeys were *Syngamus tracheea* 63.6%, *Heterakis sp.* 40.9%, 26% *Ascaridia dissimilis*. One species of nematode infestations were identified in 68.1% and two species in 31.8% of individuals.

3.2. Parasitic nematode infestations identified in guinea fowls were *Ascaridia* 33.8%, *Heterakis gallinae* 51.4%, *Capillaria sp.* 32.3% and 25.0% *Syngamus tracheea*. The degree of infestation had values of 50-250 OPG for *Ascaridia*, of 100-350 OPG for *Heterakis*, of 50-350 OPG for *Capillaria* and of 50-250 OPG for *Syngamus tracheea*.

3.3. In histological sections made from fragments of intestinal caecums taken from guinea fowl killed at the end of the experiment, the following injuries could be noticed: typhoid catarrh, hemorrhagic typhoid, necrotic epithelial denudation accompanied by lymphohistiocytic infiltration of the chorionic lining of the caecums.

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## **THE RELEVANCE OF THE ULTRASOUND INVESTIGATIONS OF THE ABDOMINAL CAVITY IN FOALS**

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**Key words:** ultrasonography, young horses, abdominal cavity

### **SUMMARY**

Abdominal ultrasound allows evaluation of the abdominal cavity organs, vascular structures, diaphragm, abdominal formations, peritoneal effusion, and possible topographical changes. The study was conducted on 16 foals (equine youth aged 2 weeks to 6 months) with various diseases which interested the abdominal cavity organs/structures.

The accuracy and specificity for the gastrointestinal parietal changes and accumulations in peritoneal space is recommended that an imaging technique with high relevance, easy and non invasive.

Ultrasound examination structures aiming to evaluate the abdominal cavity: parenchimatous organs, cavitary organs, vascular structures, diaphragm, peritoneal accumulation, changes in tone, motility and possible topographical changes of these cavitary organs (2, 7).

### **1. MATERIAL AND METHODS**

The study was performed on foals (n = 16) with different ages (between 2 weeks and 6 months), with various problems that interested organs/structures from the abdominal cavity, especially in cavitary organs: stomach, intestine, bladder (n = 11) and accumulation in the peritoneal space (n = 5).

Ultrasound examinations were performed with portable ultrasound (Mindray and Esaote Pie Medical) with linear and convex probes with different frequencies between 5 and 10 MHz.

In the diagnosis of the cavitary organs` (stomach, small and large intestine, and urinary bladder) diseases, correlated with obvious clinical signs, the ultrasound examination can easily confirm the clinical hypothesis of the clinical suspected affections (3).

The ultrasound examination of the cavitary organs was performed using linear or convex probes, of normal or high frequency (5, 6; 6,5; 7,5; 8 or 10 MHz), obtaining valuable information about their wall structure and thickness (normal 3-5 mm). The ultrasound evaluations were performed using different probes (according to the age of the foal). The clinical changes were registered and correlated with the results of the paraclinical investigations, especially with the ultrasound investigation results.

In such cases, for ensuring a maximum accuracy of the ultrasound investigations we've followed and respected strictly the steps for obtaining the most relevant images, without or with minimum artifacts images, in order to diminish the relevance of the obtained ultrasound images of the cavitory organs (4, 6).

The main causes of the artifacts inducing images are in most cases attributed to the gas accumulation, the lack of content (in case of urinary bladder evaluation).

On behalf of the tendency of maximum reducing of the improper artifactual situations, was very important to recommend prior the ultrasound examination the food diet and the proper oral fluid intake (2, 9).

## **2. RESULTS AND DISCUSSIONS**

The most important objective of our study it was to achieve and to correlate the clinical expression of the abdominal cavity and cavitory organs` diseases with the ultrasound changes, in order to find strictly correspondences between these, in confirming the diagnosis.

The normal patterns of the stomach and intestine can be easily appreciated and revealed in the ultrasonographyc image, with the compounds of the wall architecture (Figure 1 and Figure 2).

After the clinical and ultrasound investigation of the cavitory organs (stomach, small and large intestine and urinary bladder), the most easily identified and confirmed were the diseases with high degree of specificity, as the inflammatory changes, dominated by the uniform thickening of the wall, especially of the superficial components and the maintaining of the parietal tonus (in acute inflammations)/or diminishing the parietal tonus (for the chronic inflammations).

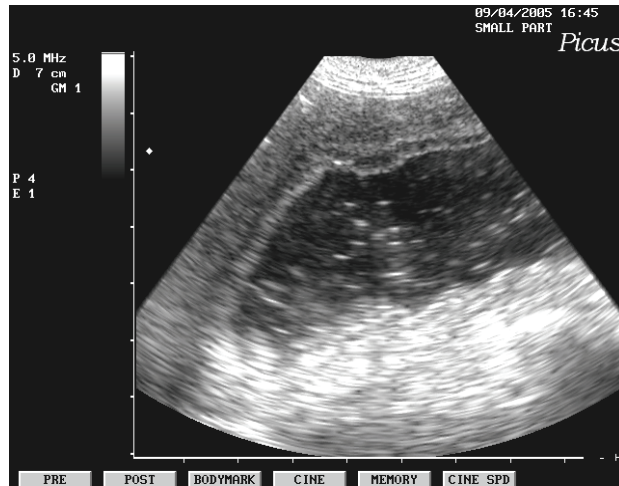
From ultrasonographyc point of the view, the most important and facile too is to identify the changes of the parietal architecture and wall thickening, specific for each cavitory organ.

In this frame, for the investigated cavitory organs, the stasis of the content can be specific clinical correlated with the ultrasound changes, as in gastric or intestinal inflammations (Figure 3, 4, 5 and 6), with obvious digestive specific simptomatology, in accordance with the degree of parietal injuries (1, 5).

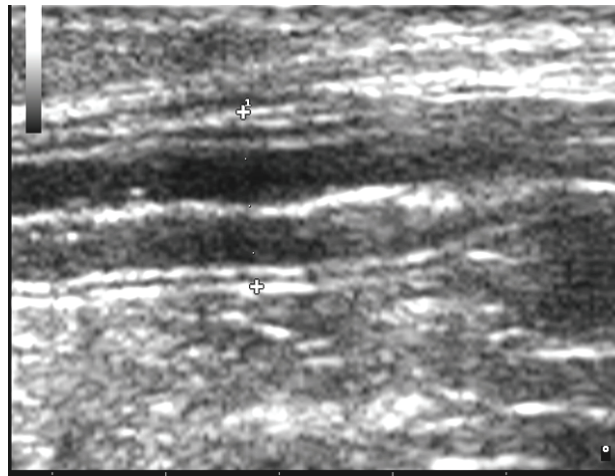
In case of urinary bladder, the distension is more easily observed by ultrasound scanning, less correlated with the clinical signs, with minimum influence to the layers architecture or thickness, but any changes in normal



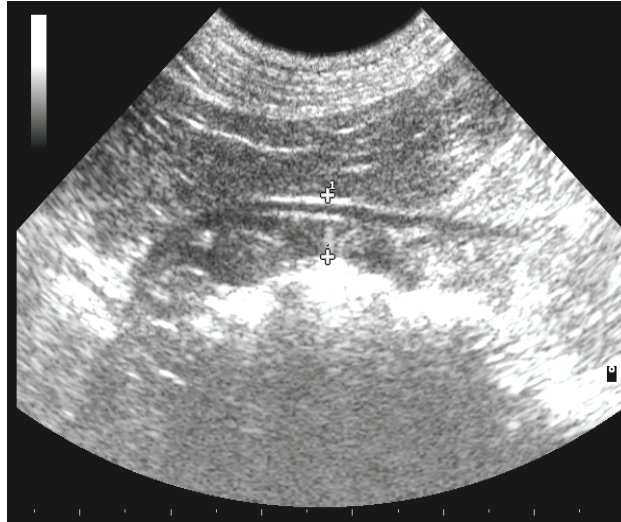
anechoic content of the normal bladders can be observed and evaluate (Figure 7 and Figure 8).



**Fig. 1.** The normal ultrasonographic aspect of the stomach. Can be easily identified the parietal components, the type and the aspect of the gastric content.



**Fig. 2.** The normal view of the parietal compounds of the intestinal wall of the small intestine – (lumen, mucosa, submucosa, muscularis and serosal compound).



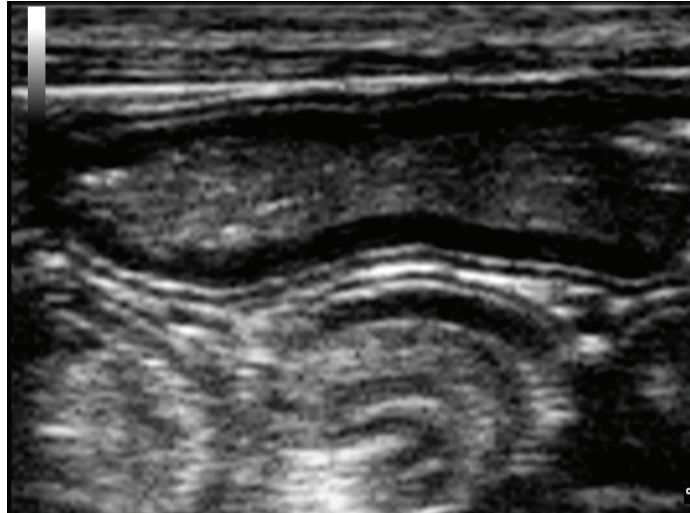
**Fig. 3.** Stomach with an obvious thickening of the wall - gastritis (reaction of the superficial compound), with keeping unaltered the parietal architecture.



**Fig. 4.** Acute gastritis in a 3,5 months foal. Obvious parietal reaction (uniforme thickening) with important reaction of parietal affected components (hypertrophy of gastric mucosa).

From clinical point of view, in most cases the quantity changes of the cavitory organs indicates alterations of the morphology and functions of these (especially parietal injuries). Changes in the quality of the content of

these cavitory organs (stomach, intestine, urinary bladder) are usually suggestive regarding the type of the registered morphologic alterations (10).



**Fig. 5.** Acute enteritis (young horse, 4 months, female). Aspect of intestinal stasis (distended by hypoechoic content – with visible lumen) and important parietal reaction (uniform thickening of the wall)

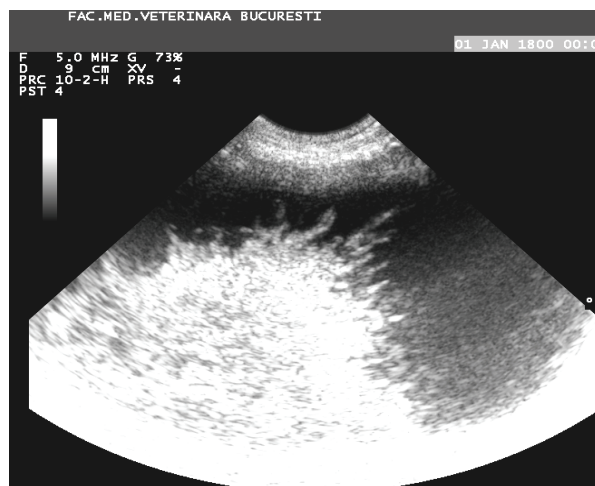


**Fig. 6.** Characteristic aspect of acute enteritis in a foal (7 weeks, half-breed, male). Intestinal stasis (intestin distended by hypoechoic content – with highlighting of the lumen) and important parietal reaction (uniform thickening).

Peritoneal fluids can be easily identified and can appreciate: the quantity, nature, echogenicity, homogeneity, particles in suspension. Ultrasound technique is extremely useful in achieving abdominal puncture. With the ultrasound technique can be assessed the amount of free liquid peritoneal space and its character (Figure 7 and Figure 8).



**Fig. 7.** Ascitic fluid (anechoic aspect) with important cellularity (numerous corpuscular elements in suspension).



**Fig. 8.** Severe peritoneal effusion, with particles in suspension – Vortex aspect (tornado) of the corpuscular elements in suspension (uroperitoneum).

### 3. CONCLUSIONS

The studies described in this paper were aimed for highlighting the main quantification and corroboration of clinical matters and ultrasound (ultrasound) changes in some internal diseases, in order to assess the relevance of ultrasound technique in the diagnosis of organs `diseases and/or systemic diseases in foals. The analysis of the results obtained correlated with those described in the literature allowed the separation following conclusions:

3.1. Ultrasound examination of abdomen generally allows identification and evaluation of the abdominal cavity organs, vascular structures, formations of intra-abdominal free fluid in the peritoneal space and / or at the hollow, and any topographic changes.

3.2. Due to peculiarities of sonography, peritoneal fluid can be identified most easily (because of transonic - being anechoic), considering the quantity, nature, echogenicity, homogeneity, presence or absence of particles (corpuseular elements) in suspension, fibrin or hematoma.

3.3. In the cavitory organs (stomach, intestine, and bladder) can be assessed the constituents' parietal (parietal components), parietal thickness, retention/loss of architecture specific parietal and parietal any changes.

3.4. At gastric, intestinal and bladder easiest and specificity are valued highly inflammatory changes (characterized by uniform thickening of the parietal type), with parietal tonus and keeping the interest components superficial reaction.

3.5. Quantitative changes/stagnation/stasis contents (gastric, intestinal, urinary retention, respectively) reveal a lesional substrate (responsible for these functional disorders) that can be easily evaluated by ultrasound.

3.6. The presence of gas in the gut (cecum, colon) can significantly limit the evaluation of these organs, and because of artifacts induced, prevents assessment of adjacent structures.

3.7. Non-invasive nature, high degree of specificity and accuracy of results obtained from carrying out ultrasound, recommended this technique in the diagnosis of parenchymatous organs diseases and/or cavitory organs - in young horses.

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## **RESEARCH ON THE DYNAMICS OF HAEMATOLOGICAL INDEXES CAUSED BY STREPTOZOTOCIN EXPERIMENTAL DIABETES IN RATS**

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**Key words:** hematology, streptozotocin, rats

### **SUMMARY**

Blood results are presented, obtained from experimental challenge with streptozotocin diabetes in male rats, Wistar breed. The experiment lasted 7 months, was made on 5 groups of animals, and we followed the dynamics of leukocytes, lymphocytes, hemoglobin, hematocrit and platelets.

For treatment, were used Eridiarom and Diavit phyto-therapeutic, original products.

Monthly data obtained are compared with a healthy control group and an untreated patient group.

Induction of experimental diabetes in rats, tracking sick animals for a long time, treatment with phyto-therapeutic original products and the results are another approach to diabetes, as a curable disease.

### **1. MATERIAL AND METHODS**

The investigations were made on 63 male rats, Wistar breed, aged 2 months, elected as class (weight 150 g), age and size should be as uniform as possible. At first we randomized took seven heads, and we formed the first batch:

Group 1 - 7 heads - healthy controls.

Other animals were intra-peritoneal injected with streptozotocin, 4mg/100 g body weight. The animals were monitored for 3 days, then we formed the experimental groups as follows:

Group 2 - consisting of 14 animals - control group, diseased and untreated.

Group 3 - consisting of 14 animals - group treated daily with one tablet of SIOFOR 1000, equivalent to 12 mg/kg and 4 g of lactose to correct taste.

Group 4 - consisting of 14 animals - group treated daily with 5,4 g ERIDIAROM/ batch.

Group 5 - consisting of 14 animals - treated daily with DIAVIT, 6 g/ batch.

Housing, feeding and drinking water of animals were the same (standard), also microclimate conditions. The investigations were made during 2007-2008.

After the first, 2nd, 3rd, 5th and 7th experimental month, blood samples were collected (from each 7-8 animals/group) for serological and biochemical tests. Individual samples were taken from internal angle of the eye, were collected in special containers, kept on ice and transported within an hour at the biochemical research laboratory.

All tests were made using specific enzymatic methods, colorimetric, samples were processed by KONELAB automatic analyzer, biochemical and immunological.

## 2. RESULTS AND DISCUSSION

**White Blood Cells (WBC):** The sick and the untreated group, is the largest, averaging 9.76 ( $10^3/\mu\text{l}$ ), closely follows the group treated with 7.58 SIOFA ( $10^3/\mu\text{l}$ ), the control groups, treated with Eridiarom and Diavit have values close to 6.52 and up to 7.0 ( $10^3/\mu\text{l}$ ).

At 2 months of treatment, the values are almost identical for all groups, with the first month of treatment. Higher values of 9.7 ( $10^3/\mu\text{l}$ ) occur in the untreated patient group, followed by the group treated with SIOFA - 8.4 ( $10^3/\mu\text{l}$ ), other groups (healthy controls, and treated with Eridiarom, record values of 7, 23 ( $10^3/\mu\text{l}$ ) respectively 7.3 ( $10^3/\mu\text{l}$ ) and the group treated with Diavit, 6.83 ( $10^3/\mu\text{l}$ ).

Table 1

Average values of leukocytes in the seven months, in the studied groups ( $10^3/\mu\text{l}$ )

Month	No. Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
1	6	4,1 - 10,9	7	9,75	7,58	6,52	6,82
2	5	4,1 - 10,9	7,23	9,7	8,4	7,3	6,83
3	7	2,1 - 19,5	10,21	15,34		8,25	7,32



5	6	2,1 – 19,5	10,2	13,67		7,93	6,7
7	6	2,1 – 19,5	11,91	12,76		8,5	7,89

At 3 months, mean values of leukocytes increased significantly in untreated patient group, but the group treated with Eridiarom and Diavit, decrease slightly, reaching below the healthy control.

At 5 months of treatment in healthy control group, the dynamics of leukocytes is 10.2 ( $10^3/\mu l$ ), but increases over the untreated patient group.

At 7 months of treatment they get to 12.76 ( $10^3/\mu l$ ) in untreated patient group, and decreases in the group treated with Eridiarom and with Diavit, below the average for the healthy group.

**Lymphocytes:** After 2 months of treatment, mean lymphocytes in healthy control group is 5.83 ( $10^3/\mu l$ ), increases for the untreated patient group, and keep almost the same values in groups treated with Siofor and Diavit, and smaller in the group treated with Eridiarom.

After 3 months, for the healthy control group the values are maintained at 6.71 ( $10^3/\mu l$ ), but greatly reduced for the untreated patient group and for the group treated with Eridiarom. Somewhat higher values 5.38 ( $10^3/\mu l$ ), are for the group treated with Diavit. In all treated groups, the values are below the average of healthy controls.

At 7 months of treatment, we observe a marked decrease in lymphocytes from healthy control group and the untreated patient group, and a very significant increase in the group treated with Diavit.

*Table 2*

Average values of lymphocytes, the groups studied for 7 months ( $10^3/\mu l$ )

Month	No Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
2	5	2 – 14,1	5.83	7.56	5.93	5,46	5,93
3	7	2 – 14,1	6,71	3,5		5,81	5,38
5	6	2 – 14,1	5,57	3,36		6,2	6,19
7	6	2 – 14,1	2,96	3,33		7,42	10,94

**Hemoglobin:** After 2 months of treatment, differences remain, meaning that hemoglobin increase in the group treated with Eridiarom.

After 3 months of treatment, the differences are increasing because the group treated with Diavit reach above the maximum, normal limits.

At 7 months of treatment, the differences are large. Hemoglobin reaches very high, the group treated with Diavit, followed by the group treated with Eridiarom. The lowest value is recorded in the untreated patient group.

Table 3

Average values of hemoglobin, in the studied groups (g/dl)

**Hematocrit:** After the first month of treatment, hematocrit falls for the diseased and untreated control group and also for the group treated with Siofor, and increase in the group treated with Eridiarom.

Table 4

Average values of hematocrit, for the studied groups (%)

Month	No Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
1	6	35 - 52	46,4	42,6	42,6	48	45,6
2	5	35 - 52	42,86	40,23	43,3	48,33	45
3	7	35 - 52	42,91	43,1		40,16	43,07
5	6	35 - 52	42,69	40,74		40,42	43,08
6	6	35 - 52	41,77	40,7		42,89	43,27

After 2 months, increased hematocrit remains high, the group treated with Eridiarom and Diavit, exceeding the average of healthy control, and also of the diseased and untreated one. At 3 months, the hematocrit is lower in all experimental lots, the lowest recorded value is in the group treated with Eridiarom.

At 5 months, is maintained at the level seen at 3 months. At 7 months, the hematocrit increases in the group treated with Diavit, followed by Eridiarom.

**Platelets:** After the first two months, the mean platelet count falls to the healthy control group, in all groups studied.

Table 5

Average values of platelets, for the studied groups ( $10^9/l$ )

Month	No. Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
1	6	500 - 1370	811.2	486	484	524	522
2	5	500 - 1370	716.33	483.33	490	523.33	523.33
3	7	500 - 1370	770.66	1310		627.66	757.55
5	6	500 - 1370	830.53	1168.62		566.46	764.7
7	6	500 - 1370	1049.69	1608.72		1180.31	928.53

At 3 months, the values are much increased for the untreated group, but for the group treated with Diavit, the values are close to the healthy control group.

Platelet growth remains high in the untreated patient group, between 5 and 7 months.

At 5-7 months, for the groups treated with Eridiarom and Diavit, their value is close to the healthy control group.

### 3. CONCLUSIONS

**WBC:** In the untreated patient group the largest value is obtained, and the lowest recorded in the group treated with Eridiarom and Diavit.

After 3 and 5 months of treatment, differences remain, but increased in the diseased and untreated group (9%). At 5 months, the values recorded in the groups treated with Eridiarom and Diavit are lower than for the healthy control group but for the patient group are very high.

**Lymphocytes:** After seven months we see a remarkable decrease in lymphocytes, in the case of diseased and untreated group, but also for the healthy control and a very significant increase in their value in the group treated with Diavit. For the healthy control, the values are still for five months, then decreased significantly to 7 months.

**Hemoglobin:** After the first month there is a slight increase in hemoglobin, for the groups treated with Eridiarom and Diavit, and a significant decrease in the untreated control group.

After 5-7 months of treatment, emphasizes the differences in the group treated with Diavit, the average value exceeds the upper limit of the species.

**Hematocrit:** After the first month, hematocrit decreases in the untreated patient group and also for the group treated with Siofor, and increase for the group treated with Eridiarom.

After 2 months, hematocrit values remain high in the groups treated with Eridiarom and Diavit.

After seven months, the values are higher in the groups treated with Eridiarom and Diavit. In the untreated patient group, the lowest average is recorded.

**Platelets:** After the first two months, the mean values decrease from the control group, in all groups.

After 3 months, greatly increased for the diseased and untreated group, and also for the group treated with Diavit; mean media reach healthy control group.

After 7 months, their value for the Eridiarom and Diavit treated groups was close to the average healthy group, but was very high in untreated patient group.

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## **RESEARCHES REGARDING ANTIOXIDANT SUPPLEMENTATION OF CRYOPRESERVED DOG SEMEN**

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**Key words:** dog, semen, motility, antioxidant

### **SUMMARY**

Semen from four dogs was frozen using a comercial extender (Uppsala), supplemented with antioxidant (Coenzyme Q - CoQ), in an attempt to evaluate the effect of this antioxidant on sperm total motility, linear motility and sperm velocities. The percentage of motility was significantly higher in samples frozen with the addition of antioxidant. Our results suggest that the Uppsala extender can be improved with the addition of CoQ.

Currently, there is a growing interest on dog semen freezing biotechnology. Advantages of using frozen semen breeding program are numerous. The most obvious benefit is the ability to store genetic material originated from dogs of great value. In the last five years there have been many attempts to improve this technology, but there are still many variables that influence the survival of dog spermatozoa (*Nunez Martinez et.al., 2007, Nunez Martinez et.al., 2006, Leibo, S.P., Bradley L., 1999*).

A particular aspect of dog sperm cryobiology is that spermatozoa from different males exhibit different responses at the same freezing protocol. Plasma membrane lipid peroxidation (LPO) is incriminated as a major factor involved in altering sperm quality. (*Ball B.A., 2008, Aitken R.J.et.al., 2007, Cassani P. et.al. , 2005*).

The particular susceptibility to oxidative degradation of plasma membrane is due to a high content of polyunsaturated fatty acids and a protective enzyme deficiency because of the loss of cytoplasm during spermiogenesis.

Polyunsaturated fatty acids (PUFA) have been detected in men sperm membrane, but also in other mammals. They give the plasmatic membrane the fluidity necessary for a spermatozoa to take part to the membranes fusions associated with fertilization. However, these molecules are vulnerable in front of reactive oxigen species (ROS).

It is shown that spermatozoa that undergo through a freezing/thawing cycle produce ROS (Alvarez J.G., Storey B., 1992, Agarwall A., Said T.M., 2005).

These ROS have a beneficial effect as long as they are produced in low quantities, but once produced in excess they can be harmful for the cell. ROS production has been associated with reduced motility, low capacity for the spermatozoa to merge with the oocyte and infertility (Michael A., *şi col.*, 2007).

One way to prevent these undesirable effects of ROS could be the addition of antioxidants compounds in freezing extenders to prevent or block oxidative stress (Donoghue A.N., *şi col.*, 1997).

## 1. MATERIALS AND METHODS

Four privately owned mongrel dogs (*Canis familiaris*) of previous known fertility (all of them had sired at least a litter in the past 2 years) were used as semen donors. Their weights ranged from 10 to 25 kg, with median age 2 years and range of 1 to 4 years. Semen was collected by masturbation in a prewarmed graduated test tube. A bitch in estrus was not present during collection. Then, sperm samples were kept for less than 10 min at 37°C in a water bath. An aliquot was removed for sperm concentration measurement and evaluation of motility using computer-assisted sperm analysis (CASA). Samples were divided in 2 subsamples, frozen using Uppsala method, one used for control and one treated with an antioxidant compound, Coenzyme Q (CoQ) in dimethyl sulfoxide (DMSO) to a final concentration of 1mM. The experiments were repeated three times. Four ejaculates from each dog were frozen.

Motility was measured, before and after cryopreservation, using a CASA system. Analysis was based on the examination of 25 consecutive, digitized images obtained from a single field using a x 10 negative phase-contrast objective. Images were taken with a time lapse of 1 sec—the image capture speed was therefore one every 40 msec. The number of objects incorrectly identified as spermatozoa was minimized on the monitor by using the playback function. With respect to the setting parameters for the program, an object with a mean velocity (VAP) < 10 mm/sec was considered immobile, whereas objects with a velocity >15 mm/sec were considered motile. Spermatozoa deviating <10% from a straight line were designated linear motile. Sperm motion kinematics measured by CASA included the following: Curvilinear velocity (VCL), mm/sec: Measures the

sequential progression along the true trajectory. Linear velocity (VSL), mm/sec: Measures the straight trajectory of the spermatozoa per unit time. Mean velocity (VAP), mm/sec: Measures the mean trajectory of the spermatozoa per unit time.

Semen frozen using the Uppsala method was processed as follows: after collection, semen was diluted 1:1 in Tris-glucose extender I (Tris 200 mM, glucose 70 mM, citric acid 63 mM, bovine serum albumin (BSA) 3%, penicillin 1000 IU/ml, dihydrostreptomycin 1 mg/ml) and centrifuged for 8 min at 700 x g. The seminal plasma was then removed and the sperm pellet resuspended in extender II at room temperature (Tris 200 mM, glucose 70 mM, citric acid 63 mM, glycerol 3% vol/vol, egg yolk 20% vol/vol, penicillin 1000 IU/ml, dihydrostreptomycin 1 mg/ml) and cooled to 5°C over a period of 1 h, resulting in a concentration of 300x10<sup>6</sup> to 400x10<sup>6</sup> sperm/mL. After equilibration, an equal volume of extender III (Tris 200 mM, glucose 70 mM, citric acid 63 mM, glycerol 7% vol/vol, egg yolk 20% vol/vol, Equex STM paste 1% vol/vol [Nova Chemical Sales Inc., Scituate, MA, USA], penicillin 1000 IU/ml, dihydrostreptomycin 1 mg/mL) was added at 5°C to a final sperm concentration of 150x10<sup>6</sup> to 200x10<sup>6</sup> spermatozoa/mL. The sperm samples were then loaded in 0.5-ml straws and frozen horizontally in racks, placed 4 cm above the surface of LN2 in an open Styrofoam box for 10 min, and then plunged directly in liquid N2. After 4 wk of storage, the straws were thawed in a water bath at 70 8C for 8 sec.

## 2. RESULTS AND DISCUSSION

Values of sperm quality in fresh sperm are given in Table 1. Only samples with at least 70% motility and 80% normal morphology were included in the study.

*Table 1*

Average sperm quality in fresh samples (means ± SD, four dogs, three ejaculates each).

TM, %	91,4 ± 1.34
LM, %	48,3 ± 18.17
VCL, mm/sec	89,6 ± 40.28
VAP, mm/sec	50,8 ± 23.50
VSL, mm/sec	36,6 ± 16.01

TM, total percentage of motile spermatozoa; LM, percentage of linear motile spermatozoa; VCL, curvilinear velocity; VAP, mean velocity; VSL, linear velocity.



Dog semen frozen using Uppsala method with addition of antioxidant gave better results of motility and sperm velocities after thawing (Table 2).

Table 2

Sperm motility postthaw in canine semen frozen in the Uppsala extender with and without CoQ supplementation (means  $\pm$  SD, four dogs, three ejaculates each).

	UPPSALA	UPPSALA+Coenzyme Q
TM, %	40,2 $\pm$ 12,73	47,3 $\pm$ 11,32
LM, %	15,6 $\pm$ 19,4	20,2 $\pm$ 10,30
VCL, mm/sec	47,6 $\pm$ 18,8	51,3 $\pm$ 9,6
VAP, mm/sec	28,5 $\pm$ 11,8	30,3 $\pm$ 8,4
VSL, mm/sec	21,1 $\pm$ 8,9	23,5 $\pm$ 7,6

TM, total percentage of motile spermatozoa; LM, percentage of linear motile spermatozoa; VCL, curvilinear velocity; VAP, mean velocity; VSL, linear velocity.

Total motility increased with 7,1% in the samples frozen with CoQ supplementation with respect to the control samples. Anyway, compared with fresh semen, total motility decreased with 44,1% in treated samples and with 51,2% in control samples. All other parameters assessed with CASA system were improved in the samples supplemented with antioxidant.

In the current study, a commercial extender was supplemented with an antioxidant in order to assess the effect of this antioxidant on sperm motility and velocities assessed with CASA system. The antioxidant used, CoQ is an energy promoting agent and antioxidant and is concentrated in the mitochondria of the midpiece, so that the energy for movement and all other energy-dependent processes in the sperm cell also depend on the availability of CoQ. The reduced form of CoQ -ubiquinol also acts as an antioxidant, preventing lipid peroxidation in sperm membranes. It was stated by Jones and Mann (1977) that sperm motility, requiring elevated energy supply and being sensitive to oxidative stress may depend on the energetic and antioxidant functions of CoQ. Several studies have established the link between CoQ, and sperm quality and function. Coenzyme Q concentration was found to be in good correlation with sperm motility and sperm count in men (Mancini *et al.*, 1994). Mazzilli *et al.* (1990) showed improvement of seminal parameters after CoQ administration to men with sperm pathology. Others have also observed an inverse correlation between CoQ concentration and sperm motility. It has been suggested that the reduced

sperm motility in infertile patients could adversely affect other fertilization functions of the sperm cell.

Total motility, along with sperm morphology are the most important parameters described for dog semen in predicting the fertility capacity of a semen sample. CASA instruments eliminates the objectivity of the human factor, therefore this results are reliable in stating that the CoQ can be added in the extender to improve post-thaw dog semen motility.

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## **INDUCTION AND SYNCHRONIZATION OF PARTURITION IN FEMALE PIGS IN ORDER TO OPTIMIZE THE TECHICAL AND ECONOMIC PARAMETERS**

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**Key words:** reproduction biotechnology, induction of parturition, prostaglandins, puerperal diseases

### **SUMMARY**

The efficiency of pig farming is depending of high production results and low cost prices. In pig raising farms, the technical and economic parameters are related with the management of reproduction proces and the quality of biological material.

In order to produce more pork meat at national level is necessary to optimize the production in pig farms and this aim can be obtained only by improving the level of results in swine reproduction.

Using the II-nd degree biotechnologies gives the oportunity for farmers to have a high-performance method in controling and improving the reproduction in swine.

Induction and synchronization of parturition in sows, at a certain day at the will of the farmer, can be used both in industrial and conventional system of pig raising, due to the advantages of this method.

The medium length of gestation in sows is known to be 114 days, but as a reality of the last years, the medium lenght is around 116 days, with variations between 108 and 124 days. This is a big problem for industrial system because a compartement in farrowing house is completed in 7-10 days, wich create difficulties related with the supervision of farrows, establishing the nursing sows, development of the piglets and weaning system and consequently the management of housing capacity.

It is recognized that the average length of gestation in pigs, is 114 days, with variations between 108 and 116 days (Dinu I., 1989 N. Cristea, 1989).

To reduce the interval between farrowings and their group, some authors claim two hypotheses:

- injecting 1 ml prostaglandin/ animal at 113 days of gestation would reduce the length of pregnancy at exactly 114 days (with some variations), and can group the farrowings depending the capacity of maternity (Feredean T., Mantea St., 1984, Boland and Crasig, 1979);

- injecting PG at 111, 112 and 113 days of gestation, all births are grouped within two days, excluding births on "Sunday" with technological, economic and organizational specific problems (Brumble I., 1986).

In this paper we proposed to determine how these assumptions can be applied to concrete and practical technological process, with all variables and possible side effects.

Another hypothesis relates to the incidence of puerperal diseases studied under prostaglandins influence, considering the ocytocic effect they produce indirectly. Eimersson and Bäckström (1976) noted a much lower percent of agalaxia in sows with induced farrowing compared to untreated.

## 1. MATERIAL AND METHOD

The work was carried out in the maternity sector of a Selection Unit in two stages, each having an experimental group and control group, sows or gilts.

Products used were:

First phase (01. 10. 2009 - 31. 01. 2010):

- Remophan - a product of synthesis of PG $2\alpha$ , with the active principle cloprostenol 0,075 mg / ml.

- Flavoliz - cloprostenol 0.175 mg / ml.

Second phase (05. 05. 2010 - 08. 06. 2010)

- Flavoliz - cloprostenol 0.175 mg / ml.

The objectives under observation were:

- The effect of induced parturition to 113 days of gestation;

- Influence of the product on the effect;

- The possible influence of race and the season on the effect;

- The effect of synchronization at 111, 112 and 113 days of gestation;

- The effect of prostaglandins on the incidence of puerperal diseases

Method of administration:

- 1 ml / animal, im, regardless of product used for the experimental group at 113 days of gestation;

- 1 ml / animal, im, 111, 112, 113 days of gestation, all animals being injected in the same day (on Thursday).

## 2. RESULTS AND DISCUSSION

A) The effect of synchronisation of parturition at 113 days of gestation

Stage I: 01. 10. 2009-31. 01. 2010

To see the possible influence of the cold season on the effect of PG2 $\alpha$  administration, was chosen this season for the time of this experiment. All animals in farrowing period (75% of the original experimental group) were injected with 1 ml Remophan and Flavoliz, i.m. at 113 days of gestation.

The results are outlined in the table 1.

Table no. 1

### COMPARATIVE RESULTS OBTAINED IN INDUCTION OF PARTURITION IN SOWS

Specification	No. animals in the experiment	STAGE I							
		Remophan injection		Flavoliz injection		Sows farrowed with different treatments			
						Remophan		Flavoliz	
		No.	%	No.	%	No.	%	No.	%
Experimental Lot	138	48	39,3	74	60,6	48	100	73	98,6
Control group	72	-	-	-	-	63 (75%)			
Total	210	122				184 (73,6 %)			

As noted in the table, from the total of 138 sows of the experimental group, 122 farrowed (88,4%), after the injection with the two type of products (Remophan - 48 heads Flavoliz - 74 heads). In terms of the effect of these products, we can see that the answer was 100% in Remophan injection group and 98,6% in the second case in Flavoliz injection group and a sow having dystocia was sacrificed.

So, the answer was prompt in both cases, differing only in time of injection and the time elapsed until the occurrence of parturition:

- Remophan product – farrowings were grouped at 24-36 hours after injection (45 sows of 48, representing 95,8%);

- Flavoliz product – farrowings were grouped around 48 hours (96,2%).

In terms of the influence of race, we found no significant differences in effect, all answering the same way to synchronization, specifying that a slight tachycardia at the race Synthetic Line – 345 Peris.

The control group also has a farrowing percentage of 75% of all animals IA, but this take place at about 115-116 days comparing with 114-115 days in the group treated with prostaglandins. Also, the frequency of long term and laborious births, about 4-5 h, was higher in the control group compared with the experimental group (1-2 h), due to indirect ocitocic effect of prostaglandins.

Only 2 sows (1,6%) of those injected with PG2 $\alpha$  had extended farrowings to 3-4 hours.

B) Stage II: 05. 05. 2010 - 08. 06. 2010

In this stage it was used only Flavoliz product in a dose of 1 ml / animal, injected at 113 days of gestation, as shown in the table below:

Table no. 2

*INDUCTION OF PARTURITION IN SOWS WITH FLAVOLIZ*

Specification	No. animals in the experiment	STAGE II			
		Sows injected with Flavoliz		Sows farrowed after injection	
		No.	%	No.	%
Experimental Lot	130	117	90	116	99,1
Control group	70	-	-	-	-
Total	200	117	58,5	116	99,1

It may be noted that mortality rates were lower during this period (72%), but were not influenced by the product used. No significant differences in terms of the effect in this period, because approximately 99% of injected animals farrowed, so the answer was the same. Also, there is observed no differences based on race or time period between injection and the moment of parturition.

In conclusion, injection of this product at 113 days of gestation, has the same effect throughout the year. The same results were obtained when administered at 111, 112 and 113 days of gestation.

Mortality rates in the control group had approximately the same values as the experimental group (71,8%), but farrowing took place around 115-117 days of gestation.

In terms of grouping farrowings in both periods, it can be said that there were differences, as follows:

- The experimental group injected with PG2 $\alpha$  – farrowed especially during the day and less at night;

- The control group - births took place, especially at night, when stress factors are minimized.

*Reducing the incidence of puerperal diseases.*

After completion of the second phase, during August and September 2010 was done a research concerning the ocitocic and luteolitic indirect effect (Mantea St, Feredeian T., 1984) to reduce puerperal disorders.

The research was conducted on a number of 186 sows as follows:

- Experimental group – 116 sows
- Control group -70 sows.

After synchronizing the parturition to 113 days of gestation, all animals in the experimental group were injected again with 1 ml Flavoliz at the end of farrowing, and each sow was clinically examined twice a day.

The main problems encountered both in the control group and to the experimental, were:

- Mastitis;
- Endometritis;
- MMA syndrome;
- Placental retention;
- Agalactia.

The results are presented in the table 3.

*Table no. 3*

*EFFECT OF INDUCTION OF PARTURITION ON PUERPERAL DISORDERS*

Specification	No. animals in the experiment	No. animals injected Flavoliz	With puerperal main diseases									
			Mastitis.		Endom.		MMA		Placental retention		Agalactia	
			No	%	No	%	No	%	No	%	No	%
Experimental Lot	116	116	3	2,6	3	2,5	2	1,7	6	5,2	7	6
Control group	70	-	2	2,8	3	4,2	2	2,8	4	5,7	5	7,1
Total	186	116	5	2,7	6	3,2	4	2,1	10	5,4	12	6,5

As can be seen from the table, the most common disease both in the control group and to the experimental, was agalactia, and the most rare was MMA syndrome, because this syndrome usually progress through one or two symptoms and very rarely in all three (Brumble I., 1986).

It may be noted that if the prostaglandin was administrated after farrowing, the incidence of puerperal disorders was reduced to the control group, especially in terms of endometritis (2,5% for the experimental group, versus 4,2% in the control group) and MMA syndrome (1.7% versus 2,8%). This was due mainly to indirectly ocitocic effect, more complete recovery of the genital tract and mammary gland, largely avoiding infection. For this effect also contributes the shorter duration of parturition, and a small number of dead piglets at birth and during birth, in the case of treatments with prostaglandins.

Also, the incidence of agalactia is lower in the experimental group compared to control group (6,03% versus 7,1%) because prostaglandins trigger prompt lactation.

### 3. CONCLUSIONS

The induction and synchronization of parturition could be more efficient for reproduction in the following ways:

It may shorten the period of gestation with 2 -3 days, thus increasing the rate of use of sows.

A compartment in the farrowing house may be completed the same day.

It may hold a more accurate monitoring of farrowings by focusing the attention and the specialized personnel in the preset day, thus reducing the loss of piglets at birth.

It can avoid farrowing during night-time and week-ends.

It can make a fair distribution of piglets at the sows and nurses taking into account the number of piglets and the number of functional nipples.

It can organize better health and veterinary actions in the compartment.

It can reduce the number of puerperal disorders.

It can be done in good condition weaning actions.

All this technology results in a smooth flow that is ultimately expressed in a better economic return to the farm.

Also in the small farm system, where the number of sows is lower, the advantages mentioned are taken into account by the fact that the farmer can arrange better the farrowing area, can appreciate better the time of farrowings and the supervision, etc.



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## INDUCTION OF PUBERTY GILTS TO REDUCE THE INTERVAL BETWEEN GENERATIONS

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**Keywords:** biotechnology ,induction of puberty, prostaglandins, gonadotropins

### SUMMARY

Induction of ovulation in sows is a phase of biotechnology to control the sexual cycle and is particularly important, both for the technological process in production units as well as for biotechnologies of II-nd and III-rd degree. To decrease the length of interval between generations, so necessary in genetic improvement and selection work to obtain a new population, it is desirable to use females for breeding as early as possible. It is known that sexual precocity is a character influenced by several factors such as race, diet, breeding system, etc. The use of hormonal treatments can induce first appearance of oestrus and ovulation in gilts before puberty, from the age of 150-160 days. The results in reducing unproductive interval of gilts until to the first fertile mating are followed by a reduction in maintenance costs and an increase in farm profitability. But using this method, it is important to make a correlation with body development knowing that during pregnancy and lactation, the entire organism of females must make big efforts, and if the body is not in good condition, could appear metabolic or hormonal disorders followed by the loss of these animals for breeding. The ability of response to treatments with exogenous gonadotropins in immature genital tract is due to increases in ovarian steroid hormone secretion, initiated by this administration.

To decrease the interval between generations, so necessary in genetic improvement and selection work to obtain a new population, it is desirable to use breeding females as early as possible. In their work, "Genetic improvement in pigs", Beris Liviu and Maria Stoicea states that the age at first estrus is actually one of the first objective measurements of capability for reproduction of gilts. A precocious puberty, reduce unproductive time that separates the end of the first mating to the first farrowing.

In a study made by Le Gault, on Large White sows, Landrace and crossbreds was found that 93.5% of females showed first estrus

accompanied by ovulation, before the age of 300 days. Also, 4% of females were before puberty at 300 days and 2% showed false heat (not accompanied ovulation). There were observed, also, genetic differences. Thus, in Landrace breed, gilts before puberty or false heat were entirely absent, in crossbreds they represent less than 1%, and in Large White it was found a rate of 6% gilts before puberty and over 3% of gilts manifested false heat, without ovulation.

The average body weight of gilts in the moment of puberty, ranged from 104-119 kg in Large White and Landrace and 87 kg to 97-98 kg in crossbreds. At the same time, the average puberty time varied more at Large White (202-241 days), very little to metise (182-183 days) and intermediate in Landrace (195 days).

## 1. MATERIAL AND METHOD

Experiences were performed on gilts before puberty, at 150-160 days, because there is insufficient data in the literature and from our previous experiences, for ovulation induction in gilts aged under 180 days. Initially we started from the level of 400 IU PMSG 200 IU HCG in a single dose treatment known to have good results for gilts over 180 days. In a first step, because it was considered that at this age the genital tract is not yet ready to start sex physiological processes, it was experienced an induction of sensitivity to gonadotropins, using synthetic steroids.

Treatment started with IM administration 0.5 ml Sintofolin in two halves every 48 hours, followed by another 48 hours of gonadotrophins in the mixture described.

These studies showed that in prepuberal gilts treated with PMSG + HCG ovulation occurs after 40 hours, so the administration of HCG was asynchronous, spanning a period of time. This leads to failures in the process of fertilization as a result of desincronization between ovulation and insemination time, of course followed by embryos of poor quality. Researches conducted worldwide has shown an increase in prostaglandin F<sub>2</sub> $\alpha$  and E<sub>2</sub> concentrations around ovulation moment although their role in this process is not fully established. According to some authors it appears that prostaglandins mediate lysis of follicular collagen, while others argue that inhibits collagen synthesis in the follicular apex thereby facilitating rupture here. Whatever the mode of action, remains clear that using cloprostenol (a synthetic analogue of PGF<sub>2</sub> $\alpha$ ), Downey and Srikandakumar managed best timing of ovulation in sows.

Starting from this idea and the results obtained in the first stage, it was proposed an another experiment to try to improve the results obtained by induction of ovulation in gilts before puberty. Our experiments were conducted on F1 prepuberal gilts, crossbreds between MA x L, aged between 150 and 160 days and weighing between 60-70 kg. They were divided into two groups: experimental and control. Both groups were treated for stimulation with increased doses of gonadotrophins, ie 800 IU PMSG, followed 72 hours of 500 U.I. HCG. Each gilt in experimental group was injected with cloprostenol (synthetic analogue of PGF2 $\alpha$ ) in doses of 175 mg every 24 to 28 hours after HCG, followed by insemination at 4 and 22 hours after the last injection with cloprostenol. Gilts in the control group were inseminated at intervals of 34 and 48 hours after HCG. All gilts were subjected to laparotomia and embryos and unfertilized eggs were collected by washing the oviducts, then were examined morphologically to assess quality.

## 2. RESULTS AND DISCUSSION

The results are showed in Tables 1 and 2.

Examining the data listed in Table 1, we can see that there are differences between treatments. Thus from gilts treated with gonadotrophins, only 45% showed oestrus compared with 75% in those with prior treatment with Sintofolin. Ovarian response is much lower than the normal ovulation rate in gilts over 180 days (given by the literature and own experiences). Unfertilized egg number increases greatly at this age, probably due to a more pronounced ovulatory asincronisation, as a result of poor hormonal balance. The large number of normal embryos in the group with pre-treatment with Sintofolin, suggests that this treatment is acting on genital tract, increase its volume and motility, favoring sperm transport and make the other target organs more responsive to the action of gonadotrophins.

Table no. 1

## Induction of puberty in gilts results of 150 days

Treatment	Gilts treated			Response to treatment		Control laparatomii		
	No.	Age	Weight			Corpora lutea	Embryos	Ovules unfertilized
	No.	Medie	Medie	No.	%	Medie	Medie	Medie
400 U.I.PMSG + 200 U.I.HCG	20	150 - 160	70 - 75	9	45	11	4,5	6,5
Sintofolin 2 x 0,5 mg to 48 h. followed by 400 U.I. PMSG + 200 U.I. HCG	12	150 - 160	70 - 75	9	75	9,2	6,0	3,2

\* Gilts that had not responded to treatment at slaughter, infantile uterus and ovaries hypoplasia.

The results listed in Table 2, confirms the literature, namely that  $PGF2\alpha$  is a necessary mediator in the initiation of ovulation. Thus, while in the control group the average rate is 8.5 corpora lutea and 4.2 follicles, using the treatment with cloprostenol, the average rises to 13.4 corpora lutea and not-opened follicles reduced to 0.85.

The treatment with  $PGF2\alpha$  analogue had also an effect of reduction of ovarian cysts that occur as a result of developments of follicles that do not suffer dehiscence.

The situation observed in the examination of ovaries is also reflected in embryos morphology after recovery. As it is shown in table 2, in the control group the percentage of unfertilized eggs (18.01%) and abnormal embryos (5.88%) is higher, resulting a low average of embryos morphologically normal (6.9%), but in the group treated with cloprostenol these unwanted percentages are reduced (6.71% unfertilized eggs and 1.41% abnormal embryos) and as a result an average of 12.35 embryos that can be used for implantation, from prepuberal gilts after hormonal stimulation. From the above results, it is evident that the exogenous  $PGF2\alpha$  analogue in the experimental group, had a good effect by inducing follicle dehiscence, after the treatment with exogenous gonadotrophines in gilts before puberty.

Also, the pre-treatment with Sintofolin, before hormonal stimulation, prepare the genital tract and had a positive effect on the occurrence of oestrus in pre-puberal gilts.

These techniques make possible the use as embryo donors the gilts before puberty, from an age at which normal reproduction is impossible. Results also provide a basis for studies starting in the future to improve this technique and to extend them to other age groups.

Table no. 2

Comparative results of two treatments with exogenous hormones

Lot	No. females 150-160 days	Stimulation treatment	Ovulation induction treatment	Ev. oestrus-site clinical	Laparotomy and morphological examination results					
					No. corpora lutea	No follicles 7-10 mm	No. cysts	No. unfertilized eggs	Embryos	
									Total	In which abnormal
M	32	800 U.I. PMSG + 500 U.I. HCG	-	100 %	272	137	28	49	221	13
E	40	800 U.I. PMSG + 500 U.I. HCG	2 x 175 mg cloprostenol	100 %	536	34	11	36	49	7

### 3. CONCLUSIONS

It is possible to induce first oestrus and ovulation in gilts before puberty, aged 150-160 days, using exogenous gonadotropins in doses of 800 IU PMSG and 500 U.I. HCG.

After this treatment, administration of a synthetic analogue of PGF<sub>2</sub> $\alpha$  (cloprostenol), increased the ovulation rate from 8.5 to 13.4 C.L. per sow and reduced the emergence of ovarian cysts from 0.87 to 0.27 on average.

Using a pre-treatment with Sintofolin, prepared the genital tract of the pre-puberal gilts, which is not completely developed at this age, for the action of exogenous gonadotrophins, and had a positive effect on the occurrence of first oestrus .

These techniques make possible the use as embryo donors, the gilts before puberty, from an age at which normal reproduction is impossible, and

to shorten in this way, the interval between generations, so necessary in the works for genetic improvement.

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## ASPECTS OF AVIAN INFLUENZA EVOLUTION IN CONSTANTZA DEPARTMENT IN THE PERIOD OF TIME FEBRUARY – MARCH 2006

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**Key words:** avian flu, wild birds, domestic poultry, hotbeds.

### SUMMARY

Avian flu is an infectious disease of birds, caused by the mutation of A type of the flu virus. The outbreaks of some grave forms were caused by H5 and H7 subtypes, but H5N1 represents a particularity because it modifies itself rapidly being able to affect human beings.

In order to be able to understand the epidemiologic aspects of the evolution of avian influenza in our country we considered that this matter could be performed through detailing and explanation of the appearance and evolution of avian influenza hotbeds in Constantza department and afterwards through corroboration of all the data we could be able to draw conclusions related to the sources of the disease, represented, mainly, by the migration of wild birds, carriers of LPAI or HPAI strains of the avian influenza virus in our country, by the interferences among the species receptive to the disease, respectively the contact between contaminated wild birds and poultry receptive to the disease. In all the avian influenza hotbeds in Constantza department we could not specify with certainty the source of contamination of poultry, but it was considered that this might have been represented by wild birds, the path of transmission being a direct one( the contact between poultry and wild birds) and also an indirect one, represented by the habitat( water surface, ponds, rivers on which birds had rested).

The risk factors must be taken first into consideration, the factors which favour the spreading of this disease: geographical area which are preferred by migratory birds, the raising of poultry in traditional system ( in the rural environment) with access to the water surface, having thus the possibility of coming into contact with wild birds, carriers of the avian influenza virus, the lack of biosecurity in the poultry farms,etc.

In Constantza department 14 avian influenza hotbeds evolved in the period February-March 2006 , in different places situated or not in the vicinity of water sources ( lakes, rivers, ponds, the Black Sea, etc), the presentation of the evolution of these hotbeds being carried out in this work in-extenso.

The initial introduction of the avian influenza virus in poultry exploitation is the most likely the result of a direct or indirect contact with wild birds. It is possible that LPAI viruses introduced by a wild reservoir might have circulated to domestic poultry without being detected, as the clinical signs are often discrete, even absent. Once introduced to poultry, the types of LPAI virus belonging to H5 and H7 subtypes can undergo mutations to produce HPAI subtypes. Although it seems that during the transformation of the LPAI virus in HPAI virus more mechanisms



intervene, the factors which are at the basis of this mechanism are still unknown. In some cases, the mutation seems to have occurred rapidly, at the initial site, after the transfer through wild birds, while in some other cases LPAI virus had circulated among poultry for some months before undergoing mutations. Therefore, it is impossible to foresee if and when such a mutation supervenes. However, it can be assumed that the more HPAI viruses circulate among poultry the higher will be the risk of mutation of the HPAI virus.

## **1. MATERIAL AND METHODS**

The study was carried on in the period February-March 2006, a period when 14 avian influenza hotbeds evolved in Constantza department, and it consists in presenting some epidemiologic aspects from these hotbeds, namely: the appearance of the disease, the causes, the diffusing of the disease, how it is transmitted, etc. The diagnosis of avian influenza was performed through laboratory examinations carried on the Sanitary-Veterinary Laboratory and for Food Safety Constantza, diagnosis which subsequently was confirmed by the Diagnosis and Animal Health Institute Bucarest. The laboratory test consisted in clinical, anatomopathological, serologic and virusologic exams on samples represented by corpses, cloacal and tracheal tampons, organs as well as sanguine serum taken from poultry suspicious of avian influenza. Serological exams consisted in ELISA tests and reaction of immunodiffusion in agar gel (AGID), and virusologic exams consisted in rapid tests for diagnosis-the test of rapid detection of antigen of avian influenza virus through immunochromatography and in the case of positive tests through RT-PCR tests which were carried out in the Sanitary-Veterinary and Food Safety Laboratory Constantza and for the positive samples the confirmation was performed by the Diagnosis and Animal Health Institute Bucarest through RT-PCR test and the isolation of the virus on SPF eggs with embryos.

## **2. RESULTS AND DISCUSSIONS**

All the positive cases of avian influenza were diagnosed in poultry raised in non-professional exploitations (in the small personal farms of the population), the system of raising poultry being characteristic to villages in our country, namely the poultry has access to water areas (lakes, ponds, rivers) or to pastures near the houses, thus coming into contact with wild birds which are incriminated in the avian influenza epidemiology.

The first suspicion of avian influenza in Constantza Department appeared on 8<sup>th</sup> of February 2006 when in **Topraisar** mortality was registered in the case of poultry from a private household, where out of 27 hens the evolution was the following: 3 dead hens, 3 hens presenting clinical signs of disease (cyanosis of crest and wattles, diarrhea)-poultry which died the next day (on 9<sup>th</sup> of February 2006) and the rest of 21 hens presented discrete clinical signs of the disease.

On 8<sup>th</sup> of February virusological exams were performed, exams which consisted in: the test of rapid detection of avian influenza virus antigen through immunochromatography from cloacal and tracheal tampons and RT-PCR test from homogeneity of organs from the 3 hen corpses, both tests turning positive for A 150. On 9<sup>th</sup> of February laboratory examination was performed to the other existent birds in the private farm where there was the suspicion of IA:

- 3 hen corpses-positive result for rapid immunochromatographic test and RT-PCR test

- 13 blood samples drawn from 13 hens-2 samples were positive for ELISA test, the other 11 being negative.

- 40 cloacal tampons-double samples drawn from 20 hens-12 samples (6 hens) turned positive for avian influenza for rapid immunochromatographic test and 28 samples (14 hens) were negative.

On 12<sup>th</sup> of February, suspicion of avian influenza is declared following the results obtained in the Sanitary-Veterinary and for Food Safety Laboratory Constantza and on 15<sup>th</sup> of February the disease is declared officially, as a result of confirmation of avian influenza by the Diagnosis and Animal Health Institute Bucarest (RT-PCR test positive; isolation of the virus on SPF eggs with embryos: positive for A 150 H5N1 subtype.

As a consequence of epidemiological investigation it was established that poultry diagnosed with avian influenza did not have access to water surfaces and also the fact that mortality among the wild birds in the area did not occur. However, it is supposed that the main source of contamination is represented by wild birds, this supposition being sustained by the arguments bellow:

- the geographical position of Topraisar village at a distance of 15 km from Techirghiol lake and of 15 km from the Danube-Black Sea Channel;

- also, losses from morbidity or mortality in the case of poultry in the neighbourhood or in other households in the village were not registered during this period;

-the fact that after only 12 days after declaring hotbed of A150 in Topraisar, avian influenza is declared in the case of a wild pigeon found dead in Mereni, a locality only 6 km away from Topraisar.

In the period of time when the control by stamping-out and subsequently all the other procedures taken to eradicate the hotbeds were being performed, other avian influenza hotbeds were declared in 13 localities in Constantza department.

In **Vlahii**, on 13<sup>th</sup> of February, the first signs of H5N1 virus appeared-in a household of this locality a number of 61 dead hens was registered in only one day out of 76 birds: 7 palmipedes and 69 hens, the mortality percentage being of 80%. The living poultry- 7 palmipedes and 8 hens presented clinical signs of disease: cyanosis of the crest and wattles (hens), nasal secretions, deviation. In that household there were also 59 pigeons which did not manifest clinical signs of the disease. The probable source of infection is represented by wild birds, this statement having as support the fact that Vlahii is approximately 300 meters away from Vederoasa Pond, area in which many wild birds had been noticed: swans, wild geese, wild ducks, herons, ember geese, without specifying their number or if there was any mortality among these wild birds as they had not been monitored.

On 14<sup>th</sup> of February 2006 another hotbed of avian influenza was registered in a household in **Ostrov**, in this case 28 gallinaceae being affected (hens and turkey hens) out of 30 existent gallinaceae in the household, the mortality rate being of 93%.The gallinaceae manifested clinical signs of disease: edema of the head, cyanosis of crest and wattles, white-yellowish diarrhea. The poultry in the household affected by H5N1 did not have access to water surfaces or ponds, being closed in the yard. Nevertheless we do not exclude the possibility of an eventual contact with wild birds, due to the fact that the presence of many wild birds was noticed in the area, birds which were in the process of migration (swans, wild geese, wild ducks), but especially because of the fact that 45 days before another avian influenza hotbed had been declared in Modelu, Calarasi department, only 10 km away from Ostrov.

In **Navodari** suspicion of avian influenza appeared on 16<sup>th</sup> of February 2006 when in a household a number of 37 gallinaceae (hens and guinea fowls) out of 72 gallinaceae died in 5 days, the mortality rate being of 51%, and the morbidity rate of approximately 100%, because most of the poultry presented clinical signs of disease (cyanosis of crest and wattles, deviation, white yellowish diarrhea).These birds did not have access to water sources but in this case too, because of the fact that prior cases of morbidity or

mortality had not been pointed out in the vicinity of the household or in town, this time too it was concluded that the main source of contamination was represented by wild birds. To support his idea we can bring more arguments, namely: very high mortality in the period of time October 2005-February 2006 in the case of wild birds in Navodari (10 known cases-seagull, bald coots, pelicans, swan, wild geese) as well as in near places, for example in Corbu-Sinoe lake (29 dead wild birds, the avian influenza virus H5N1 subtype being isolated from a swan corpse on 10<sup>th</sup> of October 2005), in Constantza city (243 dead wild birds predominating: swans, wild ducks, bald coots, seagulls, pigeons, etc).

In the same period 16<sup>th</sup> of February-17<sup>th</sup> of February 2006 the presence of avian influenza virus was signaled in **Tuzla** in three households where not only gallinaceae were affected (hens and turkey hens) but also palmipeds (7 geese); out of 61 birds 11 hens died (18%), 2 turkey hens died (3%), 2 palmipeds died (3%). In this case too the source of infection was considered to be wild birds, taking into account the following:

- the geographical placing of Tuzla – 3 km away from the Black Sea, at a distance of 3 km from Eforie Sud- a place where the presence of numerous wild birds had been signaled in the period of time November 2005-February 2006, the H5N1 virus having been isolated from a swan corpse on 22<sup>nd</sup> of February 2006 and from a bald coot corpse on 6<sup>th</sup> of March 2006;

- in Tuzla there is Tuzla pond and Techirghiol lake is only 0,5 km away;

- Tuzla is localized at a distance of 20 km from Topraisar where an avian influenza hotbed was declared on 8<sup>th</sup> of February 2006 (at a time distance of 8 days).

After only 8 days, on 24<sup>th</sup> of February 2006, in **Topalu** a suspicion of avian influenza appeared in household where from a total of 38 birds (31 gallinaceae- 8 turkey hens and 23 hens) and 7 palmipeds (ducks) the following died: 15 hens (65%) and 1 duck (14%). The remaining ones presented clinical signs of disease: cyanosis of crest and wattles, apathy, lack of appetite, etc. Cloacal tampons were drawn from living and ill poultry and the results were positive for the rapid immunochromatographic test and RT-PCR test, thus: out of 6 TC ducks- 2 positive samples (33%), 12 TC hens- 8 positive samples (66%), 2TC turkey hens – 2 positive samples (100%).

Topalu is 1 km away from the Danube and 60 km away from Vlahii where on 13<sup>th</sup> of February 2006 an avian influenza hotbed was declared. Also, in that area the presence in great number of wild birds had been signaled (the Danube flow being a migration corridor for wild birds), fact

which leads to the conclusion that also in this case the main source of contamination with H5N1 was represented by wild birds.

In other 3 places situated near one from another the occurrence of avian influenza was suspected on the same day-27<sup>th</sup> of February 2006 in Crangu, Satu Nou and Saligny.

In **Crangu** mortality related to poultry occurs in two households and in another household in the neighbourhood other 15 hen corpses had been buried a few days before. In the two households where avian influenza hotbeds had been declared as a consequence of the positive results of laboratory examination it was established that: out of 117 birds-94 hens-48 dead (51%), turkey hens-12-5 dead( 41%); 23 palmipeds (6 geese and 17 ducks)- mortality rate of 0%. Living birds manifested clinical signs of disease.

In **Satu Nou**, out of 99 birds (61 hens, 20 ducks, 10 geese and 8 turkey hens) 6 of them died on 27<sup>th</sup> of February 2006: 4 hens, 1 duck and 1 goose, the others presenting clinical signs of disease.

**Saligny**, the third place where avian influenza hotbed was declared, confronted with the following situation: mortality in poultry manifested in only household where out of 88 birds (32 hens, 4 geese, 10 ducks and 42 pigeons) 29 died: 25 hens and 4 palmipeds. The remaining poultry presented obvious clinical signs of the disease: cyanosis of crest and wattles, deviation, etc.

It is obvious that avian flu virus manifests in a very aggressive way. It was the first time when it was registered a high enough mortality among palmipeds, considering the fact that palmipeds manifest a higher resistance to H5N1 virus compared to gallinaceae. The poultry in these households did not have access to water surfaces but these localities are situated near the Danube-Black Sea Channel which is a corridor for migration for wild birds (photo 1 and photo 2).

On 6<sup>th</sup> of March 2006 a new suspicion of avian influenza in poultry occurs in **Seimenii Mici**. Out of 27 birds from a household (19 hens, 4 turkey hens and 4 geese) 5 hens died and they are diagnosed with A150 virus H5N subtype. Seimenii Mici is 0,5 km away from the Danube and the Seimeni Pond and 18 km away from Topalu where bird flu burst out on 24<sup>th</sup> of February 2006. In the same period the avian influenza virus is isolated from a bald coot corpse found in the area of "Spell of the Sea" Beach in Eforie Nord.

The next day-the 7<sup>th</sup> of March 2006-in **Harsova**-the second town in Romania where avian influenza virus was identified-the presence of bird flu

was identified after more birds in a household died and the tests indicated the presence of avian influenza virus. Mortality was registered in 6 hens out of 41 birds existent in the household: 20 hens (mortality percentage of 30%), 14 ducks and 7 pigeons. The town is on the Danube bank, palmipeds having access to the Danube, fact which strengthens the belief that the disease appeared because of the wild birds. This fact was also sustained by the owners' statement according to which they perceived the presence of a wild goose in the yard where the poultry was kept. In October 2005 the avian flu virus, H5N1 subtype was identified from a wild duck in the area of Vadu Oii-situated on the Danube bank, approximately 9 km away from Harsova. Harsova is also 25 km away from Topalu.

The 12<sup>th</sup> avian influenza hotbed in Constantza department was declared on 11<sup>th</sup> of March 2006- after mortality was registered in a household situated right in the centre of **Cernavoda** town. Out of 30 hens, 12 hens died, the other 18 which presented clinical signs of the disease were put down. The results of the laboratory examination- rapid immunochromatographic test- were positive for all the examined corpses (30 dead bodies-percentage of 100%).

Two days after declaring avian influenza hotbed in Cernavoda a new suspicion of the disease appeared in **Dunarea**, in a household. It was registered mortality in hens- 5 hens died from a total of 28 hens (17%), in that particular household there also where 5 ducks which did not present clinical signs. The locality is situated at a distance of 100 meters from the Danube and of 7 km from Seimenii Mici. In this case too wild birds were assumed to have been the source of contamination.

On 24<sup>th</sup> of March 2006, in **Deleni**, the 14<sup>th</sup> and last avian influenza hotbed in Constantza department is declared. On that date there were still active 8 avian influenza hotbeds (Topalu, Saligny, Satu Nou, Crangu, Cernavoda, Dunarea, Harsova and Seimenii Mici), while other 5 localities (Topraisar, Vlahii, Ostrov, Tuzla and Navodari) were coming out of the quarantine. The disease occurred in a household in Deleni, where 28 hens died out of a total of 70 domestic poultry (the mortality percentage was of 43%) and 5 ducks. Near the locality there are not any water courses or ponds for the birds to have access to, but it is 19 km away from Crangu. The sanitary-veterinary restrictions were suspended on 21<sup>st</sup> of April 2004, and on that date there wasn't any active avian influenza hotbed in Constantza department.

**Clinical and anatomopathological aspects associated with highly pathogenic avian influenza in domestic poultry (photo from the Sanitary-Veterinary and Food Safety Laboratory Constantza):**



Photo 1. Hens and goose dead because of infection with A150



Photo 2. Goose presenting nervous H5N1 virus clinical signs-impossibility of displacement



Photo 3. Edema of the head and neck.



Photo 4. Proventriculitis and hemorrhagic enteritis  
Cyanosis of crest and wattles.



Photo 5. Hemorrhages on the abdominal fat

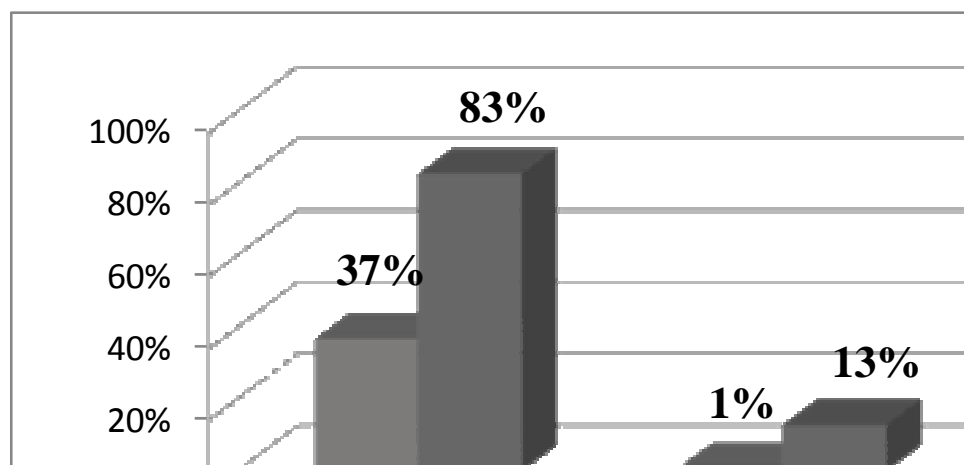


Photo 6. Edema and hemorrhages on subcutaneous conjunctive tissue in the head area.

**Tabel 1.**

**Mortality and morbidity percentage in domestic poultry from the 14 avian influenza hotbeds in Constantza department**

Nr.crt	Locality	No.of existent birds in hotbed	Dead birds		Birds presenting clinical signs of disease	
			Gallinaceae	Palmipedes	Gallinaceae	Palmipedes
1	Topraisar	27	6	-	27	-
2	Vlahii	76	61	-	69	7
3	Ostrov	30	28	-	30	-
4	Năvodari	72	37	-	72	-
5	Tuzla	61	13	2	54	7
6	Topalu	38	15	1	31	7
7	Crângu	117	53	-	94	23
8	Satu-Nou	99	5	1	69	30
9	Saligny	88	25	4	49	10
10	Seimenii Mici	27	5	-	23	4
11	Hârsova	41	6	-	27	14
12	Cernavodă	30	12	-	30	-
13	Dunărea	28	5	-	28	-
14	Deleni	70	28	-	65	5
	<b>TOTAL</b>	<b>804</b>	<b>299</b>	<b>8</b>	<b>668</b>	<b>107</b>



**Fig.1 - Mortality and morbidity percentage in domestic poultry from the 14 avian influenza hotbeds in Constantza department**



### 3. CONCLUSIONS

3.1. In the majority of cases of avian influenza diagnosed in Constantza department the main source of contamination was considered the one represented by wild birds, the path of transmitting the virus being a direct one, through the contact of poultry with wild birds but also an indirect one represented by habitat (water surfaces, ponds, rivers on which infected birds rested).

3.2. The fact that avian influenza hotbeds appeared in Constantza department even in places where there had not been a water course on which migratory birds could have rested suggest the fact that these wild birds in their searching for food travel great distances, above localities, and thus there is the possibility that the dejections of the wild birds contaminated with the flu virus to have reached the households and therefore contaminating the poultry.

3.3. In the majority of the hotbeds-Vlahii, Ostrov, Tuzla, Topalu, Cernavoda, Dunarea-the existence near the localities of water sources where the poultry had access and where there is a permanent contact between the poultry and wild birds- led to the fact that the main source of infection was represented by wild birds which were carriers of avian influenza virus.

3.4. The symptoms of the disease differ according to the species, thus gallinaceae manifest the disease more prominently compared to palmipedes.

3.5. The period of incubation of the disease varies from a few hours to a few days, the clinical forms of the disease occurring from the superacute evolution –deviation, lack of appetite and death in a few hours- to acute evolution –depression, sleepiness, cyanosis of crests and wattles, edema of subcutaneous conjunctive tissue in the head and neck area, white-yellowish diarrhea, dropping wings, incapacity of rising to their feet.

3.6. The laboratory examination performed for the diagnosis of avian influenza in The Sanitary-Veterinary and for Food Safety Laboratory Constantza- consisting in rapid tests of detection of antigen of avian influenza virus through immunochromatography and RT-PCR test for the detection of the viral genome specific for avian influenza proved to be efficient and certain, the confirmation of the diagnosis of avian influenza by the Diagnosis and animal Health Institute Bucharest, at least in the case of poultry, being of 100%.

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## STUDY ON THE ROLE OF WILD BIRDS IN THE EVOLUTION OF AVIAN INFLUENZA IN CONSTANTZA

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**Key words:** avian flu, wild birds, avian influenza virus HPAI and LPAI.

### SUMMARY

Avian influenza represents a great danger for the entire world, but especially for Romania, because of geographical particularities, the Danube Delta in particular and the phenomenon of migration of birds which are considered the natural reservoir of avian influenza virus.

The viruses of avian influenza produce infection in a great variety of wild birds and domestic poultry, the important source of the virus being represented by the wild birds, from which the virus is transmitted to the poultry. Among the wild birds, the main carriers for the subtypes of avian influenza virus are represented by the migratory water birds from *Anseriforme* order (ducks, geese) and *Charadriiforme* (seagulls and other shore birds).

Water birds are above all aimed by this virus, especially the ducks, geese, swans, seagulls and some limmicals (shore birds). Among the species belonging to these countries some of them are more frequent in our country: the great duck (*Anas platyrhynchos*), small duck (*Anas crecca*), laughing seagull (*Larus ridibundus*), winter swan (*Cygnus Cygnus*), bald coot (*Fulica atra*), cormorants (*Phalacrocorax carbo*). Ducks represent the natural "reservoir" of this virus, but in its lower pathogenic form. Ducks are known to be relatively resistant to this H5N1 strain—they rather act as carriers.

Generally, in wild birds only LPAI viruses are detected, excepting the cases when HPAI is spread by the infected poultry, thus isolation of the HPAI strain was performed during the evolution of an avian influenza hotbed of poultry in a geographical area near the poultry infected with HPAI avian flu virus.

In Constantza department the H5N1 subtype of the avian influenza virus was isolated from a number of 6 wild birds (2 swans, 1 bald coot, 1 wild pigeon and 1 owl) out of 565 birds (percentage of ~1%) which were examined to establish the avian influenza diagnosis, in the period of time October 2005-June 2006.

Avian influenza represents a great danger for the entire world, but especially for Romania, because of the geographical particularities, such as The Danube Delta and the phenomenon of the bird migration, factors which are considered the natural reservoir of the avian influenza virus. In Romania, there are two large migration channels of the shore birds: from the north (especially from the Baltic Sea) and from the south (The Nile Delta, Dakkar and Africa below the Sahara desert) towards the Danube Delta or other meadows in the south of the country. On the map we can notice the dynamics of the migration in Dobrogea area, the main routes being highlighted in red and in yellow the secondary routes, of less importance for

migration, which are in fact deviations of some restraint effectives of birds from the traditional passing routes. The main coast routes or the ones situated next to the Black Sea coast are used mainly by the shore birds and by the ones adapted to the aquatic ecosystems as well as by a considerable number of small size and medium size species (passeriforms), which prefer these lines to the ones within the territory because of the fact that the resistance to going forward, given by the relief particularities, is considerably lower along the coast and above the sea compared to the inner side characterized by a steeper and more irregular relief.



Photo 1. The migration lines in Dobrogea ( according to Rudescu).

## 1. MATERIAL AND METHODS

Researching was conducted between October 2005-June 2006 on 565 wild birds, some of which having clinical signs (being in agony), and others being dead. The latter were subjects to laboratory examination to establish the avian influenza diagnosis. The birds were drawn from different areas of Constantza department. The laboratory examinations were carried out through the test of rapid detection of antigen of avian influenza virus. The test consisted in immunochromophotography on samples represented by cloacal and tracheal tampons and was carried out in The Sanitary-Veterinary and for Food Safety Laboratory in Constantza,

and for the positive samples the confirmation came from The Diagnosis and Animal Health Institute in Bucharest through RT-PCR testing and isolation of the virus on SPF eggs with embryos.

## 2. RESULTS AND DISCUSSIONS

The laboratory examination performed on samples taken from dead wild birds in Constantza department showed that a very little number of birds had been infected with H5N1- out of 565 birds examined in the period October 2005-June 2006 the bird flu virus was isolated only from 6 birds (1 wild goose, 2 swans, 1 bald coot, 1 wild pigeon and 1 owl) (Table 1), resulting a percentage of approximately 1%, low enough considering the fact that 14 hotbeds of avian influenza evolved on the territory of Constantza department in that particular period of time. The fact that more than 565 dead bodies were tested in order to identify H5n1 virus in approximately 6 months and the results were negative might lead to the hypothesis that there is the possibility that the wild birds diagnosed with the virus H5N1 to have been contaminated by sick domestic birds or by their dejections. This possibility may be sustained by the fact that the isolation of avian influenza virus from wild birds was performed after the appearance of bird flu cases among domestic birds. Thus:

On 22<sup>nd</sup> of February 2006 the A150 virus, H5N1 subtype was isolated from a swan corpse found on the beach of Eforie Sud (Photo 5). Eforie Sud is situated at a distance of 3 km from Tuzla, where a hotbed of avian influenza was declared on 16<sup>th</sup> of February 2006 as a consequence of isolation of the H5n1 virus from domestic poultry.

On 6<sup>th</sup> of March 2006 the A150 virus, H5N1 subtype is isolated from a bald coot corpse found on “the Sea Spell” beach in Eforie Nord ( Photo 3);

On 17<sup>th</sup> of February 2006 the A150 virus, H5N1 subtype is isolated from a wild pigeon corpse found near Mereni, a locality situated at a distance of 6 km from Tropraisar where the first hotbed of avian influenza of domestic poultry from Constantza department had been declared on 8<sup>th</sup> of February 2006.

In the city of Constantza the A 150 virus, H5N1 subtype is isolated on 25<sup>th</sup> of March 2006 from an owl corpse (Photo 8 and Photo 9)- and avian influenza is also declared in Navodari, the nearest town from Constantza city on 16<sup>th</sup> of February 2006.

Another example can be represented by the isolation of the avian influenza virus from two wild birds corpses found on 10<sup>th</sup> of October 2005

in different areas: a swan corpse found in the Sinoe lake area and a wild goose corpse found near Vadu Oii (a locality on the Danube bank), the death of these birds occurring after the declaration of the first avian influenza hotbed in our country-on 7<sup>th</sup> of October 2007, in Ceamurlia de Jos, in Tulcea department.

The bellow conclusions can be drawn:

- 1) Wild birds infected with a subtype of avian influenza virus of low pathogenity transmitted this virus to domestic poultry in the bodies of whom the virus suffered mutations and transformed into a high pathogen virus which led to the occurrence of clinical signs of disease and to death.
- 2) Subsequently, other wild birds contacted the highly pathogen virus from the infected domestic poultry, determining the sickness and death of wild birds.

**TABEL 1.** Tabel situation of wild birds investigated through laboratory examination in Oct.2005- June 2006

No	Area	Wild birds investigated through laboratory examination in Oct.2005-June 2006										
		Swan	Pelican	Bald coot	Seagull	Pigeon	Wild goose	Wild duck	Cormorant	Blackbird	Sparrow	Others**
1	Constantza	16	2	63	53	43	4	17	1	3	4	45/ 1P Owl
2	Mangalia area *	22	1	117	6	1	1	5	3	-	-	9
3	Techirghiol	-	-	-	-	2	1	1	-	1	-	1
4	Vadu Oii	-	-	-	1	-	1P	-	-	-	-	-
5	Năvodari	1	3	2	1	2	1	-	-	-	-	-
6	Corbu Lake	1	-	-	1	2	-	-	-	-	-	1
7	Sinoe Lake	3 / 1 P	-	-	-	-	5	16	-	-	-	-
8	Eforie Sud and Nord	5/ 1P	-	26/ 1P	3	-	-	3	-	-	-	5/
9	Cernavoda	-	-	-	-	-	1	3	-	-	-	-
10	Cumpăna	-	4	-	4	-	-	2	-	-	1	3
11	Tuzla	2	-	-	-	-	-	-	-	-	-	1

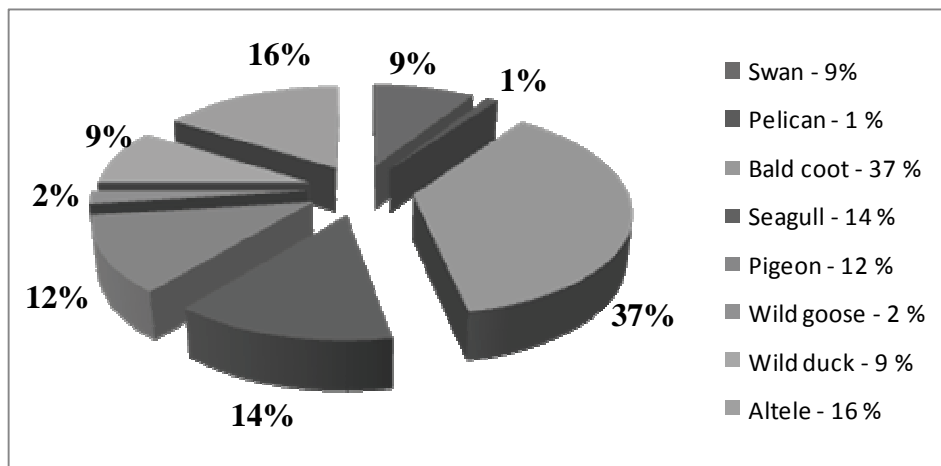
12	Medgidia	-	-	-	6	8	-	1	-	1	1	4
13	Mereni	-	-	-	-	1P						
13	Others				3	10		3				5
	TOTAL	51	7	208	78	68	13	51	4	5	6	74

Total of examined birds: 565

\*Mangalia area: Mangalia, Neptun, Venus, Saturn, Jupiter

\*\* crow, starling, magpie, jay, wood-pecker, stork, heron

P=positive



**Fig.1- Graphic situation of wild birds investigated through laboratory examination for diagnosis of avian influenza in Oct 2005-June 2006 (9%- wild duck; 2%-wild goose; 12%-pigeon; 16%-other; 14%-seagull; 9%-swan; 1%-pelican ;37%-bald coot).5**

**Wild birds corpses from which samples were taken for virusological exams for avian influenza diagnosis**

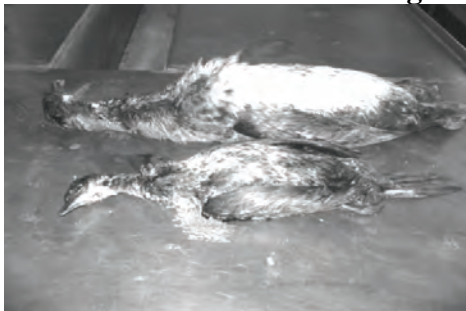


Photo 2. Cormorants



Photo 3. Bald coots

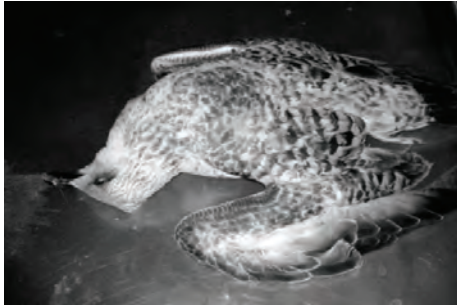


Photo 4. Seagull



Photo 5. Swan



Photo 6. Tracheal tampon prelevation from a seagull

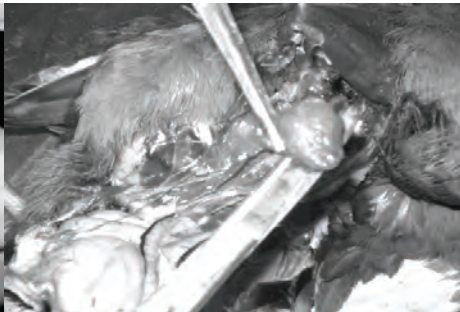


Photo 7. Organ prelevation  
from a bald coot corpse



Photo 8 and photo 9- Owl corpse-from which H5N1 avian influenza virus  
was isolated on 25<sup>th</sup> of March 2006





Photo 10- Wild ducks

### 3. CONCLUSIONS

3.1. Wild birds are carriers of a viral LPAI subtype and represent the main source of infection of domestic poultry. In the bodies of domestic poultry these LPAI viruses suffer mutations responsible for the modification of the chemical structure of hemagglutinin, with exaltation of pathogenicity and transformation of LPAI forms in HPAI forms, which pass from domestic poultry to wild birds. The wild birds which are infected with HPAI viruses manifest the disease clinically and in the end they die, but not before transmitting the infection to other domestic poultry and wild birds.

3.2. Wild birds are considered to be carriers of the bird flu virus of high pathogenicity, often manifesting a natural resistance, a fact which explains the occurrence of the disease and finally the death of the bird at very long distances of place and time from the avian influenza hotbed from which apparently they got the infection. The fact that wild birds infected with the flu virus don't manifest disease signs, being more resistant, allows them to move across long distances, thus transporting and eliminating the virus, leading to other wild birds or domestic poultry being contaminated.

3.3. Generally, only LPAI viruses can be detected in wild birds, except the cases when HPAI is spread by infected domestic poultry, therefore the isolation of HPAI strain occurring during the evolution of avian influenza hotbed in domestic poultry, in a geographical area near the

poultry infected with HPAI bird flu virus (as it was mentioned in chapter Results and discussions).

3.4. From all the birds diagnosed with avian influenza the H5N1 virus was isolated in the high pathogenic variant.

3.5. The table showing the lesions in wild birds is less expressed compared to the anatomopathologic lesions in domestic poultry; but there are situations when the contacted lesions were characteristic of avian influenza (owl: hemorrhagic lesions on serous of thorax-abdominal cavity, fat on the basis of the heart and organs, hemorrhagic proventriculitis, hemorrhagic duodenitis, hemorrhagic pancreatitis, etc).

3.6. Water birds are above all aimed by this virus and among these, especially ducks, geese, swans, seagulls and some limnicoles (shore birds). From all the species belonging to these groups, the following are more frequent in our country: the great duck (*Anas platyrhynchos*), small duck (*Anas crecca*), summer swan (*Cygnus olor*), laughing seagull (*Larus ridibundus*), lapwing (*Vanellus vanellus*). Ducks represent the natural “reservoir” of this virus, but in his less pathogenic form. Ducks are known to be relatively resistant to this H5N1 strain, they are rather the “carriers”.

3.7. Although a very high mortality was established related to wild birds before and during the evolution of bird influenza hotbeds in Constantza department, the avian influenza virus was isolated from a small number of wild birds. From the shore birds, especially the seagulls which are considered target species with a high risk of transmitting avian influenza, the virus was not isolated, although a number of 78 bird corpses had been examined (14% from the total of examined wild birds).

3.8. The fact that there was a great number of avian influenza hotbeds of domestic poultry in a relatively short period of time ( 14 hotbeds in 2 months), compared to a very small number of wild birds cases from which the flu virus had been isolated ( 6 bodies out of 565 investigated), may be explained by the fact that these birds were either infected with a low pathogenic LPAI virus ( that could have passed undetected during the rapid immunochromatographic tests), which transmitted mutations to poultry and transformed in HPAI flu virus, either our country was conveyed in transit by wild birds which were not carriers of avian influenza virus in such a high proportion as it had been expected to be.

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## MORPHO-CLINICAL ASPECTS IN EXPERIMENTAL TRICHINELLOSIS OF THE DOMESTIC PIG INFECTED WITH *TRICHINELLA BRITОВI* (partial results)

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**Key words:** Experimental *Trichinellosis*, *T. britovi*, gruntings, clinical manifestations

### SUMMARY

In this first part of the experimental paper the results of the morpho-clinical exam are presented, as peculiarities of the pigs experimentally infected with *Trichinella britovi*.

The working materials consisted of nine gruntings, aged three months and an average weight of 34 kg, which were infested with mice meat containing 400-500 *T. britovi* larvae per gram of meat, 110-120 self-isolated in cysts larvae being calculated for every kilogram of in vivo gruntings.

The results are described on the entire observation period and detailed with numbers, graphics, 9- points photographic images, regarding: the appetite, skin modifications, the body temperature, enteritis and diarrhea, manifestations of myositis, congestive-hemorrhagic lesions of the cloven hooves, Pneumonia lesions, neurological manifestations, located at SNC (world premiere in pig *Trichinellosis*, observed in human *Trichinellosis*) and the negative effects of growing fat.

The paper ends with twelve conclusions.

The results of the hematological exams will be presented in a following paper, highlighting the parameters specific to *Trichinellosis*, as well as those of the cytho-hysto-chemical and hysto-pathological of the musculature during the migration until the self isolation in the cyst of the *Trichinella spp* larvae.

It is well known that *Trichinellosis* is a serious zoonosis which manifests itself acutely, sub-acutely, chronically and a-symptomatically, depending on the number of the secluded *Trichinella* larvae eaten in the same time with the pork, wild pork, bear and other animals, meat that has not been subjected to thermal sterilization or congealment.

While in human medicine, Professor Butila and not only him, have expressed their opinion, even since 1964, that “*Trichinosis- the diagnosed clinical disease – represents a tiny percentage of the total amount of undiagnosed or with sub-clinical evolution infestations*”, in the animal medicine, especially the domestic pig, symptoms like the **appetite**- absent or reduced- **diarrheas** -sometimes with blood, **walking difficulties**, accompanied by **pains** (squealing), pains **at pressure in different anatomical areas**, Kyphosis position, **hoarse squeaking**, **sub-fever** and even **fever**, **skin congestions on the body** and, **most frequently, at the ears level to almost cyanosis**, horripilations- common in some feverish, allergic states- **neurological and heart disorders**, are not brought out into discussion for the probability of diagnosing the *Trichinellosis*, even in favourable conditions from an epidemiological point of view (the existence of rats, cannibalism etc.)

The considerably big number of animals (domestic pigs, wild boars, bears, foxes) positive at the trichinoscopic exam, with a high intensity through the extremely big number of cysts with *Trichinella* in the striated skeleton musculature, as well as the serious evolution, with clinical manifestations that usually lead to the death of the humans, convince us that, at least in domestic pigs, there were cases of clinically manifested *Trichinellosis*, but undiagnosed, being attributed to the allergic states, intoxications, the keeping conditions, with shelters that have rough pavement etc.

### **The purpose of the paper**

Given the fact that in human medicine there are numerous theses communicated at national and international level, in which data are presented that create an anatomical-clinical picture and a laboratory diagnosis of the *Trichinellosis* (synthesized in a volume elaborated by FAO/WHO/OIE, edited by Jean Dupouy-Camet, the President of the International Committee for *Trichinellosis*), we proposed that, through an experimental infestation of a group of young pigs, to sketch an image regarding the morpho-clinical aspects of the porcine *Trichinellosis*.

A beginning of this idea was made at V.A. Cluj-Napoca , by a group of people (Miruna Oltean, Adriana Titilincu, Dupouy-Camet, A. Fenesan, V.Cozman), in co-operation with Dupouy-Camet- from France, through the study of some bio-chemical, hematological and serological modifications at the experimental infestation with *Trichinella britovi* of pigs.

In this paper we present the anatomical-clinical peculiarities of the disease, regarding the aspects accessible to the practitioners, like: the appetite, the body temperature, evacuation and the consistency of the excrements, the clinical manifestations of myositis (mastication, aphonia, heavy walking, pains), congestive- bleeding lesions at skin level, the ears, determination of the *Trichinella* larvae and adults by coproscopy , and through the biopsy of the auricular and coccygeal muscles, the trichinoscopy is made, during the experiment the differences in the growth were registered, compared to the witnesses.

In a later paper we shall present the results of the hematological exam through pointing out the parameters specific to *Trichinellosis* (eosinophils, neutrophils, muscular enzymes- creatininfosfochinaza, (CPK) and lactodehidrogenaza (LDH)-as well as of the cytho-hysto-chemical and hysto-pathological exams of the different categories of striated muscles, in which the larvae have isolated themselves, staged on a 60 days period, until the completion of the larvae isolation period.

## 1. MATERIALS AND METHODS

### *Working materials*

There were nine infested gruntlings, three months aged, having an average weight of 34 kg, taken from a specialized unit, disease free, from a lot of 950 heads, which constituted a witness lot for registering the growth and health state; the experimentally infested gruntlings were isolated in a place with no epidemiological risk, fed very well with the same fodder recipe as the witness' lot and individualized by eartags.

The infesting material was formed of mice, infested, in their turn, with *Trichinella britovi*, confirmed by molecular tests (PCR), obtained through artificial digestion, in order to maintain the respective species of *Trichinella* in the laboratory of the Diagnosis and Animal Health Institute from Bucharest; we mention that, in Romania, the domestic pig, the wild boar, the bear, are parasitized with *Trichinella britovi*, too- a confirmation made through the TRICHIPORSE PROGRAMME, elaborated and financed by the European Community in the period 2002-2004, at the International Reference Centre for *Trichinella* of the Superior Health Institute in Rome-Professor Eduardo Pozio; at the mice from IDSA, through the determination of the infesting degree was established that the intensity of the *Trichinella* parasite was of 400-450 larvae per gram of meat, which means 4000-4500 isolated larvae per 10 grams of mouse-without skin, head and internal organs; for a young pig of 34 kg, 110-120 isolated larvae were administered for 1 kg of in vivo weight.

The mice that constituted the source of infestation for the pigs had been infested 45-50 days before and at the trichinellosopic control, a complete self- isolation of the *Trichinella* larvae was observed, which were able to re-infect other animals.

### **The working methods**

1.1. The infestation of the gruntlings was made the same as in natural conditions, through the oral administration of a mouse that contained 4000- 4500 larvae, for each animal that ate and swallowed, without rejecting the sample.

1.2. The observation regarding the health state was made daily, in the morning and in the evening, when the body temperature, the presence of appetite and the defecation (the consistency of the excrements) were controlled.

1.3. In the 5<sup>th</sup>-15<sup>th</sup> days post-infection, the excrements were gathered in order to discover the presence of the adult forms of *Trichinella*, eventually larvae eliminated by females, then, they were controlled in the intestines' content of the sacrificed pigs.

1.4. Depending on the disease evolution and, especially on its gravity, up to 30 post-infection days 2 or 3 gruntlings were sacrificed, one of them being in agony.

1.5. For the sacrificed pigs, according to Table 1, muscle samples were drawn, double for each group with a view to perform the trichinelloscopic exams in the Parasitology laboratory and cytho-hystopathological exams in the morpho-pathology laboratory from IDSA. The muscle samples were taken from: the tongue, the larynx, the masseters, the auditory, the oculars, the diaphragmatic pillars, inter-ribs the fore and hind legs, coccigians, heart; for the cytho-hysto-chemical and hysto-pathological exams were also drawn portions with obvious lesions from the duodenum and jejunum, as well as from the brain and lungs.

1.6. For a self-evident image of comparing the negative effects determined by the evolution of the *Trichinellosis*, the witness lot is considered the lot from which the experiment animals were extracted, in this regard we present the minus growth at the pigs infested opposed to their brothers.

## 2. RESULTS AND DISCUSSIONS

### 2.1 The appetite

The appetite of the gruntlings infected with *Trichinella britovi*, was characterized on the entire period of the experiment by disorexia, respectively, by the lowering and disappearance of the appetite (anorexia) in the period of 3-45 days post-infection, with a maximum curve between 15-30 days, after which was observed a gradual return at the normal appetite up to 54 days, we specify that in the period between 15-35 days, the animals preferred liquid fodder because of the myositis in the mastication and swallowing area, after the localization of the *Trichinella spp* larvae. In this period, dominated by diarrheas, the appetite for water has grown.

### 2.2 Cutaneous manifestations

Within six post-infection days passive hyperaemia phenomena appear, as a result of some disorders in the returning blood circulation, through the accumulation of some amounts of blood in the venous tree, which characterizes a stasis hyperaemia; the colour of the blood becomes red-bluish-cyanotic-sometimes blackish, oedematous, (visible at human at the eyelids and face level); at the infested pigs it was observed on the whole body, but, most frequently- at most of the pigs involved in the experiment, at the head and the auditory pavilion extremities level.



**Fig. 1.** Allergic dermatitis-  
congestive aspect



**Fig. 2.** Auditory stasis oedema

The above mentioned phenomenon coincides with the migration period of the *Trichinella* larvae, which penetrate the intestinal wall and enter in the blood and lymph circulation, initially peripheral, then general, and the explanation of the clinical manifestations at the cutaneous level consists in the hypersensitivity of the body towards the *Trichinella* larvae, and in a strong allergic reaction, determined by the destruction of the larvae which can not locate preferentially in the muscular fibre, becoming erratic in other tissues or organs.

The fact that the *Trichinella* larvae are gradually eliminated in the intestines by the mother-female within a period of 5-7 days and up to 30-35 days, the evidenced clinical phenomenon can be considered an anaphylactic shock, extended to the period mentioned.

The venous hyperaemia at the auditory level constituted a serious difficulty for taking blood samples because the area became oedematous and the blood could not flow, invading the tissue.

### **2.3. The Fever**

The fever, determined through rectal thermometry has begun to grow since the second week, after 2-3 days after the debut of the *Trichinella* larvae migration and knew an ascending curve until the end of the 3<sup>rd</sup> week, continuing with maximum values of 40.6- 41.3 °C to the end of the 5<sup>th</sup> week., then, it entered in a descending curve, becoming normal after 40 post-infection days.





Fig. 3 Auditory stasis oedema

Table 1

The evolution of the body temperature at the gruntlings experimentally infected with *Trichinella britovi*

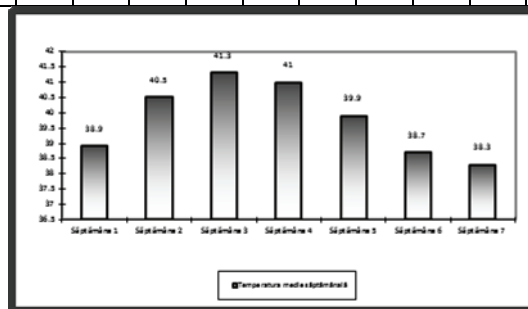
Date of infestation: 05.06.2011

Temperature taken between 5-7 p.m.

Week	Day	Data (2011)	Day No.	1	2	3	4	5	6	7	8	9	Daily average T0	Weekly average T0
				R2c	Rs	Vs	G2c	V1c	R1c	V2c	G1c	G5		
1	V	06.05	1	38,5	38,7	38,6	39,0	39,2	38,5	38,4	37,6	37,8	38,3	38,9
	S	07.05	2	38,4	38,2	38,7	39,1	39,0	38,8	38,5	37,9	37,8	38,3	
	D	08.05	3	38,6	39,0	38,6	39,0	39,2	38,8	38,8	37,8	38,2	38,5	
	L	09.05	4	39,2	39,3	38,8	39,4	39,4	39,2	39,0	38,4	38,5	39,0	
	M	10.05	5	39,1	39,7	39,2	39,6	39,6	39,5	39,4	38,8	38,6	39,2	
	M	11.05	6	39,6	39,8	39,6	9,5	39,8	39,8	39,7	39,5	38,9	39,5	
	J	12.05	7	39,8	40,0	39,7	39,7	40,2	40,2	40,0	40,2	39,0	39,7	
2	V	13.05	8	40,2	39,8	39,7	39,9	40,4	40,1	40,3	40,5	39,0	40,0	40,5
	S	14.05	9		40,2	40,0	39,6	40,6	40,5	40,6	40,2	39,5	40,1	
	D	15.05	10		39,6	40,4	40,1	40,8	40,6	40,5	40,6	40,1	40,3	
	L	16.05	11		39,8	40,2	39,7	40,9	40,8	40,9	40,9	40,6	40,8	
	M	17.05	12		40,3	40,7	40,6	40,9	71,0	41,2	40,6	40,2	40,6	
	M	18.05	13		40,6	40,8	40,7	40,8	41,2	41,1	40,5	40,0	40,7	

Week	Day	Data (2011)	Day No.	1	2	3	4	5	6	7	8	9	Daily average T0	Weekly average T0
				R2c	Rs	Vs	G2c	V1c	R1c	V2c	G1c	G5		
	J	19.05	14		40,5	40,6	40,7	41,3	41,2	41,3	41,3	40,3	40,9	
3	V	20.05	15		40,9	40,9	40,9	41,4	41,1	41,2	41,2	41,4	41,1	41,3
	S	21.05	16		40,0	41,2	41,2	41,3	41,3	41,1	41,2	41,6	41,1	
	D	22.05	17		41,5	41,4	41,5	41,2	41,4	41,4	41,3	41,4	41,4	
	L	23.05	18		41,4	41,4	41,6	41,3	41,1	41,4	41,2	41,3	41,3	
	M	24.05	19		41,6	41,6	41,4	41,1	41,0	41,2	41,1	41,1	41,3	
	M	25.05	20		41,5	41,5	41,5	41,4	41,0	41,3	41,0	41,2	41,2	
	J	26.05	21		41,6	41,4	41,6	41,3	41,1	41,1	41,0	41,1	41,3	
4	V	27.05	22			41,6	41,5	41,2	41,1	41,1	41,1	41,0	41,2	41,0
	S	28.05	23			41,7	41,7	41,2	40,9	41,2	40,7	41,2	41,1	
	D	29.05	24			41,8	41,8	41,8	40,9	41,1	41,0	41,0	41,3	
	L	30.05	25			40,9	41,5	40,9	40,8	41,2	40,6	41,0	41,0	
	M	31.05	26			41,5	41,7	41,2	41,2	40,8	41,1	40,5	41,1	
	M	01.06	27			41,8	41,6	41,4	41,1	40,7	40,0	40,2	40,9	
	J	02.06	28				41,6	41,2	41,4	39,6	39,7	40,3	40,6	
5	V	03.06	29				40,5	40,4	40,6	40,2	39,6	39,8	40,1	39,9
	S	04.06	30				40,2	40,5	41,2	40,1	39,7	39,8	40,1	
	D	05.06	31				40,4	40,6	41,1	39,8	39,8	39,5	40,4	
	L	06.06	32				40,6	40,7	40,5	39,8	39,6	38,2	40,2	
	M	07.06	33							39,5	39,6	39,2	39,4	
	M	08.06	34							39,3	39,5	39,0	39,3	
	J	09.06	35							39,2	39,5	38,8	39,5	
6	V	10.06	36							39,4	39,1	38,6	39,4	38,7

Week	Day	Data (2011)	Day No.	1	2	3	4	5	6	7	8	9	Daily average T0	Weekly average T0
				R2c	Rs	Vs	G2c	V1c	R1c	V2c	G1c	G5		
7	S	11.06	37								39,4	39,0	39,0	39,1
	D	12.06	38								39,3	38,8	39,0	39,0
	L	13.06	39								39,5	38,3	38,7	37,7
	M	14.06	40								39,4	38,1	38,5	38,6
	M	15.06	41								39,5	38,7	38,6	38,9
	J	16.06	42										38,4	38,4
	V	17.06	43										38,2	
7	S	18.06	44										38,5	
	D	19.06	45										38,4	
	L	20.06	46										38,4	
	M	21.06	47										38,2	
	M	22.06	48										38,0	
J	23.06	49										37,2		
8	V	29.06	54									38,2		



**Fig. 4.** The debut of the body temperature growth was accompanied by horripilation, the maximum fever limits being registered between the 14<sup>th</sup> and 42<sup>nd</sup> days of disease evolution.

The weekly average temperature

The graphic representation with the average values of the body temperature of the gruntlings experimentally infected with *Trichinella britovi*.

#### 2.4. Enteritis, accompanied by diarrheas

The enteritis, accompanied by diarrheas, characterizes the enteral period of the infection, respectively through the fixation of the encysted larvae in the epithelium of the mucous membrane of the intestines belonging to the duodenum and the jejunum, passing through shedding hair corresponding to the period L1-L5, the maturation and differentiation of the sexes, the laying by the females of the larvae which pass through the intestinal wall in the lymphatic and blood vessels, was present, with different intensity, at all the pigs.



**Fig. 5.** 30 days after infestation. The consequences of the enteritis (dehydration, loosing weight, dirty posterior



**Fig. 6.** 30 days after infestation .Intestines without food content and congestive haemorrhagic aspect



**Fig. 7.** 53 days- intestines with food content, without congestive haemorrhagic aspect

Through the mechanical, toxic and inoculating action of the *Trichinella spp* larvae, there are breakings of enterocytes determining inflammatory processes with serious consequences and also on the obstruction of the nutritive elements, necessary to the body; the inflammations have exudative character with serous, fibrin and haemorrhagic characters; serous exudates, to which is added that of the membranes, determines the detachment of the epithelial cells of the membranes, producing the exfoliation catarrh, the most frequent form of the catarrhal inflammation (Paul I., 1998), which also explains the long period of diarrhoeas registered at the pigs in the experiment.

The explanation of the prolonged enteritis and of the diarrheas with fluid content, sometimes with streaks of blood consists in the rather large period of larvae elimination (7- 35 d.p.i.), which cross the intestinal wall to start the migration.

At the pigs that exceeded 35-37 days post infection, the diarrhoeas stopped, once they gained their normal appetite.

#### 2.5 Myositis. Clinical manifestations

Myositis was present with different intensity in all the pigs within the period of 20-45 days post infection, through: muscular pains, squeals when pressing, hoarse squeaking, kyphosis position, heavy mastication.



**Fig. 8.** 30 dpi Kyphosis position, back pains at pressure



**Fig. 9.** 45 dpi, Kyphosis, consecutive to the legs and dorsal-muscular pains

## 2.6 Congestive-haemorrhagic lesions

Congestive-haemorrhagic lesions at the level of the cloven hooves similar to those in human *Trichinellosis*.

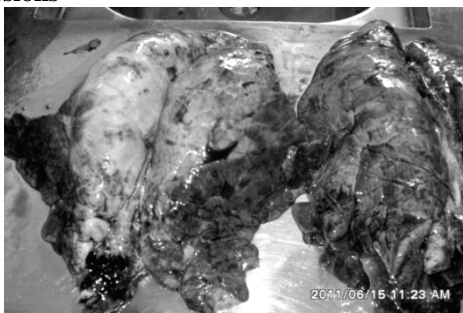


**Fig. 10.** Congestive-haemorrhagic lesions situated under the hooves



**Fig. 11.** At 39 days. Animal in agony; the necropsy revealed pulmonary lesions

## 2.7. Pneumonia lesions



**Fig. 12.** Pneumonia lesions- the apical and diaphragm lobes 39 days after infestation.  
Pneumonia lesions

## 2.8. Neurological manifestations

The neurological manifestations of the pigs artificially infested with *Trichinella britovi* are exemplified in the figures 13 and 14 in which the animal number 5, the female V1c, 30 d.p.i. manifests imbalance in walking, dromomania, sensorial deficiency, characterized by losing sight (blindness), striking other animals whom it pushes (to see the modifications of the scapular belt fig. 13 or the walls, which it also pushes), in which can be seen that the animal squeals-having its mouth open.



**Fig. 13.** Neurological manifestations at SNC level. **Fig. 14.** Dromomania, blindness

**2.9 Negative effects determined progressively by the evolution of porcine *Trichinellosis***

The negative effects determined progressively by the evolution of the *Trichinellosis* in the pigs experimentally infested were established by the weight of the sacrificed pigs, comparatively with the increases obtained with their fellows from the lot existent in the donor unit.

In the next table there were taken into consideration the following elements: the sex of the animals, the individual eartag, the sacrificing date and the number of post-infection days, weight at scarification, the average weight of the witness lot at the moment of sacrificing of every infested animal, the deviation and the minus percentage.

*Table 2*

Table with the fatties (gruntlings) subjected to the experimental infection

Date of the experimental infestation: 05.06.2011

Average weight in the infestation day: 34 kg

Differences of weight opposed to the witnesses

No of anim.	Sex	Eartag	Date of sacrifice	Days p.i.	Sacrification weight (kg)	Average weight Witnesses(kg)	Deviation in minus (kg)	% in minus	Obs.
1	2	3	4	5	6	7	8	9	10
1	M	R 2n	05.13.2011	7	32,500	39,880	7,380	18,5	
2	M	R 5s	05.26.2011	20	23,700	50,880	27,180	46,5	

3	M	G s	06.01.2011	25	24,600	55,000	30,400	44,7	
4	M	Y 2n	06.06.2011	30	26,200	59,200	33,000	44,3	
5	F	G 1n	06.06.2011	30	24,200	59,200	35,000	40,8	
6	F	R 1n	06.06.2011	30	20,800	59,200	38,600	35,3	
7	F	G 2n	06.15.2011	39	19,500	66,760	47,260	29,2	
8	F	Y 1n	06.15.2011	39	22,250	66,760	44,510	33,6	
9	M	Y s	06.29.2011	54	31,400	79,360	47,960	39,6	

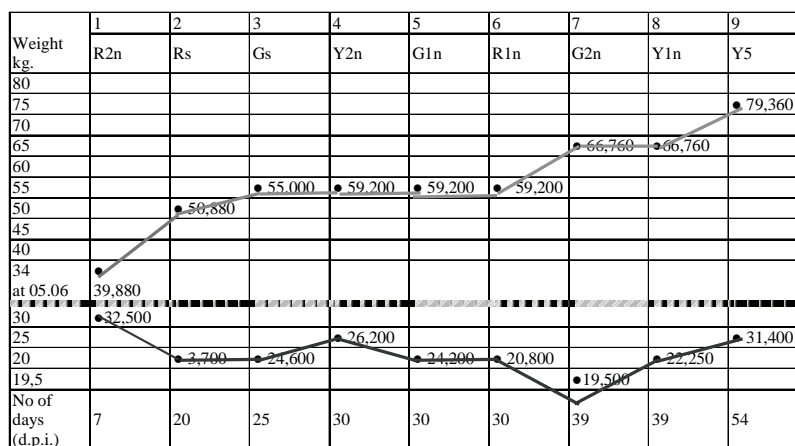
Legend: R, G, Y- the colours of the eartags: red, green, yellow  
S=simple, 1n= 1 notch, 2n= 2 notches

Table 3

Table with the fatties (gruntlings) on the experimental infection

Date of the experimental infestation: 05.06.2011

Average weight in the infestation day: 34 kg



**NB** If we consider the losses of the pigs with clinical forms of *Trichinellosis* on a period of 40-45 days of illness, after which they resume their appetite, we can record a loss of almost 40% from the weight increase, to which we add one pig dead of nine, the waste of fodder, we evaluate losses of over 50% from the value of a normal pig at sacrificing.



### 3. Conclusions

3.1. Similar to human *Trichinellosis*, in which the history, the epidemiological aspects, the clinical manifestations lead to a presumptive diagnosis of *Trichinellosis*, also in the case of the domestic pig, there are some clinical elements that lead to the probability of this diagnosis: of course, the confirmation of the disease is made through laboratory tests, stated nowadays in human medicine and the diagnosis of *Trichinellosis* is to be outlined *in vivo* at pigs when the results presented in this paper will be supported by the results of the researches in progress.

3.2. From a clinical point of view, there is a real similarity between the human *Trichinellosis* and the one we made, experimentally, in the domestic pig.

3.3. The clinical manifestations presented in this paper and exemplified iconographically, mostly met also in humans are:

the decrease or disappearance of the appetite-anorexia-in the period between 3-45 days post infection, with a maximum curve between 15-30 days; difficulties in gripping, chewing, ingestion of the coarse fodder because of the myositis in the chewing and swallowing area; in this period dominated by diarrheas, the appetite for liquid is high.

3.4. At six post infection days (d.p.i) passive hyperaemia phenomena appear through accumulation of venous blood, characterizing a stasis hyperaemia obvious at the head level, especially of the ears and eyelids.

3.5. The fever, determined through rectal thermometry, increases, in average, since the second week to 39.5, then, weekly, at 40.9, 41.3, 39.5, in the 5<sup>th</sup> week when it decreases at 38.4, 38.2 in the 6<sup>th</sup>-8<sup>th</sup> weeks.

3.6. The enteritis, accompanied by serous, serous-mucous diarrheas, sometimes with blood streaks, is the result of the *Trichinella* larvae migration through the intestinal wall, the little lymph and blood circulation

to the place of choice-the muscular fibre (myocytes) on the whole period of 7-35 days post infection.

3.7. The clinical manifestations of myositis installed with acute forms in the period of larvae location in the myocytes (20-45 days) is manifested through: heavy walking, extended decubitus, muscular pains when pressing with the hand, hoarse squealing, kyphosis position, difficulties in swallowing etc.

3.8. Congestive-haemorrhagic lesions at the hooves level, similar to those of humans.

3.9. Pneumonia lesions located at the apical and diaphragm lobes

3.10. Neurological manifestations in pigs in the figures 17 and 18- which represents news for animals, but very much exemplified in humans.

3.11. In the end we presented the effects regarding the losses in the weight increase on the whole period of the disease evolution, through graphic tables- which lead to the conclusion that *Trichinellosis*, through its clinical and even sub-clinical (a-symptomatic) evolution determines important economical losses.

3.12. We have the hope that through our results we gave a signal for intensifying the research in the field of *Trichinellosis* on two considerations: it is a serious zoonosis and it determines important losses in animal production.

#### ACKNOWLEDGEMENTS

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## **RESULTS OBTAINED WITH THE TREATMENT OF PARALITIC MIOGLOBINURIC MYOPATHY IN HORSES CLINICAL CASES**

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During 2010-2011 I had a number of 8 horse owners from the neighbouring villages that came at the veterinary clinic situated in Crevedia, Dambovita asking for a diagnostic and treatment of their animals.

Upon arrival at the sites where horses were located I noticed the following facts:

- the horse shelters were in very good order
- horse feeding was well done, with quality food
- most of the horses were heavy traction races, adults over 4 years old , one of them 8 months old male
- the horses were having a sedentary life, once subjected to an effort the signs of illness appeared, regardless of the season or weather conditions.

From the anamnesis resulted that the animals never suffered from this condition. The disease appeared suddenly after they were pulling a carriage for a distance of approx. 100-200 meters. They were slowing down, became restless, rigid, unsure walking, especially affecting the rear feet, accelerated breathing with dilated nostrils, abundant localised or generalised sweating, muscular tremors and melanuria. The animals that kept walking fell to the ground, were removed from the carriage and difficultly walked home.

The animals were treated for 2-3 days by the veterinary personnel from the respective village without any results of improvement.

At the time of my examination the animals were in lateral decubitus for 1-2 days, abundantly sweating, repeated tremors, melanuria, cyanotic conjunctive, hardly breathing, dispnea, tachycardia. One of the horses was standing, having the masseter muscles swollen and disfigured.

The diagnostic was determined from the anamnesis and clinical exam.

**TREATMENT:**

- Xilazine I.V. 3-4 ml/kg of bodyweight
- large venisection 5-8 liters
- perfusion: fiziological serum 2 liters, glucose 5% 500 ml, calcium borogluconate 150 ml, vitamin C 100 ml –twice a day for 5 days
- sodium selenyte 1/1000 50 ml S.C. once
- vitamin E 20 ml I.M. 3 times, 2 days apart
- Artrivet forte 30 ml I.V. the first day, 20 ml I.V. the second day, 10 ml I.V. the third and fourth day, 5 ml I.V. the fifth day.

The first day following the beginning of the treatment the horses were allready standing on their feet without showing any more signs of illness. The treatment was applied for a period of 5 days. The illness never reappeared.

The photographs are taken at approx. 1 year ater the treatment. Below each photo is the name of the owner and the name of the horse.

The efficiency of the treatment is very good and for this reason I recommend it to all praticians to use it with confidence.



Owner: Nicolae Lica, Brezoaiele, Dambovita 21.02.2011



Owner: Alexandru Ionita, Bărbuceanu —Butimanu 11.03.2010



Owner: Păun Marin, Ciocănești —Dâmbovița 08.12.2011



Owner Nicoleta Paun, Manesti, Dambovita 12.04.2010



Owner: Marin Paun, Ciocanesti, Dambovita 10.11.2010





Owner: : Tudor Ilie Crevedia —Dâmbovița 07.01.2011



Owner: : Gheorghe Nicolae Gulia —Tărtășești 09.03.2010



Owner: : Păun Marin Ciocănești —Dâmbovița 12.06.2009

## **ZOONOSIS -IMPAIRED NERVOUS SYSTEM IN HUMAN TRICHINELLOSIS**

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**Keywords:** trichinellosis ,headache, vertigo.

### **SUMMARY**

The emergence of a neuro-psychiatric symptomatology consisting of: headache, fatigue, dizziness, drowsiness, agitation, anxiety, photophobia, meningism can take various forms of occurrence in human trichinellosis, ranging from clinical hidden forms to severe damage to the cerebral cortex.

In a group study consisting of 327 patients diagnosed with trichinellosis in Infectious Diseases Hospital Brasov we watched the incidence of various neuro-psychiatric manifestations, laboratory constants electroencephalo-graphic and computer tomography registered and we analyzed the frequency of headache associated or not with vertigo reported to the clinical forms of disease.

The danger of associated zoonotic diseases is increasing measures were at the national and local level in order to establish the places where animals can be accepted and to prevent environmental pollution. Currently research on zoonoses is carried out in teams/communities of a medical or veterinary profile. Both the I.O.E. and the European Commission For Consumer Health and Protection have presented recently documents through which the national authorities are encouraged to use harmonized schemes for obtaining data that is comparable and representative in evaluating the sources and evolution tendencies of zoonose and zoonotical agents. The common order of the Ministry Of Food And Agriculture and the Health Ministry no. 474/1993 concerning the preventive health services that is assured by the veterinary and human health representatives for the prevention and treatment of animal transmitted diseases.

In trichinellosis the nervous system disorders are multiple. Headache, sometimes very intense is accompanied by dizziness, marked agitation or drowsiness. It may occur delirium, convulsions, meningism, encephalitis, peripheral polyneuritis, hemiplegia with tendon reflexes changes.

In the first phase of the disease from a morphologically point of view are mentioned some changes of the meninges and the nervous substance. Thus, in severe cases there are bleeding points in the brain white matter, microscopically represented by blood suffusions.

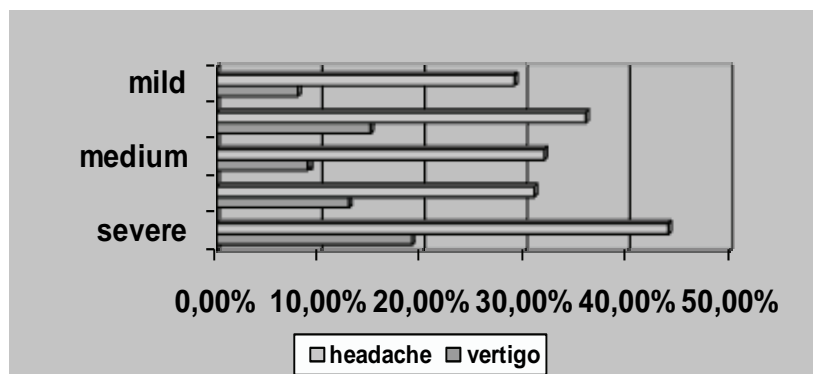
### 1. MATERIALS AND METHODS

The study was conducted on a group of 327 patients diagnosed with trichinellosis in Infectious Diseases Hospital Brasov and it followed the incidence of various neuropsychiatric events, knowing that many of these events occurred in trichinellosis are nonspecific, being found in many other diseases. These were: headache, fatigue, dizziness, vertigo, agitation, drowsiness and they are mentioned in the literature. Headache occurs in 33.33% of the examined cases and vertigo in 12.23% of the cases.

In our study the incidence of headache in the medio-severe and severe forms has almost the same percentage to the incidence of headache in mild and sub medium forms of the disease of 33%. (See diagram no. 1) We have reported the incidence of headache and vertigo in the clinical forms of trichinellosis. The second diagram shows that in the severe form the highest percentage of patients who had headache (44%) associated or not with vertigo (19%) is recorded. I haven't thoroughly examined the headache intensity considering it a subjective parameter.

When the intensity of these symptoms dominates the clinical picture, raising the suspicion of trichinellosis is very difficult.

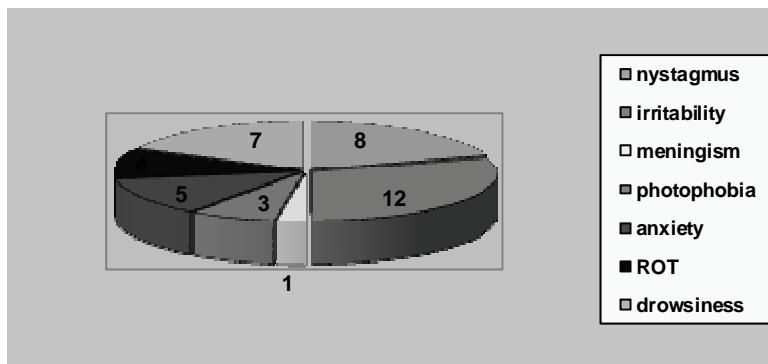
**Diagram no. 1.** The incidence of headache and vertigo in clinical forms of trichinellosis



They have an increased life-threatening and the medical history taken from confused patients or from the family is often collected with great difficulty.

Epizootical and epidemiological data are often inconclusive and sometimes absent. Thus, in cases with: focal or diffuse paralysis, meningitis, encephalitis, meningoencephalitis, subarahnoid bleeding, stroke, polyneuritis, radiculopathies, sacral spine pain only a hematological exam with a high eosinophilia can direct the diagnosis to trichinellosis and subsequently carry out the investigations to additional laboratory analysis. But it must be known that in almost all forms of trichinellosis the psychiatric manifestations are reported as minor events and they can reach major manifestations with: apathy, dizziness, depression, delirium or even coma. Of the 327 cases of trichinellosis studied, only 40 (12.37%) of the patients showed: agitation (12 persons) (30%), nystagmus (8 persons) (20%), somnolence (7 persons) (17, 5%), anxiety (5 persons) (12.5%), live tendon reflexes (4 persons) (10%), photophobia (3 persons) (7.5%), meningism (one person) (2.5 %). (see diagram no. 2).

**Diagram no. 2.** Other neurological and psychiatric manifestations in trichinellosis



Computer tomography (CT), magnetic resonance imaging (MRI) remain reserved for the cases of scientific studies or according to the possibilities and need for severe forms, particularly with cardiac, renal or neurological complications.

In case of damage to the central nervous system (CNS) CT scan shows multiple, tiny, hemispheric hypodensities by 3-8 mm in the white

matter, observed by using intravenous contrast substance. In our study performing a CT scan to a small number of cases (2), did not signal changes in the central nervous system. In the literature there are cases (between 8-24% of investigated patients) where the lumbar puncture revealed *Trichinella* larvae and numerous eosinophils. The electroencephalogram performed in our prospective study to 9 from 21 patients, showed a pathological path-wave peak discharge, high voltage in the case of two children suffering from idiopathic epilepsy.

## 2. RESULTS AND DISCUSSIONS

Capo V. and Despommier D.D. describe in trichinellosis three types of central nervous system damage: perivascular infiltrates with small hemorrhages and endothelial proliferation without alterations in cerebrospinal fluid, which correspond to nonspecific clinical manifestations, which occur in 10-20% of cases: headache, lethargy, confusion, decrease or absence of tendon reflexes; granulomatous lesions with lymphocytes, plasma cells, gigantic cells nodules containing the parasite, accompanied by alterations in cerebrospinal fluid; these lesions are manifested by severe specific brain disorder, which are fatal in 33% of cases; acute meningoencephalitis accompanied by cerebral edema with inflammatory infiltration of leptomeninge and cortex, with clumps of eosinophils and evidence alterations of cerebrospinal fluid( Capo and Despommier ,2000).

Meltzer D.M. and Bockmann A. - 1957, Morh W. - 1961, Obodowska-Zysk W. - 1961, are describing cases of encephalitis during trichinellosis. Bals M. and Botgros V. report a case of trichinellosis with right hemiplegia and dysarthria(Bals and Botgros,1960). Obodowska-Zysk W. - 1961, mentions two cases of meningoencephalitis and hemiplegia and Kerestely I. indicate the occurrence of meningeal reactions.( Kerestely I. et all. , 1966 ). Paresis or paralyzes of peripheral nerves have been reported by Ciorapciu.( Ciorapciu and Ciuplea, 1960). Also in 1962 Edwards J.L. and Hood C.J. and all conclude that in addition to parasite antigens which are a cause of meningoencephalitis's phenomena it is also necessary sensitizing the altered brain proteins through the mechanism of self harm. Rosalyn S. et all. show that the headache is present in 50-60% of the cases infected with *Trichinella spiralis*; the same incidence of headache is found by Swat G. et all. in 50-55% of the cases with trichinellosis. Compared with our observations Nemet

Codruta et al. recorded from 1.71% of the studied cases events like: nystagmus (4), anxiety and depression (2), agitation and irritability (2), meningism (also one case), dysarthria (1). Nemet Codruta et al. recorded immediate neurological complications in 18% of patients from the study, these complications seemed like polyneuritis, facial paralysis, sacral spine pain, Jacksonian seizures, strokes (Nemet and Minea,2000). In our study there was no recorded case with immediate or delayed neurological or psychiatric complications. No case in the study group has not shown signs described in the literature as: meningitis, encephalitis, focal or diffuse paresis, subarachnoid bleeding or strokes. Recent research came up with concrete arguments supporting the presence of encephalitis and polyneuritis syndrome, hemi paresis, mental delirium, excitation, erratic movements, meningitis, sub cortical infarction, transient coma.

Mawhorter S.D. et al. describe neurological signs of meningitis or encephalitis in 10% to 24% of patients with severe trichinellosis. The pathogenic mechanism for making the neurological signs and symptoms include cerebral edema, hemorrhages, embolism and perivascular infiltrates. The literature describes cardio-neurological syndrome, which manifests itself in a few days after the general symptoms and before muscle invasion. Cardio-neurological syndrome was studied on patients with neurotrichinellosis by CT, simultaneous with myocardial damage indicated by changes in the ECG tracing and changes of CPK-MB isoenzymes. This syndrome includes: diffuse encephalopathy, focal neurologic deficits at the same time, witch on brain CT appear as small hypodensities affecting the cerebral white matter; heart disease by myocardial injury and / or myocardial infarction, sinoatrial node dysfunction, congestive heart failure, everything in a high hypereosinophilia circumstance (more than 4000 eosinophils /mm<sup>3</sup>). The mechanism of neurological and myocardial injury has proven to be fundamentally ischemic, as a consequence of arteriolar microthrombosis in the brain and myocardium, which were highlighted at post-mortem histopathology ( Taratulo andVenturiello, 1997). In our study we found no such case. Dalessio D.J. et al. report neurological damage from 10 to 24% of the trichinellosis cases, of which 53-96% meningoencephalitis, 40-73% paresis or focal paralysis, 39-71% delirium, 20% areflexia, 17% meningitis, 2% psychosis.

### 3.CONCLUSIONS

3.1. A high percentage of patients in addition to neck muscle myalgia and headache have associated vertigo and / or photophobia. The study showed that photophobia associated to cervical myalgia was reported only in the severe clinical form and constitutes the criterion of seriousness that usually emphasizes a current or ongoing neurological damage, while vertigo and headache, accompanied by cervical myalgia decay from the medio-severe and severe form (56% and 23%), to sub medium form (24%). The mild form of trichinellosis had associated to cervical myalgia the headache, a selectively parameter in proportion of only 10%.

3.2. The incidence of the headache in medium, medio-severe and severe forms of disease has the same percentage like the one from the sub medium and mild forms of the disease (33%). In the severe forms of trichinellosis it has been registered the largest percentage of patients who had headache (44%) and / or vertigo (19%).

3.3. Other neurological and psychiatric events such as: agitation, nystagmus, drowsiness, anxiety, meningism, photophobia were reported in a percentage of 12.37% of the cases analyzed.

### ACKNOWLEDGMENTS

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## **THE USE OF HUMAN ONE DAY THERAPEUTIC CONTACT LENS IN CORNEAL DISEASES OF DOGS**

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**Key words:** contact lenses, corneal erosions, dog

### **SUMMARY**

Ophthalmic diseases are very common in veterinary practice. The eye has a major importance for the organism, being the structure that assures the entrance of the greatest amount of information from the environment.

The cornea is the transparent and fibrous outer coat of the eye which plays a big role in maintaining the visual acuity. Because the cornea is in permanent contact with the exterior, corneal lesions are the most frequent ocular diseases.

The aim of this study is to present a modern therapy for corneal diseases in dog, the use of human one day therapeutic contact lenses. This is an alternative for the more aggressive techniques such as grid or punctate keratotomy, superficial keratectomy or third eyelid flap.

Human one day therapeutic contact lenses (HTCL) are soft, they adhere to the surface of the cornea and are permeable for collyres. Another advantage is that after applying the contact lens, the corneal healing can be observed in progress.

Corneal diseases in dogs are very frequent and painful. The eye blinking, movements and cleaning processes increase the local pain in corneal erosions, anterior corneal plagues, corneal burns, anterior keratitis and indolent corneal ulcers, traumatic corneal ulcer secondary to foreign bodies.

Hence, the pain prevents corneal healing, therapeutic contact lenses act as a bandage, adding a desirable comfort to the patient.

### **1. MATERIAL AND METHODS**

This study has been performed in 30 dogs which presented at the Faculty of Veterinary Medicine Bucharest, Ophthalmology Clinic, between August 2010 and September 2011 with various corneal diseases. Their treatment was based on the use of human one day therapeutic contact lenses along with local collyres with antibiotics, mydriatic and anticollagenase drugs such as acetylcysteine.

Corneal diseases had no breed, age or sex predisposition. The study was performed on 6 months to 12 years old German Shepherd, Bichon, Pekingese, Pug, Caniche, Shi Tzu, English Bulldog, Boxer, Brac of different genders.. The dogs included in this study had different corneal lesions, including: indolent ulcer, corneal dystrophy, bullous keratopathy, anterior keratitis, corneal burns, traumatic corneal ulcer.

The ophthalmic examination was represented by ophthalmoscopy, illumination with a pen torch, examination of the cornea using the magnifying loupe, fluoresceine test. Ocular signs were photophobia, blepharospasm and ocular discharge. Many of the dogs examined had been on different treatments before coming to the Ophthalmology Clinic, but with no results. Based on the severity of the injuries, the patients were given generally antibiotics for 7 to 10 days after applying the HTCL.

In order to apply the therapeutic contact lenses, we only needed to anesthetize the cornea using drops of benoxicaine collyre; the animals could be sited in lateral decubitus or they could take the sit position. In patients with indolent ulcers, debridement of the nonadherent epithelium was performed prior to the lens insertion, using a sterile cotton-tipped applicator.

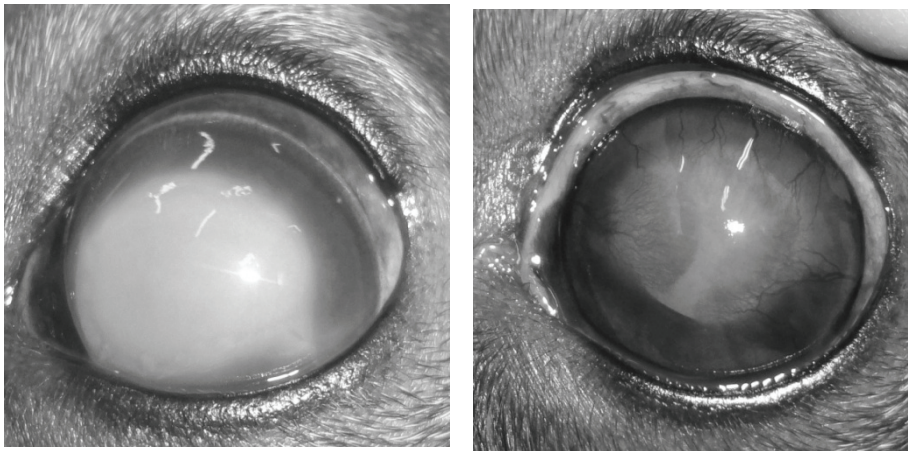
For handling the bandage lenses, we used a small plastic forceps to avoid damaging them. The contact lenses must be lying under the third eyelid for a better contact with the corneal surface, paying attention not to have any air bubbles between them. Closing the dog eyelids and performing a gentle massage improve the adherence of the contact lenses. The lenses may be changed at every 5-7 days or anytime it is required (e.g. they may be lost) until complete recovery.

## 2. RESULTS AND DISCUSSION

### *Case no. 1*

A five year old Brac male, named Deer, presented at the Ophthalmology Clinic because the owner observed that for about three days the dog was keeping his left eye closed, with excessive lacrimation, pain, red conjunctiva. After we took the anamnesis, we found out that the owner had recently painted the walls in his house and that the dog could have come in contact with chemicals. We proceeded with the ophthalmic examination and the clinical signs were: photophobia, blepharospasm, epiphora and positive fluoresceine test, the cornea was fixing the dye on the entire surface

(see Fig. 1). We put the diagnosis of chemical burn and the treatment was represented by inserting a therapeutic contact lens, along with the use of one antibiotic collyre (eg. Ciplox) and artificial tears for three weeks and a mydriatic (Tropicamida) for three days. After 7 days, we could see an improvement, the corneal lesion was smaller and the ocular pain was gone. After 30 days of HTCL, we saw that the lesion healed, but with neovascularization (see Fig. 2).

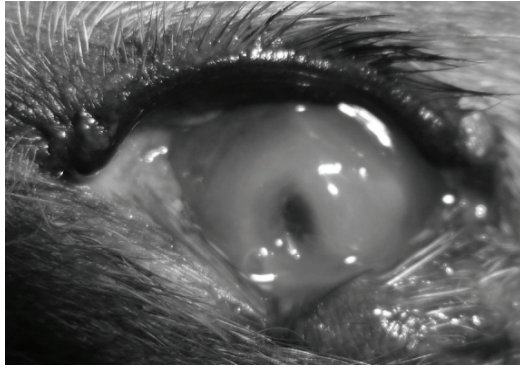


**Fig. 1,** Before HTCL **Fig. 2,** After 30 days with HTCL  
OD Corneal chemical burn ( DEER, Brac, 5 years); Fluorescein test was positive in 85% of the cornea (left); the corneal healing was with pigmentation (right)

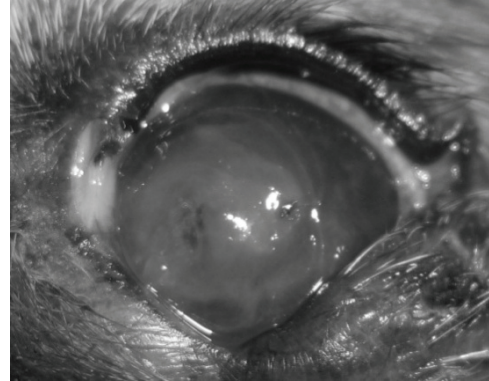
#### *Case no. 2*

An one year old mixed breed male was brought by his owner at the Ophthalmology Clinic because three days ago he had a fight with another dog and he got perforated his left cornea. At the ophthalmic examination the clinical signs were: photophobia, blepharospasm and ocular discharge. Using further diagnostic tests, we saw also a central corneal lesion, corneal edema, positive fluoresceine test, small, short blood vessels at the corneo-scleral limb and the presence of cicatricial ectropion (Fig. 3). Based on the diagnostic of corneal ulcer with vascularisation, the treatment was represented by the use of HTCL, along with local collyres with antibiotics, artificial tears and anticollagenase drugs such as acetylcysteine. After 5 days of treatment we could see a reduction of the ocular signs (Fig. 4), and after

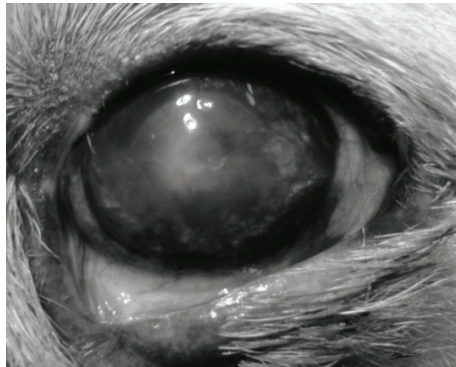
only 10 days the cornea regained its transparency, with the remaining of a minimal corneal scar (Fig. 5).



**Fig. 3,** Before HTCL  
OS Corneal plagues ( NESU, Metis, 1 year)



**Fig. 4,** After 5 days with HTCL



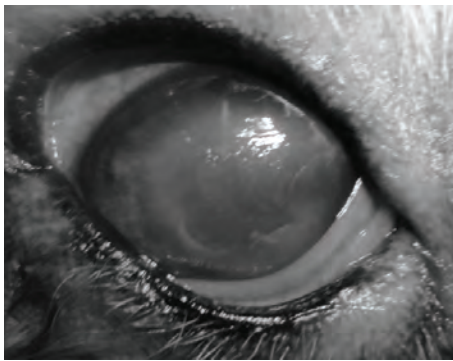
**Fig. 5,** After 10 days with HTCL there was only a minimal central scar

*Case no. 3*

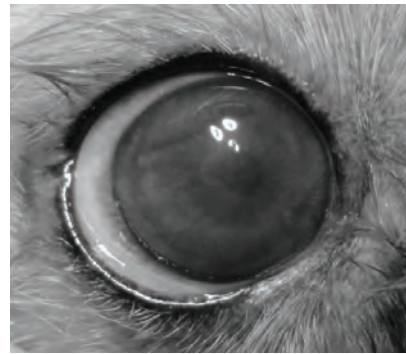
Bichon maltese female 8 years old. Mouse was brought by her owner at the Ophthalmology Clinic as a few days ago the dog came in contact with some construction materials and since then she had excessive lacrimation in her right eye and a big pain when trying to touch it. At the ophthalmologic examination the ocular signs were: epiphora, blepharospasm, photophobia and positive fluoresceine test, the cornea was fixing the ophthalmic dye on its entire surface. The anterior corneal epithelium was denudated and there were no blood vessels (see Fig. 6). The diagnostic was of chemical corneal burn and we decided to insert a therapeutic contact lens. Before doing this, we removed the nonadherent epithelium using a sterile cotton-tipped

applicator, after the cornea was anesthetized using drops of benoxicaïne collyre. The recommended local treatment was represented also by the use of an antibiotic collyre and artificial tears for 14 days.

After 10 days of treatment, Mouse came for a new investigation and we observed that there were no more epiphora, blepharospasm, photophobia, the fluorescein test was negative and the neovascularization was present. In this case, the cornea healed “ad integrum” (Fig. 7).



**Fig. 6,** Before HTCL  
OD Corneal chemical burn ( MOUSE, Bichon, 8 years)



**Fig. 7,** After 10 days with HTCL

Fluorescein test was positive and the anterior corneal epithelium was simply denude (left)

Fluorescein test was negative, neovascularization was discrete and the corneal healing was "ad integrum" (right)

For the dogs in our study, the recovery was achieved "ad integrum" for 25 cases (see Mouse) or with sechela (corneal scar and/or pigmentation) in 5 cases (see Deer, Nesu). The recovery period depended on the severity of the lesions, the breed and the age of the dogs.

Exclusive use of local and general therapy resulted in healing the lesions in a variable period of time between 15 and 60 days. The recovery period was reduced to half (7-30 days) by completing this therapy with the use of HTCL.

### 3. CONCLUSIONS

3.1. HTCL are indicated for: indolent corneal ulcers, corneal erosions, traumatic corneal ulcers, secondary corneal foreign bodies' wounds (aristae), chemical corneal burns.

3.2. Applying HTCL only require local anesthesia with drops of benoxicaïne collyre.

3.3. HTCL cannot be used in case of desmetocoele, following the third eyelid ablation, in scarring of the third eyelid, in keratoconus or when the corneal surface is too flat, making impossible the lenses adherence.

3.4. By using both local drops and contact lenses in corneal diseases, the recovery period is reduced to half. (7-30 days)

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## OPHTHALMIC EXAMINATION FEATURES IN EXOTIC COMPANION BIRDS

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**Key words:** exotic, bird, ophthalmic examination

### SUMMARY

The research has been realized during a period of 2 years (2009-2011) on 34 cases of exotic companion species. The study aimed the ophthalmic examination features of exotic companion birds. From a total number of 34 exotic examined cases, the birds represented 32,35%. They were all examined according to the standard procedure, using standard tests and the results consisted in the existence of a tight connection between the eye anatomy and the ocular disease encountered.

There is an increasing medical attention given to the new exotic companion species, so the diagnostic and treatment procedures became for the veterinary practitioner a truly challenge.

Although many of the ocular diseases resemble those met in dog, they are difficult to diagnose, due to a lack of anatomy knowledge and the impossibility of realizing some diagnostic methods in general practices.

Ophthalmic examination features of the exotic companion birds are based on very good knowledge of their eye's anatomy and most frequent diseases, especially infectious diseases, and of their wild behaviour and different methods of contention. There are few references in medical literature concerning ocular diseases in this species, mostly been journal papers.

In birds' eye examination, one must consider the absence of the menace response, the voluntary contraction of the pupil (striated muscular sphincter), the presence of the scleral ossicles that limit the eye movements, the anangiotic retina, the vascular pecten and the fovea centralis.



## 1. MATERIAL AND METHODS

Ophthalmic examination in birds is very difficult, because of the lack of the menace response and their wild behavior. To examine the bird's eye the clinician must avoid sedatives or anesthetic agents; those could interfere with the lacrymal system and the occurrence of the reflexes. During the examination, the examiner should limit stress by manipulating the bird in a darkened room.

The cases taken in consideration belonged to different bird species: the budgerigar (*Melopsittacus undulatus*), the Ara parrot, the woodcock (*Scolopax rusticola*), the owl (*Bubo bubo*), the long-eared owl (*Asio otus*), the Eurasian tawny owl (*Strix aluco*), the little owl (*Athene noctua*), the Eurasian hobby (*Falco subbuteo*, *Falco columbarius*).

A total number of 12 bird species were examined according to a standard procedure with usual methods used in the examination of small animals.

The methods used in this study consisted in the evaluation of the main eye's reflexes, the examination with a light source, with a magnifying loupe, the Schirmer tear test, the fluoresceine dye test, the red phenol thread test, performing ophthalmoscopy, measuring intraocular pressure, the slit lamp and bio-microscope examination, and other complementary examinations.

## 2. RESULTS AND DISCUSSION

The examinations showed 3 bird cases with normal eye function, 3 cases with infectious diseases, 3 traumatic cases and 1 tumoral/congenital disease. From the total number, 5 were unilateral affections and 4 were bilaterals.

Palpebral reflex has been assessed by touching the skin of the lateral and medial angle of the eyelid, and then the nictitating membrane will quickly and completely cover the cornea, both eyelids will move slowly, although the inferior one covers a larger area of the cornea (Fig. 1).

The comparative aspect of both eyes has been evaluated in each case (Fig.2) The differential diagnosis between congenital and acquired *phthisis bulbi* should be made on anamnesis and complete eye examination, being less obvious than in mammals to differentiate due to the scleral ossicles.

In one case of an owl, *Asio otus*, a tumoral disease was suspected, but histo-pathological examination showed the evolution of a poxvirosis, seen as a blepharitis with a prolipherative aspect.

In another case of a budgerigar, a traumatic/inflammation etiology of the nictitating membrane was suspected, but the association with the central nervous system signs showed the involvement of a tumoral process (Fig. 3).

Using light source examination one could see the direct pupillary reflex in a darkened room, but spontaneously pupillary movements can appear due to a voluntary control (Fig. 4). The indirect reflex may not appear considering the complete separation of the optic nerve fibers.



**Fig 1.**—Examination of the bird (*Strix aluco*) in its environment evaluating the menace responses



**Fig. 2**—Comparative aspect of both eyes modified right eye with pthisis

Magnifying loupe examination has been very helpful in examining very small eyes and diagnosing discrete corneal lesions.

Schirmer Tear Test is used especially in medium and large species (Fig. 5). Values may vary: in psittacines 3.2-7.5 mm/min without local anesthesia and 1.7-4.5 mm/min with local anesthesia; 4.1-14.4 mm/min without anesthesia and 2-4.2 mm/min with anesthesia, at phalconiformes; and 10.7-11.5 mm/min without anesthesia in acipitriiformes (Table 1).



**Fig. 3**—A budgerigar (*Melopsittacus undulatus*) with exophthalmia due to the inflammation of the posterior segment, tumoral disease was suspected



**Fig. 4**—Light source examination, evaluating the pupillary reflex in *Asio otus*

Red Phenol Thread Test is replacing the Schirmer tear test in very small eyes.

Fluoresceine Dye Test is compulsory in the differential diagnosis of ocular diseases and choosing the right treatment (Fig. 6 and Fig. 7).

*Table 1*

*Reference values for Schirmer tear Test (Williams D. (2007))*

Species	Schirmer Tear test	Red Phenol Thread test
Birds (Psittacines)	5 ± 3 mm/min.	22 ± 4 mm in 15 sec.



**Fig. 5**—Schirmer tear test in a barn owl,



**Fig.6** - Fluoresceine dye test positive,

*Asio otus*

the dye is retained by the ocular secretions



**Fig.7**—Fluoresceine dye test in *Asio otus* —negative for both eyes

The slit lamp is a valuable instrument, but very expensive and usually not found in general veterinary practices.

Tonometry has been used to assess normal values of intraocular pressure. The reference values measured with Tonopen are: in raptors 11-16 mm Hg, in psitacines 20-25 mm Hg. Tonovet has been used in a study on 31 raptors, getting an average value of 9 mm Hg in small owl and 40 mm Hg in large species (Table 2).

*Table 2*

*Reference values for intraocular pressure (Williams D. (2007))*

Species	Tonometry values (mm Hg)
Birds (raptors)	20 ± 4
Owls	11 ± 4

The indirect or direct ophthalmoscopy (Fig. 8 and Fig. 9) requires drug induced pupil mydriasis.

Birds' ciliary muscles are consisted mainly of striated fibers, then tropicamide and atropine, or even simpaticomimethic agents are inefficient in producing mydriasis; so this is obtained through general anesthesia or by local application or intracameral injection of tubocurarine (d-tubocurarine chlorure) 20 mg/ml.

Direct ophthalmoscopy is used mainly in medium and large birds and

indirect one in small species. The vascular pecten and its shape has been evaluated in each case (Fig. 10).

The posterior segment is frequently affected especially due to the eye's large diameter, its fixation in the orbit and inflexibility due to the scleral ossicles.



**Fig.8** Examination of the posterior segment in *Strix aluco*, non visible due to anterior changes



**Fig.9** Direct ophthalmoscopy in *Asio otus*



**Fig.10** - Fundus aspect in *Asio otus* —the folded vascular pecten

### 3. CONCLUSIONS

3.1 Ophthalmic examination is difficult especially in small species as it requires an adequate contention, but also because of the lack of medical references regarding comparative anatomy and the standard tests values (the Schirmer tear test, the red phenol thread test, the tonometry).

3.2 An adequate ophthalmic examination requires anatomy and behavior knowledge, sometimes anesthesia of the patient for different techniques.

3.3 Considering the normal voluntary pupillary reflex, the impossibility of assessing mydriasis with atropine and tropicamide, the eye inflexibility given by the scleral ossicles, the fact that small trauma can cause important changes of the posterior segment; and the special features of the anangiotic retina with the presence of the different sized vascular pecten may be misinterpreted as pathological, together with misdiagnosing a tumoral disease instead an infectious one (poxvirosis).

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## **EXPERIMENTAL POST-INFLUENZA *STREPTOCOCCUS PNEUMONIAE* 6B PULMONARY INFECTION IN MICE**

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**Key words:** histopathology, mouse, post-influenza, *S. pneumoniae*, whole body plethysmography

### **SUMMARY**

Mouse models represent an important tool for gaining new insight in secondary pneumonia due to pneumococcal infection following influenza, which is one of the main culprits affecting human and animal health alike. The aim of this study was to investigate an intranasal model of hyaluronidase-supplemented *S. pneumoniae* 6B infection in a C57BL6 mouse, post-influenza scenario. For this purpose, the mice were nebulized with PR8 influenza and bacterial infection took place at 9 days after PR8 infection. Whole body plethysmography-determined breathing parameters, bacterial presence and anatomic pathology of the lungs were assessed on days 2, 4, 6 and 10 after bacterial infection. Body weight and clinical signs of pneumonia were monitored daily throughout the experiment. We concluded that *S. pneumoniae* 6B in conjunction with hyaluronidase causes non-lethal subclinical pneumonia in C57BL6 mice, after PR8 influenza infection, and this affects breathing parameters determined by whole body plethysmography; a useful in-vivo method for assessing respiratory disease.

It is well known that pulmonary disease due to secondary bacterial infection following influenza, especially pneumococcal, is one of the main culprits affecting human health (Diatopoulou *et al.*, 2010). This pathologic burden is of concern to veterinary medicine also, where literature reports secondary infection after equine (Yamanaka *et al.*, 2006) and canine influenza (Larson *et al.*, 2011). Although mouse models of bacterial pneumonia are extensively studied, a “one size fits all” concept cannot be applied, especially for post-influenza secondary pneumonia, due to variability of the murine, viral and bacterial strains alike (Bakker-Woudenberg, 2003; Mizgerd and Skerrett, 2007). One problem is that most serotypes of *Streptococcus pneumoniae*, except for 1 and 3, seem to be naturally avirulent in immunocompetent mice when given intranasally (Bakker-Woudenberg, 2003). Of the virulence factors of streptococci, hyaluronidase has been shown to enhance experimental pathogenicity of *S. pneumoniae* (Zwijnenburg *et al.*, 2001). Microbiology and histopathology,

the “golden standard”, offer precise information in monitoring experimental pneumonia however, these methods are biased by animal euthanasia necessity, thus increasing lot numbers (Bakker-Woudenberg, 2003; Mizgerd and Skerrett, 2007). Whole body plethysmography (WBP) has been proven to be a valuable non-invasive method for monitoring respiratory disease in mice and has the advantage of producing multiple sets of data from one animal as disease progresses (Hamelmann *et al.*, 1997; de Hennezel *et al.*, 2001; Bonville *et al.*, 2006).

This study was designed to investigate the clinical and pathological changes elicited by intranasal challenge with *Streptococcus pneumoniae* serotype 6B and hyaluronidase, of C57BL6 mice that have been previously infected with influenza virus A/Puerto Rico/8/34. The experimental design was done with the imperative of the 3R's in mind and thus clinical evaluation by body weight, signs of illness and WBP—determined breathing parameter came to complete pathology, that was assessed by bacterial culture and pathologic anatomy.

## 1. MATERIALS AND METHODS

The study protocol was approved by the Internal Ethics Committee of the “Cantacuzino” Institute. All animal care was in accordance with Council Directive 86/609/EEC.

*1.1 The “3R's” principle* Replacement - this study could not be reproduced by in vitro methods, Reduction - only 4 mice per group were used, and Refinement - study was conducted by qualified personnel with non-invasive methods complementing classical ones and procedures that assured animal welfare during the experiment.

*1.2 Infectious agents* Mouse adapted influenza virus A/Puerto Rico/8/34 (PR8) was thawed, total protein titrated at 3584 hemagglutinating units and serially diluted to  $4 \times 10^{-4}$ ,  $4 \times 10^{-5}$ ,  $4 \times 10^{-6}$  for nebulization. *Streptococcus pneumoniae* (*S. pneumoniae*) serotype 6B (6B), cultures were washed and set at  $0.5 \times 10^{11}$  CFU/ml in 0.9 % NaCl solution for intranasal infection.

*1.3 Animals and infection protocol* Female C57BL6 mice of 8-10 weeks old were assigned to 4 groups for mild influenza infection: PR8\*1 for the  $4 \times 10^{-6}$  dilution, PR8\*10 for the  $4 \times 10^{-5}$  dilution, PR8\*100 for the  $4 \times 10^{-4}$  dilution and SB for the control group - sham nebulization with saline buffer (SB). Mice were infected with 0.25 ml of PR8 9 days prior to the *S.*



*pneumoniae* 6B challenge. PR8 nebulization was performed using a 4 chambered WBP system with aerosol delivery (EMMS, Hants, UK). On day 9, after influenza nebulization, the mice were subjected to intranasal instillation of bacteria. For this purpose, mice were mildly anesthetized with intramuscular ketamine (100 mg/kg). All the animals were first given 10 µl (300 IU) of hyaluronidase and then 20 µl of *S. pneumoniae* 6B ( $0.5 \times 10^{11}$  CFU/ml) suspension so that each animal received  $10^9$  CFU of bacteria.

*1.4 Clinical follow-up of infected animals* Mice were weighed and clinical signs of pulmonary disease (ruffled fur, lack of movement, hunched posture, closed eyelids, dyspnea) were monitored daily.

*1.5 Breathing parameter assessment* The same 4-chamber WBP system, as previously described was used to record flow-derived parameters (FDP) at baseline values before influenza infection and on days 2, 4 and 6 after pneumococcal challenge. Values for tidal volume (TV), frequency of breathing (f) and minute volume (MV) were analyzed.

*1.6 Bacterial detection and pathologic investigation* Animals were euthanized for microbiology and pathology after *S. pneumoniae* 6B infection in the following manner: 1 mouse/group on day 4; 2 mice/group on day 6; 1 mouse/group on day 10. Bacterial identification was based on cultural characteristics and specific hemolysis. Necropsy, with special attention to gross examination of the thoracic cavity, was performed. Lungs were removed, immersed in 10% neutral buffered formalin and submitted to routine histopathology slide preparation. Hematoxylin-eosin stain was performed and slides were examined under light microscope. Perivascular or peribronchiolar cuffing, a common finding in *S. pneumoniae* lung infection (LeVine *et al.*, 2001) was scored I, II and III according to Table 1.

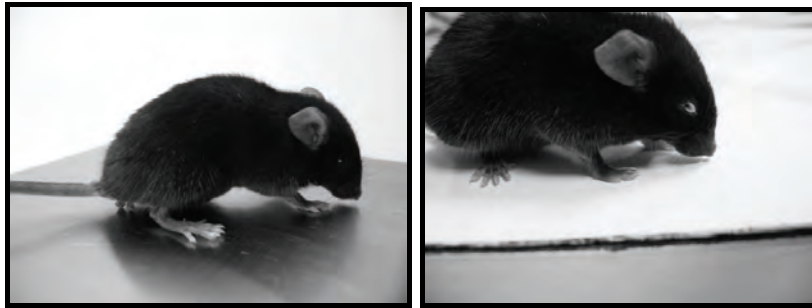
Table 1

Perivascular/Peribronchial cuffing (PVC/PBC) score

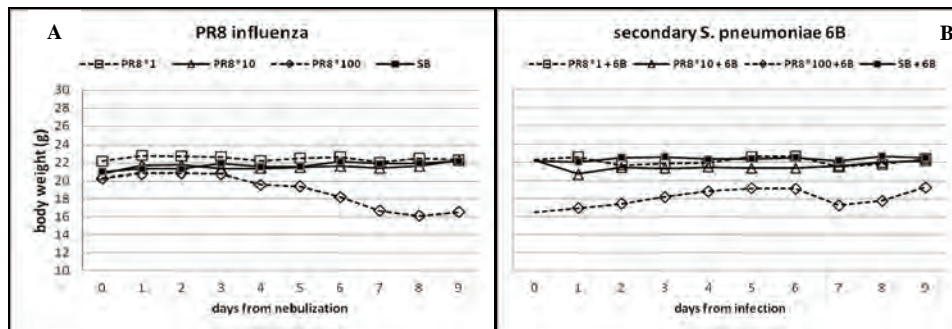
Score	Legend
PVC/PBC I	Few inflammatory cells, loosely arranged around vessel/bronchiole
PVC/PBC II	Many inflammatory cells, with some degree of concentric organization around vessel/bronchiole. Less than 3 rows of cells.
PVC/PBC III	Numerous inflammatory cells, with evident concentric organization around vessel/bronchiole. More than 3 rows of cells

## 2. RESULTS AND DISCUSSION

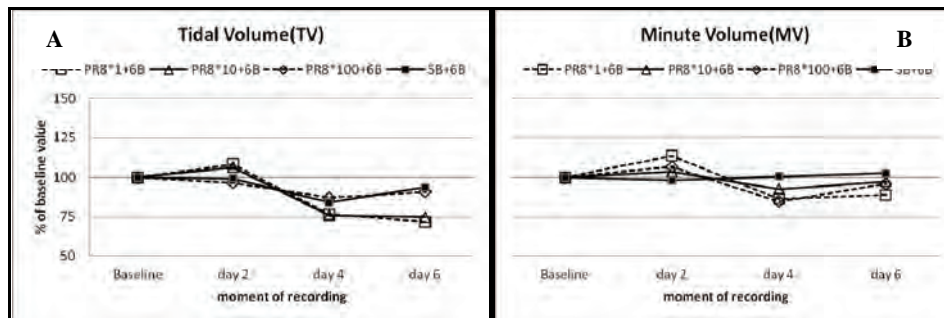
**2.1 Body weight and clinical signs of infection** Following PR8 infection only mice in group PR8\*100 showed a pronounced drop in body weight and clinical signs of disease (ruffled fur, lack of movement, hunched posture, closed eyelids, dyspnea), as seen in Fig. 1., whilst other groups showed no drop in body weight and no symptoms of disease. Body weight progression after PR8 infection is summarized in Fig. 2A. After intranasal *S. pneumoniae* 6B challenge, mice did not show weight loss or clinical signs (Fig. 2B). On the contrary, group PR8\*100+6B reached initial body weight.



**Fig. 1.** C57BL6 mouse, on day 8 after infection with influenza virus A/Puerto Rico/8/34 (H1N1) —PR8 from group PR8\*100 ( $10^{-4}$  dilution), exhibiting hunched posture, ruffled fur, dyspnea(left) and ocular secretions (right).



**Fig. 2.** Effect of infection with A) mouse adapted influenza virus A/Puerto Rico/8/34 (H1N1) —PR8 and B) post-influenza intranasal hyaluronidase (300 IU) and  $10^9$  CFU *S. pneumoniae*, serotype 6B, on body weight of C57BL6 mice. PR8\*1:  $10^{-6}$  dilution; PR8\*10:  $10^{-5}$  dilution; PR8\*100:  $10^{-4}$  dilution; SB: saline buffer



**Fig. 3.** Effect of post-influenza intranasal hyaluronidase (300 IU) and  $10^9$  CFU of *S. pneumoniae*, serotype 6B (6B), on A) tidal volume (TV) and B) minute volume (MV) of C57BL6 mice. Data are presented as % of the baseline values. PR8\*1:  $10^{-6}$  dilution; PR8\*10:  $10^{-5}$  dilution; PR8\*100:  $10^{-4}$  dilution; SB: saline buffer

**2.2 Breathing parameters** FDPs showed a slight elevation of TV on day 2 after *S. pneumoniae* 6B infection in PR8\*1+6B and PR8\*10+6B groups. A general decrease in TV was seen by day 4 but was more evident in PR8\*1+6B and PR8\*10+6B. On day 6 PR8\*100+6B and SB+6B groups returned close to baseline values whilst PR8\*1+6B and PR8\*10+6B continued with low TV (Fig. 3A). MV was slightly increased on day 2 in all groups except for SB+6B. By day 4 a general decrease in MV was observed except for SB+6B group. On day 6, only PR8\*1+6B group still had a decreased MV, the remaining groups returning to baseline value (Fig. 3B).

**2.3 Bacterial presence** *S. pneumoniae* 6B positive samples were obtained from 1 blood sample from day 4 after pneumococcal challenge in PR8\*1+6B group, 2 blood samples from day 6 both in PR8\*100+6B group, one of which also had a positive lung culture, and from 1 sample from day 6 in SB+6B group. All samples collected on day 9 following pneumococcal infection were negative. Bacteriology results obtained after *S. pneumoniae* 6B secondary infection are presented in Table 2.

#### 2.4 Pathological anatomy

**2.4.1 Gross pathology** Gross inspection of lungs in all mice led to the identification of active hyperemia. No lesions were found in other organs investigated. In mice infected with the highest titer of PR8 virus, pulmonary sequelae comprising focal to coalescent “dry” grey-red patches were observed at all harvesting time-points.

Table 2

Microbiologic blood (underlined) and **lung** (**bold underlined**) samples from influenza recovered C57BL6 mice intranasally instilled with hyaluronidase (300 IU) and  $10^9$  CFU *S. pneumoniae*, serotype 6B. Samples are collected on days 4, 6 and 10 after *S. pneumoniae* instillation. Data are expressed as positive samples/total number of samples collected from group. PR8\*1:  $10^{-6}$  dilution; PR8\*10:  $10^{-5}$  dilution; PR8\*100:  $10^{-4}$  dilution; SB: saline buffer.

Group	Moment of sampling after <i>S. pneumoniae</i> 6B infection (days)					
	4		6		10	
PR8*1 + 6B	<u>1/1</u>	<b><u>0/1</u></b>	<u>0/2</u>	<b><u>0/2</u></b>	<u>0/1</u>	<b><u>0/1</u></b>
PR8*10 + 6B	<u>0/1</u>	<b><u>0/1</u></b>	<u>0/2</u>	<b><u>0/2</u></b>	<u>0/1</u>	<b><u>0/1</u></b>
PR8*100 + 6B	<u>0/1</u>	<b><u>0/1</u></b>	<u>2/2</u>	<b><u>1/2</u></b>	<u>0/1</u>	<b><u>0/1</u></b>
SB + 6B	<u>0/1</u>	<b><u>0/1</u></b>	<u>1/1</u>	<b><u>0/1</u></b>	<u>0/1</u>	<b><u>0/1</u></b>

2.4.2 *Histopathology* Of the lesions that had ubiquitous distribution throughout samples (hyperemia, emphysema and bronchiectasis), a background picture was imagined and thus, these phenomena will not be presented along with other lesions observed in samples, but rather mentioned in the lack of. Lesions encountered at 4, 6, and 10 days after *S. pneumoniae* 6B infection are synthesized in Table 3. Suggestive lesional aspects encountered after pneumococcal infection are displayed in Fig. 4.

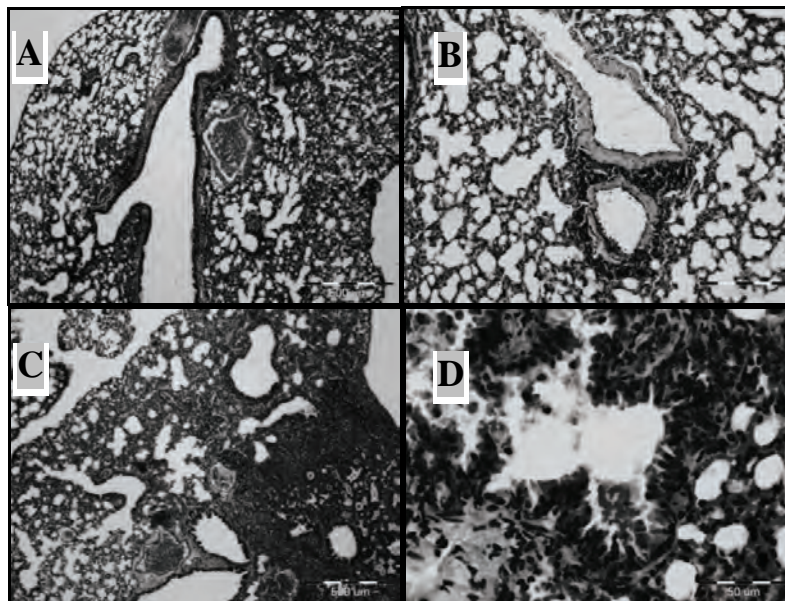
Table 3

Lesional aspect found in lung from influenza recovered C57BL6 mice, intranasally instilled with hyaluronidase (300 IU) and  $10^9$  CFU *S. pneumoniae*, serotype 6B. Samples were collected on days 4, 6 and 10 after *S. pneumoniae* instillation. PR8\*1:  $10^{-6}$  dilution; PR8\*10:  $10^{-5}$  dilution; PR8\*100:  $10^{-4}$  dilution; SB: saline buffer.

Group	Moment of euthanasia after <i>S. pneumoniae</i> 6B infection (days)		
	4	6	10
PR8*1 + 6B	severe hemorrhage, acute interstitial pneumonia, epithelial necrosis, PVC III, PBC I	acute pneumonia, necrosis, PVC II	interstitial epithelial no lesions
PR8*10+ 6B	moderate hemorrhage, interstitial pneumonia, PVC I, PBC II	interstitial pneumonia with epithelial necrosis, PVC I, PBC II	interstitial pneumonia in resolution, epithelial necrosis, PVC I, PBC I

PR8*100+ 6B	moderate chronic pneumonia, necrosis, PVC III	hemorrhage, interstitial epithelial	moderate chronic pneumonia, necrosis, PVC III	hemorrhage, interstitial epithelial	chronic pneumonia, PVC II	interstitial PVC I, PBC
SB + 6B	PVC I		PVC I		PVC I, no emphysema	

Analysis of histopathology results showed acute pneumonia lesions in groups PR8\*1+6B and PR8\*10+6B. Chronic interstitial pneumonia was found in PR8\*100+6B group. These results can be correlated with decrease in TV for acute pneumonia groups PR8\*1+6B and PR8\*10+6B and no marked decrease in TV seen in group PR8\*10+6B. A PR8 dose dependent effect can be seen in acute pneumonia groups with lesions lingering on until day 10 in PR8\*10+6B compared with no lesions seen in group PR8\*1+6B. SB+6B group showed just PVC I, at all days of investigation. This shows that PR8 pre-infection determines increased pathogenicity of *S. pneumoniae* 6B and is consistent with the resolution of lesions in independent pneumococcal pneumonia (LeVine *et al.*, 2001).



**Fig. 1.** Representative lesions from post-influenza intranasal hyaluronidase (300 IU) and  $10^9$  CFU *S. pneumoniae*, serotype 6B infected C57BL6 mouse lungs. A) Emphysema and bronchiectasis, with PVC I, in PR8\*1+6B group at day 4, 40x. B) Emphysema and PBC II in PR8\*10+6B group at day 4, 100x. C) Area of pulmonary consolidation, interstitial pneumonia and emphysema with bronchiectasis, in PR8\*100+6B group at day 6, 40x. D) PBC II with epithelial necrosis in PR8\*10+6B group at day 6, 400x.

The results show that in non-lethal PR8 influenza infected C57BL6 mice, *S. pneumoniae* 6B causes a clinically unapparent pneumonia, which affects breathing parameters to some extent but does not cause clinical signs of disease or weight loss. PR8 does not seem to have the supraacute lethal synergistic action with *S. pneumoniae* 6B as it was shown to have with the serotype 2, *S. pneumoniae* D39 strain (McCullers and Rehg, 2002). Bacterial presence in lungs or blood did not offer conclusive evidence on the influence of PR8 pre-infection on pneumococcal disease progression. This could be due to the late moment of investigation as there is evidence that *S. pneumoniae* is cleared from lungs within 48 hours (LeVine *et al.*, 2001). Positive samples from lungs and blood on day 6 were in correlation with the severity of pulmonary lesions at that point. The increase in TV on day 2 and subsequent decrease below baseline values on days 4 and 6 in mice pre-infected with doses of PR8 that do not elicit clinical influenza signs, can be correlated at least in part, to the severe outcome observed with *S. pneumoniae* D39 at similar doses of PR8 (McCullers and Rehg, 2002). The initial increase in TV can be correlated with the bronchiectasis encountered in both SB and PR8 pretreated groups and could be an effect of hyaluronidase. Hyaluronidase has been shown to induce airspace enlargement when administered with elastase or alone when given intravenously (Tazaki *et al.*, 2006). Previously, de Hennezel *et al.* hypothesized that minute volume (MV) is a valuable parameters for monitoring lung disease and evidenced that pneumococcal pneumonia leads to a MV decrease as a direct consequence of decreased TV (de Hennezel *et al.* , 2006). We also observed a decrease in TV but MV was not affected directly by this, probably due to the compensatory role of breathing frequency.

### 3. CONCLUSIONS

3.1 *S. pneumoniae* 6B in conjunction with hyaluronidase causes non-lethal subclinical pneumonia in C57BL6 mice when administered at 9 days after PR8 influenza infection. Acute lesions of interstitial pneumonia and decreased tidal volume can be seen in mice receiving PR8 in concentrations that do not elicit clinical signs or weight loss during influenza infection.

3.2 Acute pneumonia in mice, even in sub-clinical forms, affects tidal volume in mice but not minute volume, probably due to the compensatory effect of breathing frequency.

3.3 Whole body plethysmography can be a useful in-vivo method for assessing respiratory disease.

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## **RAMS SCRAPIE GENETIC SUSCEPTIBILITY IN A TRADITIONAL ROMANIAN SHEEP FARM**

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**Key words:** TSEs, genotyping, polymerase chain reaction (PCR)

### **SUMMARY**

The paper describes the rams genotype profile, in a traditional Romanian sheep farm from southeast area. The results proved the highly variation of susceptibility in non-genetically selected herd. Even more, the number of rams susceptible and highly susceptible to scrapie was significant 14 rams (56%) in class 2, making difficult the genetic selection. In the same herd were identified 1 ram (4%) in class 1, 8 rams (32%) in class 3, 1 ram (4%) in class 4 and 1 ram (4%) in class 5. The sheep PRNP gene genotyping by PCR with melting curve analysis revealed the presence of all five classes of scrapie susceptibility.

TSEs refer to a group of fatal neurodegenerative disorders affecting mammals and human beings [23, 29, 32]. Scrapie is a naturally occurring transmissible spongiform encephalopathy (TSE) of sheep and goats, caused by a misfolded prion protein (PrP<sup>Sc</sup>) that is protease resistant and infectious [29]. That was the first spongiform encephalopathy whose transmissibility was demonstrated [10].

The first European scrapie reports were in England (1732) and Germany (1759). Romanian official report of scrapie was in 2002 [43].

Disease often occurs in animals aged 3-4 years and rarely under 18 months age. Field and experimental studies suggest that the spread of scrapie among sheep occurs mainly by horizontal transmission. Rams role in



disease spread is recognized often the disease occurrence being related with the purchase of breeding rams [28].

Genetics studies revealed a strong association between classical scrapie and *PRNP* polymorphisms at codons 136, 154 and 171 [7, 17, 18, 22, 33, 39, 42, 43, 48, 49]. The combination of this polymorphism gives rise to 5 *PRNP* codon haplotypes or alleles and 15 *PRNP* diploid genotype combinations, commonly found in sheep. The sheep susceptibility to scrapie varies widely between genotypes: from greatest resistance (ARR/ARR) to extreme susceptibility (VRQ/VRQ) [3, 4, 17, 18]. According to the UK National Scrapie Plan, the sheep genotype susceptibility is classified in 5 classes [12]. The sheep-genotype scrapie resistant is ARR/ARR (Class 1).

The sheep genetic ARR/AHQ, ARR/ARH, ARR/ARQ (Class 2) are resistant to scrapie but they need a special attention in selection programs.

The sheep with lower genetic resistance to scrapie are ARQ/ARH, ARQ/AHQ, AHQ/AHQ, ARH/ARH, AHQ/ARH, ARQ/ARQ (Class 3).

The sheep sensitive to scrapie are ARR/VRQ (Class 4), and the highest sensitive are AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ (Class 5).

The main purpose of the study was to analyze the data on genotype frequencies in rams from a traditional Romanian sheep farm, by the *PRNP* gene polymorphisms at codons 136, 154, 171 and to estimate the genotype-specific relative risks to become a detected scrapie case.

## 1. MATERIALS AND METHODS

### **Biologic material**

Blood samples, collected from 25 rams, have been submitted to the study of ovine prion protein gene (*PRNP*) genotypes. The samples originated from a traditional southeast Romanian farm. All blood samples were collected in 3 ml tubes containing K3-EDTA and stored at -20°C before DNA extraction.

### **DNA extraction**

Samples were processed using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) for isolation of nucleic acids from mammalian whole blood (200 µl) and mammalian tissue (200 µl brain homogenate) for LightCycler®PCR. The kit was used in accordance with the manufacturer's instructions.

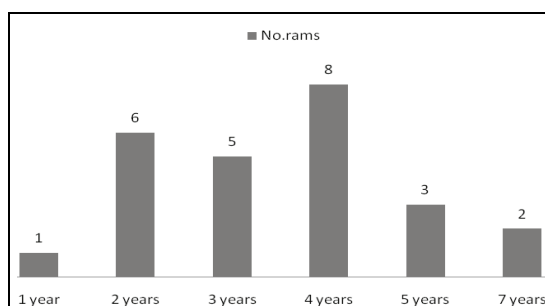
### **Genotyping of ovine prion protein gene (PRNP) variants by PCR with melting curve analysis**

The protocol used in this study was as described by LightCycler® Scrapie Susceptibility Mutation Detection Kit (TIB MOLBIOL, Berlin, Germany), used with Roche FastStart reagents LightCycler® FastStart DNA Master<sup>PLUS</sup>HybProbe (Roche, Mannheim, Germany). Melting curve analysis was performed using LightCycler® 2.0 Real-Time PCR System (Roche, Germany).

## **2. RESULTS AND DISCUSSIONS**

The studied farm was in southeast Roumanian area and has 625 animals in which 600 sheep and 25 rams. The rams had 1-7 years aged, were from Palas Merinos breed, own breeding (Fig.1).

**Figure 1.** Age distribution of rams

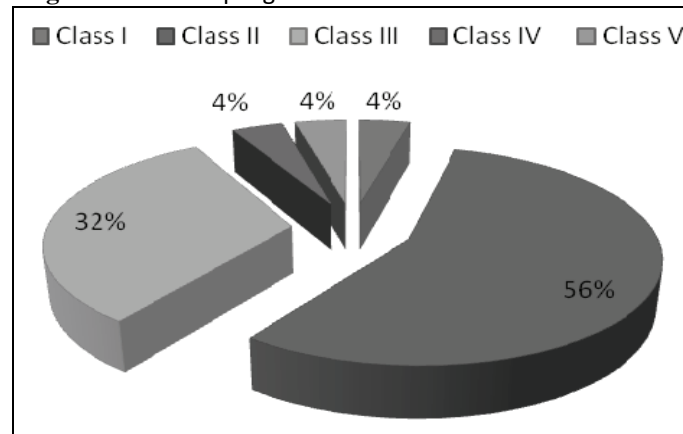


The sheep PRNP gene genotyping by PCR with melting curve analysis revealed the presence of all five classes of scrapie susceptibility (Fig. 2).

Most of the rams were in class 2 (56%), meaning sheep genetic resistant to scrapie, but asking a special attention to be used in selection programs. In class 1 (genotype ARR/ARR) we found 1 ram (4%), which makes very difficult the genetic selection. We recommend to make guided mating using rams from class 2 heterozygous (ARR/\*) only with sheep from class 1(ARR/ARR) and so, there are 75% chances for the “new generation” to be in class 1. However precautions should be taken to prevent transmission of scrapie by secondary sources, looking to the experimentally transmission study results [34].

In the same herd were identified 8 rams (32%) in class 3, 1 ram (4%) in class 4 and 1 ram (4%) in class 5.

**Figure 2.** The scrapie genetic risk classes distribution in rams



In previously Romanian genotyping studies, focused on specific sheep breed (Turcana breed, Sibian ecotype), were obtained similar results in the herd structure about males: 19, 25% genotype ARR/ARR class 1, but different results in other classes: 27.68% class 2; 40.69% class 3; 5.53% class 4 and 6.84% class 5 [9]. In studies performed in herds from outside countries the data are heterogeneous for all classes: the genotype ARR/ARR (class 1) frequency has 21.3% in Great Britain, 15, 9% in Norway, 11% in Canada and 9.3% in Japan [4, 17, 19, 22].

All haplotypes have been found in the studied herd rams. The ARQ variant was the most frequent (56%). The ARR variant was present but in one ram. The VRQ haplotype frequency in rams was 8%, this being highly similar with data previously published, where this haplotype missed or registered low rate [1, 2, 3, 8, 18, 19, 35, 38].

It has been identified 9 genotypes of PRNP gene. The genotypes AHQ/AHQ, AHQ/ARH, ARH/ARH, ARR/VRQ, AHQ/VRQ, VRQ/VRQ have not been identified.

The frequency of PRNP risk genotypes, susceptible and highly susceptible to scrapie (classified in risk groups 4 and 5, respectively), have been at 4% ARH/VRQ, 4% ARQ/VRQ. PRNP genotypes also associated with scrapie susceptibility (risk group 3) showed a frequency of 16% AHQ/ARQ, 4% ARH/ARQ and 16% ARQ/ARQ.

**Table 1.** Frequency of scrapie susceptibility classes\* and PRNP genotypes\*\* in rams from a Romanian farm

<b>Palas Merinos Breed ♂</b>		
	<b>No. Rams</b>	<b>%</b>
<b>Classes*</b>		
<b>1</b>	<b>1</b>	<b>4</b>
<b>2</b>	<b>14</b>	<b>56</b>
<b>3</b>	<b>8</b>	<b>32</b>
<b>4</b>	<b>1</b>	<b>4</b>
<b>5</b>	<b>1</b>	<b>4</b>
<b>Haplotypes</b>		
ARR	1	4
AHQ	6	24
ARH	2	8
ARQ	14	56
VRQ	2	8
<b>Genotypes**</b>		
ARR/ARR	1	4
ARR/AHQ	2	8
AHQ/AHQ	0	0
ARR/ARH	1	4
AHQ/ARH	0	0
ARR/ARQ	10	40
AHQ/ARQ	4	16
ARH/ARH	0	0
ARH/ARQ	1	4
ARQ/ARQ	4	16
ARR/VRQ	0	0
AHQ/VRQ	0	0
ARH/VRQ	1	4
ARQ/VRQ	1	4
VRQ/VRQ	0	0

\*In accord with UK National Scrapie Plan classification.

\*\*Genotypes were obtained by PCR with melting curve analysis.

### 3. CONCLUSIONS

The sheep PRNP gene genotyping by PCR with melting curve analysis revealed the presence of all five classes of scrapie susceptibility, proving the highly variation of susceptibility in non-genetically selected herd.

Most of the rams were in class 2 (56%), meaning sheep genetic resistant to scrapie, but asking a special attention to be used in selection programs.

In class 1 (genotype ARR/ARR) we found 1 ram (4%), which makes very difficult the genetic selection.

The results of this study confirm the importance of sequence-based genotype investigations in the sheep-breeding programs. Also, data support the scrapie control and eradication programs based on the gradual elimination of haplotypes associated with scrapie susceptibility and the use in breeding of the ARR/ARR genotype rams.

In our study the presence of one ram in class 1 makes very difficult the genetic selection, so we recommend to make guided mating using rams from class 2 heterozygous (ARR/\*) only with sheep from class 1(ARR/ARR) and so, there are 75% chances for the “new generation” to be in class 1.

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## **OBSERVATION CONCERNING THE EFFECTS OF MEDETOMIDINE-MICRODOSE ON MIDAZOLAM-KETAMINE INDUCED ANESTHESIA IN DOGS.**

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**Key words :** anesthesia, medetomidine, midazolam-ketamine, dogs.

### **SUMMARY**

The study was designed to compare quality of anesthetic induction, recovery, analgesia, muscle relaxation, duration of immobilization, and ease of endotracheal intubation between midazolam-ketamine-medetomidine (MKM) and midazolam-ketamine (MK) induced anesthesia in five dogs. Midazolam (0.25 mg/kg of body weight, IV) and ketamine (5 mg/kg, IV) with or without a microdose of medetomidine (5 µg/kg, IV) were administrated to dogs. Baseline measurement of heart and respiratory rate were taken before drug administration. All measurement was repeated again at 5, 10, 20, and 30 minutes after drugs administration. Endotracheal intubation was attempted beginning one minute after the last drug was administration. Analgesia was evaluated by tail clamp and needle prick testing.

Intravenous administration of midazolam — ketamine (MK) hydrochloride has widespread use in clinical practice for anesthetic induction in healthy and cardiovascular compromised dogs. Anesthetic induction with MK may require repeated administration and may be prolonged with excessive chewing, delaying in this way rapid endotracheal intubation in excited dogs. MK induced anesthesia also has been used for chemical restrain in dogs. However, duration of anesthesia may be inadequate after a single dose of MK. Administration of midazolam (0.2 mg/kg of body weight, IV) and ketamine (5 mg/kg, IV) did not result in successful immobilization in some dogs and caused excitement in others (Kolata, 1996).

Medetomidine hydrochloride is a potent  $\alpha_2$ -adrenoceptor agonist that induced sedation, analgesia, and muscle relaxation in dogs. In dogs, reliable, profound sedation is produced by IM administration of 40 µg of medetomidine/kg. Adverse cardiovascular effects associated with this

amount of medetomidine include bradycardia, second-degree block, and hypertension (Short, 1996). Because medetomidine is a potent sedative with adverse cardiovascular effects, a relatively small amount, or microdose of medetomidine (5µg/kg) may minimise these effects (Cullen, 1996, Thurmon *et al.* 1996). Addition of microdose of medetomidine may improve the quality of anesthetic induction and endotracheal intubation and extend duration of immobilization compared with MK administration alone (Oană *et al.*, 2008).

Therefore, the purpose of this study was to compare the anesthetic and cardiorespiratory effects midazolam-ketamine-medetomidine (MKM) with midazolam-ketamine (MK) induced anesthesia in dogs.

## 1. MATERIALS AND METHODS

The study was performed on five healthy dogs presented for minimal invasive surgical procedure (wounds suture, skin growth removal), in the Surgical Clinic of the Faculty of Veterinary Medicine Cluj Napoca. Before anesthetic procedures in all dogs from this study, heart rate (HR), respiratory rate (RR) were measured, and body temperature was determined by rectal checking. A 5.1cm, 20-gauge catheter was placed in the cephalic vein for drug administration. To alleviate the pain associated with catheter placement, skin over dorsal cephalic vein was infiltrated with a 1% solution of lidocaine hydrochloride.

After baseline measurement, dogs received midazolam (0.20 mg/kg, IV), ketamine (5 mg/kg, IV), and medetomidine (5 µg/kg, IV; MKM-group dogs) (Cullen, 1996, Kolata, 1996).

Anesthetics were administered individually in the following sequence: midazolam, ketamine, medetomidine. All three drugs, delivered one at a time, were administered within 180 seconds. Cardiorespiratory variables including HR and RR were measured 5, 10, 20, and 30 minutes after last drug administration. Eye position and rectal temperature were recorded at each time interval. All dogs continued to breathe air from room after anesthetic induction (Thurmon *et al.* 1996).

Induction, anesthesia, and dog recovery quality of MK and MKM-group dogs were also evaluated on a scale of 1 to 3. Additional variables recorded included duration of lateral recumbency, analgesia, and endotracheal intubation. Analgesia and intubation were recorded as

central, slightly ventral, or ventral. All qualitative measurement were performed after measurement of cardiorespiratory variables (Hellyer *et al.* 1991, Thurmon *et al.* 1996).

Depth of analgesia was monitored by applying pressure with a 5-cm Backhaus towel clamp around the tail. The clamp was kept locked in place for 15 seconds or until a gross movement was elicited. The clamp produce a blunt, squeezing, noxious stimulus without penetrating the skin with the sharp tips. The area of the tail was clipped before the first measurement of analgesia. Depth of analgesia was also tested by use a injection needle 20G. Skin covering the hindquarters, abdomen, shoulder, and neck areas was pricked with the needle. Muscle fasciculation, limb withdrawal, or head moving was considered as a lack of anesthesia(negative result)

Endotracheal intubation was performed beginning one minute after the last drug was administered. If intubated, the endotracheal tube was removed. If there was a tight jaw tone, chewing motions or excessive licking during intubation, the procedure was aborted.

## 2.RESULTS AND DISCUSSIONS

After administration of anesthetic, HR and RR significantly decreased from baseline values in MKM-group dogs; by 30 minutes after administration these variables returned to baseline values. In MK-group dogs RR did not change significantly from baseline values, although HR was significantly increased from baseline values after anesthetic administration. After drug administration HR was significantly lower in MKM-group dogs, compared with MK-group dogs. The highest HR was 240 beats/min at 10 minutes after administration of MK alone. The lowest HR was 50beats/min at 20 minutes after DKM administration. RR was significantly lower in MKM-group dogs compared with MK-group dogs at 5,10, 20 minutes after anesthetics (Kolata,1996, Oană *et al.*, 2008). Rectal temperature remained within reference range values, but decreased similarly over time in both groups, more obviously in the MKM-group.

All dogs assumed lateral recumbency within two minutes after anesthetic drugs administration. Anesthesia induction was smooth in all MKM-group dogs. As a result of inconsistent endotracheal intubation, induction was not so smooth in MK-group compared with MKM-group dogs. Endotracheal intubation could not be achieved in two dogs

administrated MK alone, because they had excessive jaw tone and excessive chewing motions. The rest of three dogs from MK-group dogs were intubated, had tight jaw tone and strong coughing, accompanied by gagging against the endotracheal tube. Endotracheal intubation were easily achieved with minimal coughing and swallowing against the endotracheal tube in all five dogs from MKM-group.

Quality of anesthesia was superior in MKM-group dogs compared with MK-group dogs. Excessive salivation, head shaking, body tremor, were commonly observed in MK-group dogs (Oană *et al.*, 2008). One DK-group dog vocalized and defecated, and another one did not maintain lateral recumbency and attempted to stand. These signs were not observed in any dogs from MKM-group. Duration of lateral recumbency was significantly longer in MKM-group dogs compared with DK-group (Short, 1996).

In MKM-group dogs, eye position rotated ventrally but returned to a central position as depth of anesthesia decreased. In contrast, eye position was always central in MK-group dogs. Time from head lifting to sternal positioning and time from head lifting to walking with minimal ataxia were not significantly different between the groups dogs. Time from induction to walking with minimal ataxia was significantly longer for MKM-group dogs. Recovery qualities were similar in both groups (Vaha-Vahe, 1989).

Changes observed in MKM-group dogs were attributed to decreased RR indicate that the microdose of medetomidine potentiated respiratory depression caused by MK (Kolata, 1996). Results of others studies indicates that RR significantly decreased from baseline values after MK administration in dogs. Microdose of medetomidine, potentiated respiratory depression of MK induced anesthesia associated with severe hypoxemia. Because severe hypoxemia can occur during MKM treated dogs oxygen insufflation is strongly recommended to prevent potential hypoxemia when this combination of anesthetic is used. In addition, because some veterinarian will intubate the dogs after administration of MKM is also recommended assisted or controlled ventilation to be used during the first few minutes of anesthesia (Short, 1996).

Heart rate significantly increased from baseline values and tachycardia (240 beats/min) was observed in 3 dogs with MK induced anesthesia. The increase in HR may be attributed to general CNS awakening or excitation (Thurmon *et al.* 1996).

Severe bradycardia (30-40beats/min) which is typically associated with high amounts of medetomidine (20-40  $\mu\text{g}/\text{kg}$ ) did not develop in the dogs of this study. The lowest HR recorded after MKM induced anesthesia was 50 beats/min. This may be the net cardiovascular effects between development of bradycardia associated with medetomidine and tachycardia associated with midazolam and ketamine (Vaha-Vahe, 1989).

Midazolam and ketamine are used in dogs by small animals veterinarians as an alternative to anesthetic induction with thiobarbiturates, propofol and methohexital. In a study six of seven dogs that were given midazolam (0.20 mg/kg, IV) and ketamine (5.5 mg/kg, IV), could not be intubated, despite a smooth transition to unconsciousness status (Hellyer *et al.* 1991). They required an additional one fourth of the original amount of midazolam and ketamine to be administered before endotracheal intubation to be achieved. Similarly in the present study, two from five DK-group dogs could not be intubated, and strong coughing and gagging reflexes were observed in the remaining MK-group that were successfully intubated. Medetomidine administration after MK causes sedation, muscle relaxation and analgesia in dogs. In the study presented here, administration of microdose (5  $\mu\text{g}/\text{kg}$ ) of medetomidine after MK significantly improved the quality of anesthetic induction and extended the duration of endotracheal intubation, analgesia, and lateral recumbency compared with DK alone. Improved induction quality was indicated by a rapid and smooth transition from consciousness, with an extended period of easy intubation (Short, 1996). Recovery qualities were similar in both treatment groups.

### 3.CONCLUSIONS

3.1. Administration of a microdose of medetomidine provides a useful adjunct to midazolam-ketamine induced anesthesia in dogs.

3.2. Medetomidine improve quality of anesthetic induction, ease of endotracheal intubation, and extended duration of analgesia and lateral recumbency in anesthetized dogs.

3.3. The addition of a microdose of medetomidine to MK causes sedation, muscle relaxation and analgesia in dogs.

3.4. Microdose of medetomidine, when used with MK, also extended the duration of analgesia, indicates that this combination is not only an

alternative for anesthetic induction but is also suitable for short duration of immobilization for non invasive and slightly invasive surgical procedures in dogs.

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## LAMENESS, HOOF CARE AND TRIMMING IN HOLSTEIN COWS

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**Key words:** lameness, cow, hoof, trimming.

### SUMMARY

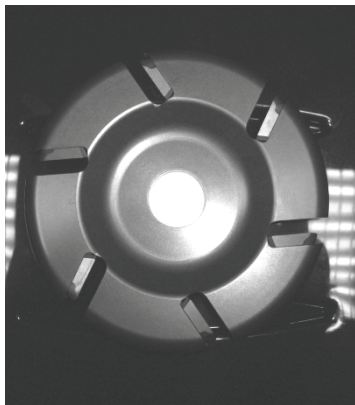
Lameness in Holstein cows is a multifactorial disease and is directly related to how cows are managed. Some of the contributing factors are nutrition, hygiene, cow comfort (freestall management), walking surfaces, time spent standing on concrete, hoof health, and hoof trimming. Prevention is more economical than treatment, but close observation and prompt treatment of lame cows will decrease the duration and thus the cost of each case. It is commonly recommended that all cows be trimmed twice per year, once when the cow is dried off and once during mid lactation. Functional hoof trimming is designed to balance the effects of wear and growth and will be explained more fully later in the paper.

Cow lameness is a continuing problem on dairies around the world. The three most common reasons for premature or involuntary culling of dairy cows are reproduction, mastitis, and lameness. The economic loss from lameness is due to premature culling, lost milk production, milk discard due to treatment with antibiotics, treatment costs, and reproductive inefficiencies.

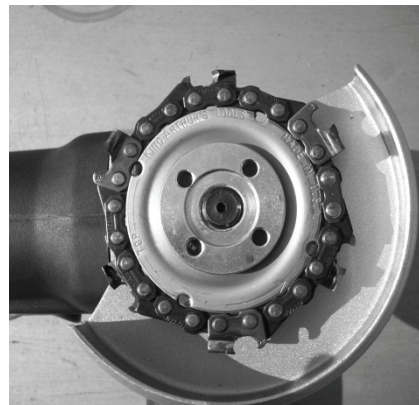
The incidence of lameness is higher during the winter than during the summer. As would be expected, there was large farm to farm variation, which indicates that management makes a big difference (Raven *et al.* 1989). 99% of the lesions causing lameness occurred in the feet with 92% occurring in the rear feet. Of the lesions in the rear feet, about 68% were on the lateral (outside) claw, 12% on the medial (inside) claw, and 20% on the skin. The most common lesions (58%) were sole ulcer and white line disease. These are the direct result of insult or injury to the corium and are lesions we generally attribute to "*laminitis*" (Hahn *et al.*, 1986). The goals of functional trimming are to distribute the weight evenly between the 2 claws of the foot, to leave sufficient horn to protect the corium, and to trim the claws to their normal shape and proportion.

## 1.MATERIALS AND METHODS

The observations were performed on a herd with 200 milking cows from Holstein breed. For this study we used 2 different discs: rotoclip aluminium disc with carbide steel blades fitted to them (Fig.1.) and tungsten carbide tipped chain saw disc (Fig.2.). A correct functional hoof trimming were done with these disks using a standard sole thickness of 5 mm in the toe area. Hoof tends to grow at a rate of about 5 mm per month. The shape of the hoof is a result of a balance of growth and wear.



**Fig.1** Rotoclip disc

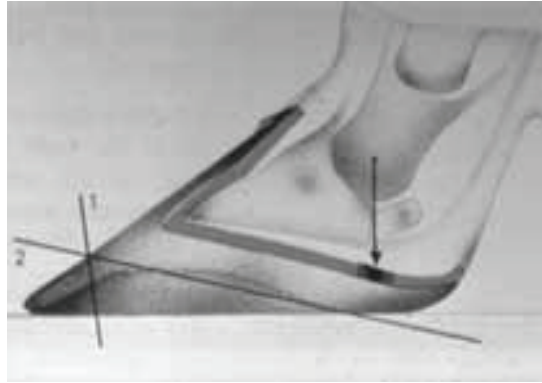


**Fig.2.** Chain saw disc.

The following procedure was applied for the rear claws because they are most often the problem. Trimming the front claws would be done in the reverse order (lateral claw first) since when lameness occurs on the front feet it is more often the medial claw that is involved. Trimming occurs in 6 steps; the first 4 steps are corrective and the last 2 steps are therapeutic or curative (Raven *et al.* 1989):

1. Cut the medial claw to length with a pincers, 7.5 cm (Fig.3.). The measurement is made from the start of the hard horn just distal to the periople to the toe. The cuts are made perpendicular to the solear surface.





**Fig.3.**Trimming of the distal edge of claw (by E.T.Raven)

This is the most crucial measurement and cut of the entire procedure. If the toe is cut too short then the corium of the toe will be exposed to trauma when the cow walks. It is better to cut slightly too long than too short. We trimmed the solear surface of the medial claw with the discs, taking more of the sole off of the toe than the heel.

The heel should be spared as much as possible and in many cases will not need to be trimmed at all. Leave ample sole to protect the corium, 5-7 mm. The weight bearing surface should be flat and in the correct plane, i.e. 90° to the leg.

2. Using the medial claw as a guide, we measured and trimmed the toe on the lateral claw to the same length. The front wall of the lateral claw may be slightly longer than the front wall of the medial claw. Pare the sole of the lateral claw to the correct thickness. The toes of the medial and lateral claws should be flat across the toes and flat from toe to heel.

3. Shape the axial sole so that the sole slopes toward the interdigital space. Do not reduce the weight bearing surface any more than is necessary. Trim less rather than more.

4. Balance the heels. The weight bearing surfaces should be flat across the heels.

5. If the lateral claw is diseased then the heel will have to be trimmed lower than the sound, medial claw. Specific conditions that would warrant lowering the heel on the lateral claw would be sole ulcer, abscess, or white line disease. Allowing the lateral claw to rest will facilitate healing. Sometimes it will be necessary to apply an orthopedic block to the sound claw to get more weight off of the diseased claw.

6. Remove loose horn and pare away all the hard edges. Only healthy horn should be left. Be careful not to trim too much off of the lateral claw. Preserve the heels, especially the medial rear heel as much as possible.

## 2. RESULTS AND DISCUSSIONS

We mentioned above the hoof tends to grow at a rate of about 5 mm per month. The shape of the hoof is a result of a balance of growth and wear. The toe will tend to wear slower since it is harder and the heel will wear faster since the horn is softer. Overgrowth will generally result in a hoof that is too long and the toe (the toe angle is too shallow). We are basically trying to create a flat surface for the cow to walk on that will minimize trauma to the corium. By functional trimming we will establish appropriate weight bearing in the claws on all four legs. Most cows will need to be trimmed 1-2 times per year. Cows with problems may need to be trimmed more often. Heifers should be trimmed about 2 months before calving to help shape the claws for the first lactation. Most producers on a regular hoof trimming program trim cows at dry off. Most cows could also use a mid-lactation trimming or at least an evaluation to determine if trimming is necessary (Blowey *et al.* 2007, Stanek *et al.* 1998). Cutting the medial claw to length with a pincers, is the most crucial measurement and cut of the entire procedure. If the toe is cut too short then the corium of the toe will be exposed to trauma when the cow walks. It is better to cut slightly too long than too short. The medial claw was a guide, to trim the toe on the lateral claw to the same length. The toes of the medial and lateral claws should be flat across the toes and flat from toe to heel. Proper sloping of the sole will take pressure off of the typical site at the caudal part of the lateral sole and will open up the interdigital space so that manure and other debris are less likely to accumulate. On dairies, where the cows do not have to walk very far and hoof trimmers may "rest" the lateral heel by trimming it about 3-5 mm lower than the medial heel. On most of dairies where the cows walk greater distances and have more wear on the heels. "Resting" the heels if they are already thin is not advisable. If the lateral claw is affected then the heel will need to be trimmed lower than the sound, medial claw. Specific conditions that would warrant lowering the heel on the lateral claw would be sole ulcer, abscess, or white line disease. Allowing the lateral claw to rest will facilitate healing. Sometimes it will be necessary to apply an orthopedic

block to the sound claw to get more weight off of the diseased claw. Remove loose horn and trim away all the hard edges. Only healthy horn should be left. Be careful not to trim too much off of the lateral claw. Preserve the heels, especially the medial rear heel as much as possible.

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

*Sole and white line hemorrhages* originate from damage to corium with the blood being incorporated into the horn as it grows. These will become visible several weeks after the insult depending on the thickness of the sole and rate of growth. A sole abscess or white line abscess can occur if the hemorrhage becomes infected. *Sole separation* results when the corium is damaged transiently, produces weak or damaged horn and then normal horn production resumes. *Sole ulcer* is a continuous opening in the sole horn that exposes the corium. The typical site is the rear middle part of the sole, which corresponds to the rear part of the pedal bone. The prognosis for sole ulcers depends on the damage to the horn producing tissue and the condition of the other claw. *Toe ulcer* occurs when the sole is worn too thin at the toe, or the toe drops inside the horn due to laminitis, or if the toe is accidentally trimmed too thin. Toe ulcers will always require an orthopedic block on the opposite claw. *White line disease* starts with fissures due to hemorrhage and poor quality horn formation. Rocks and gravel can become embedded in these and cause further problems. The problems with the stones or gravel are more likely the result of white line disease rather than the cause of the disease. *Heel horn erosion* is the formation of holes or fissures in the heel horn. Normal heel horn should be smooth. One hypothesis is that manure slurry contributes to heel horn erosion (the British refer to this condition as slurry heel). This generally does not cause lameness unless it is severe. This condition has also been associated with interdigital dermatitis. *Papillomatous digital dermatitis* (*footwarts, heel warts, digital dermatitis*,) is a superficial dermatitis that occurs most often on the rear feet at the commissure of the interdigital space near the heels. It is thought to be a multifactorial disease with bacterial involvement. Response to topical antibiotics is good but recurrence is common. *Interdigital phlegmon* (*foul in the foot, footrot*) is a bacterial disease that is generally caused by a synergism between two bacteria. It has a very characteristic smell and causes necrosis in the interdigital skin. It can invade the deeper tissues if not treated early with antibiotics (Blowey *et al.* 2003, Masson *et al.* 1988).

### 3. CONCLUSIONS

3.1. The correctly trimmed, normal foot should have a flat weight-bearing surface (wall and sole) and should be flat across the toes and heels and flat front to back.

3.2. The correctly trimmed, normal foot should also be perpendicular to the leg of the cow when viewed from the front or the rear.

3.3. Thermogenesis due to mechanical hoof trimming by using these modern disks are negligible if correct trimming is performed.

3.4. Loss of mechanical sole hoof stability leads to corium contusion, to breaking of the thin sole horn or breaking within the white line between sole and wall and to subsequent infection of the corium.

3.5. The hoof trimming by using chain saw disc are more precise, efficient allow fine adjustment and is not so reacting like rotoclip disc.

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## **AEROMICROFLORA SIGNIFICANCE IN RAISING OF BROILER CHICKENS**

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**Key words:** bird, aeromicroflora, pathogenic processes

### **SUMMARY**

This paper aims aeromicroflora's impact on the health of broilers. In a hall with 14,450 chickens, were followed from start to delivery microclimate factors. It was found amid a high humidity has increased over rules of aeromicroflora and a close relationship between E. coli isolated from air and isolated from the bodies. The air contains a wide variety of pathogens, which make up aeromicroflora. They come from manure, litter, feed, secretions and excretions as well as normal or pathological (Decun, 2007). Aeromicroflora is closely correlated with the concentration of dust in the air. Maximum load of the shelter air should be between 25,000 and 250,000 germs/m<sup>3</sup>air. (Decun, 2005). Veterinary technology supports a maximum load of 200,000 but germs/m<sup>3</sup> air (Drăghici, 1991). In the present study were followed microclimate factors and microbial load especially correlation with the pathogenic processes involved pathogens.

### **1. MATERIAL AND METHODS**

Research has been conducted on a farm in western country. In this study the number of subjects was 14,450 chickens, and length of investigations was 5 weeks, during which measurements were made of microclimate: temperature, humidity, air flow rate, ammonia, aeromicroflora. It also followed the deaths and injuries bodies by making necropsii and seeding the culture media of the samples from the corpses with injuries.

Methods used to determine the microclimate factors were the usual termohigrometeres respectively, anemometers, Dräger pump and gravity sedimentation method for aeromicroflora (Mitrănescu, 2002). All measurements were performed at three points in the hall throughout the experiment.

The culture media were chosen to establish different infectious agents involved in pathogenic processes, namely Agar for NTGMA, Levine environment for coliforms and Chapman environment for staphylococci.

Exposure time was 10 minutes for Levine and Agar, and 5 minutes to Chapman. Since the first harvest the number of colonies formed units (CFU) was too high, making it almost impossible to count colonies, the following determinations halved exposure times. Plates were left at the thermostat, and then were read with colony counter.

Bodies were necropsies after specific method. In the experiment were examined an estimated 29 bodies a week. The sampling was performed and antibiotic susceptibility.

## 2. RESULTS AND DISCUSSION

After measurements were obtained the following results:

1. Temperature was within acceptable limits. There were obtained temperature variations than those provided for rearing technology.

2. Humidity exceeded normal values accepted throughout the experiment.

3. Speed air flow was in line with pup age and remained constant throughout the measurements

4. Ammonia increased from week to week, but variations were not large and were classified as normal.

5. Aeromicroflora had variable values during the experiment. Total maximum allowable bacteria count is 200,000/m<sup>3</sup> of air, in which coliforms should not exceed 20%, or 10 000 CFU, and staphylococci not exceed 50%, or 100 000 CFU.

Given these limits were observed as follows:

In the first week values for the total number of bacteria, coliforms and staphylococci were within limits, being even lower limit.

In the second week NTGMA values were higher than in the first week, as the CFU 1136882, coliforms were located at the lower end, and staphylococci were much above the permissible.

In the third week there were increases in all three categories, the total number of germs being 5,653,469. They coliforms exceeded the upper limit, the number 10 532 CFU. The same thing happened when staph colonies observed when the number of CFU reached 2698841.

In the fourth week the total number of bacteria CFU was 2931616, coliforms fell below the limit, staphylococci decreased but even so they were worth three times the maximum permissible ie a total of 319 881 CFU.

Mortality also varies in quite wide limits, leaving 234 dead in the first week and reached 79 in last week.

Following necropsies of 117 bodies were found 56 corpses with clear colisepticemia injuries while the other had injuries that do not allow us to suspect any diagnosis.

Were isolated a number of 40 strains of *Escherichia coli*. After the examination of *E.coli* strains by PCR, it was establish that the air isolated were identical in 98% to those cadavers isolated.

As a result of antimicrobial susceptibility testing were found resistance germs to antibiotics that are commonly used in practice, and we observed sensitivity only to florfenicol and oxytetracycline.

### 3.CONCLUSIONS

3.1. Germs in the air cargo from Week II were constant over hygiene rules allowed.

3.2. It was found in 98% of cases a correlation between the types of *E. coli* isolated from the air and bodies.

3.3. Usual medication increased antibiotic resistance was due to the application of indiscriminate working protocols.

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## **SURGICAL TREATMENT OF CHRONIC PROLIFERATING EXTERNAL OTITIS IN DOGS BY MODIFIED TOTAL EAR CANAL ABLATION TECHNIQUE**

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**Key words:** ear, otitis externa, surgical treatment, total canal ablation

### **SUMMARY**

Total ear canal ablation is a radical surgical technique required if the external auditory canal presents stenosis due to the prolonged evolution of an inflammatory process at this level.

The purpose of this study was to evaluate the efficacy of modified total ablation of the ear canal to treat chronic proliferative otitis externa. We chose this method as a single method of surgical treatment of otitis externa without concomitant bulla osteotomy because the dogs had no otitis media simultaneously.

The study was conducted at the Department of Surgery, Faculty of Veterinary Medicine Cluj-Napoca on a number of 12 dogs of different ages and breeds who had unilateral chronic proliferative otitis externa.

Postoperative evolution of the studied cases was favorable, at 3 weeks postoperatively healing was recorded in all 12 animals. We have not seen recurrences in any of the studied animals.

Dogs with chronic ear infections can acquire irreversible changes at the ear canal and timpanic membrane level (Cole *et al.*, 1998).

Irreversible inflammatory changes are leading eventually to the obliteration of horizontal and vertical ear canal and dystrophic calcification of aural cartilages (McCarthy *et al.*, 1995; Matthiesen *et al.*, 1990).

Ear canal ablation is total surgical excision of the entire ear canal and is recommended in patients with chronic proliferative otitis externa, ear canal neoplasm or severe trauma affecting auricular canal (Bruyette *et al.*, 1993; Beckman *et al.*, 1990; Sharp, 1990).

The most common complications consecutive this surgery technique include facial nerve trauma, fistulizations, and wound infection (Krahwinkel *et al.* 1991; Smeak, 1990).



Total ear canal ablation as single method of surgical treatment is not recommended in patients with otitis externa and concurrent otitis media (Sharp, 1990).

## 1. MATERIAL AND METHODS

The study was conducted at the Department of Surgery, Faculty of Veterinary Medicine Cluj-Napoca on a number of 12 dogs of different breeds and ages who have been diagnosed with unilateral chronic proliferative otitis externa.

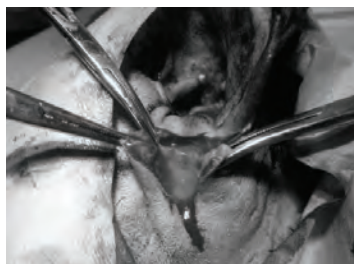
The diagnosis of proliferative otitis externa was established by corroborating the results of clinical examination, otoscopy and contrast radiography. We also performed X-rays to rule out otitis media because most animals with otitis externa have secondary otitis media.

The animals were subjected to a modified total ear canal ablation without resorting to lateral bulla osteotomy because there was no secondary otitis media.

Before surgery we collected discharge from the ear canal to carry susceptibility tests to establish appropriate postoperative treatment.

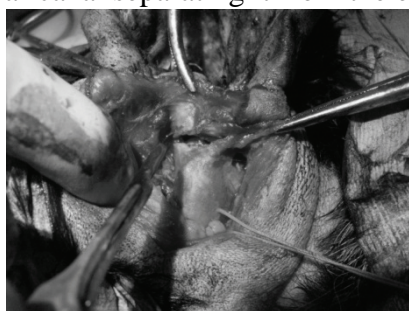
The animals were subjected to neurolept-analgesia with xylazine (1 mg / kg) and ketamine (10-20 mg / kg). After that we cleaned the auricular and the periauricular area. We also performed a lavage of the external ear canal to minimize the risk of contamination during surgery.

After the restraining and application of the operator field we made a "T" incision with the horizontal line situated on the back of the vertical ear canal and the vertical perpendicular line continued over a distance equal to the length of the vertical portion of the ear canal (fig. 1). We mention that the horizontal incision was made at 0.5 to 1 cm from the tip of the tragus to keep the external shape of the ear canal opening. The subcutaneous tissue was blunt dissected and was pulled rostral and caudal of the ear canal while avoiding the parotid gland located ventrally.



**Fig.1.** Appearance of the "T" incision at the skin level

The horizontal incision located on the skin has been extended rostral and caudal at the auricular cartilage level until we cut the entire circumference of the ear canal separating it from the ear concha (fig. 2).



**Fig. 2.** Horizontal cutting of the ear canal in the distal portion

By blunt dissection we dissected the periauricular tissue, step by step, until we reached the external acoustic meatus area where we identified the junction of the annular cartilage with bony external meatus (fig. 3). We cut the ligament between the two anatomical structures and we removed the entire ear canal.

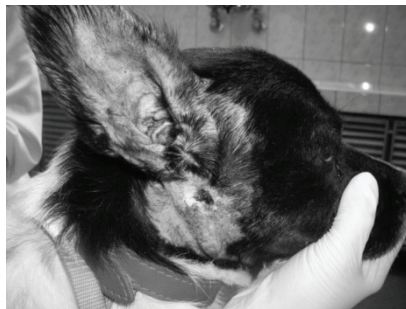


**Fig. 3.** Isolation of the ear canal and identification of the external acoustic meatus

With blunt scissors and a curette we removed the skin from the external acoustic meatus.

After completion of hemostasis we applied a drain to the external acoustic meatus that was fixed by suture to the skin. Operative wound was sutured in separate points with Dexon 3-0. The skin was sutured in separate points with Mersilk 1 and to protect the wound we applied each animal a Elizabethan collar.

Animals undergoing surgery were kept under surveillance for seven days during which we administered injectable antibiotics according to pathogen susceptibility results. To combat pain and inflammation we have given each animal analgesics (Novasul) for 4 days. Surgical wound was treated topically providing drainage until the deeper layers healing has occurred. All animals were declared healthy 3 weeks after performing surgery (fig. 4).



**Fig. 4.** Wound appearance two weeks after surgery

## **2. RESULTS AND DISCUSSION**

Surgery was performed in good condition in anesthetized animals by NLA and there were no side effects at the used products.

Cleaning the ear canal to obtain appropriate antiseptisy was difficult in all cases due to stenosis at the level of this anatomical structure.

Making the "T" shaped incision on the skin in the manner presented above allowed us partial detachment of the ear canal and keeping a annular portion of 0.5-1 cm of the ear cartilage which provided normal ear shape. We preferred this method to stabilize the ear because previous studies<sup>5</sup> show that after classical total ear canal ablation, especially in dogs with erect ears, occur a drop of the ear concha changing irreversibly the appearance of the

dog. By applying this method we were able to keep the original form of the ear in all studied dogs.

Periauricular tissue dissection to isolate the ear canal was difficult in all cases because of pronounced fibrosis of the tissue. The dissection was accompanied in all cases by diffuse, significant, bleeding easily controlled.

Cutting the ear canal at the external acoustic meatus level was easily performed in all cases because this area is not affected by the pathological process.

A very important aspect of the surgery was the curettage of the skin from the bony portion of the ear canal. This curettage was performed to the periosteum to be sure that we removed all the skin and no secretory glands remain at this level which could affect postoperative progress of patients. Removing the skin near the tympanic membrane was performed carefully so as not to traumatize it. Bleeding resulting from removing the skin was minimal in all cases and was controlled easily.

Postoperatively all animals showed edema for 3-4 days in the periauricular area. Medication with antibiotics for 7 days according to antibiogram had very good effects there were no wound infections. The drained maintained for 7 days in all animals allowed drainage of all exudates resulting from reparative processes thus avoiding the formation of sequesters that can later lead to chronic fistulas.

Because the operative wound was protected for 2 weeks with an Elizabethan collar the animals have not produced damage to the wound and the healing was not affected.

Animals were declared healthy 3 weeks after surgery when we found regression of all repair phenomena, wound healing and a crust located in the place where the draine was attached to the skin.

### **3. CONCLUSIONS**

3.1. Modified total ear canal ablation unlike TECA associated with LBO is suitable in patients that have chronic proliferative otitis externa without concomitant otitis media.

3.2. Keeping a ring of cartilage from the distal portion of the ear canal allows keeping the ear shape and position by a better surgical reconstruction of the base of the ear.

3.3. Because the presence of cartilaginous ring positively influences postoperative evolution of patients we recommend that they have a length of more than 0.5 cm to consolidate the base of the ear.

3.4. Where you cannot keep a portion of the ear canal, ear base reconstruction must be made with periauricular tissue and auricular cartilage.

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## **SURGICAL MANAGEMENT OF CERUMINOUS GLAND CARCINOMA IN DOGS**

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**Key words:** dog, neoplasm, ceruminous carcinoma, surgical treatment

### **SUMMARY**

Ceruminous gland carcinoma in dogs is a malignant tumor that affects ceruminous glands from the ear canal.

The purpose of this study was to evaluate the efficacy of surgical treatment in dogs with ceruminous gland carcinoma.

The study was conducted in the Department of Surgery, Faculty of Veterinary Medicine Cluj-Napoca, on a number of 8 dogs of different breeds and sexes, aged between 5 and 9 years who had pedunculated tumor in the ear canals.

The tumors were identified by clinical and otoscopic examination and the confirmation of the diagnosis was performed after histopathological examination.

The treatment was surgical by excision of the tumor along with a portion of peritumoral normal skin. Postoperative evolution of animals was different depending of animal and the amount of excised portion of the ear canal. At 4 weeks postoperatively all animals were declared clinically healthy. In one patient the tumor recurred 6 months postoperatively requiring a second surgery.

Neoplastic diseases in dogs ear are rare compared to the incidence of skin cancers in other regions. Approximately 2-6% of dogs presented for ear surgery have a ear tumor.

Neoplastic process usually starts in the epithelial structures of the ear or its annexes but can also start from the cartilage or from the infected bone.

Neoplasia involves often auricular concha and the external ear canal while the middle and internal ear are rarely affected (Withrow *et al.*, 2007).

Ceruminous gland carcinoma occurs in old dogs and is less aggressive in this species than in cats (Morrison, 1998).

The concurrence of otitis media and ceruminous gland-related tumor development has also been reported in dogs (Moisan *et al.*, 1996).

Surgical treatment of ear tumors depends on the location, type and behavior of tumors and the disease progression. Radiation therapy was found to be safe and effective in this type of tumors (Théon *et al.*, 1994).

Laser surgery is very effective when it is available (Rest, 2004).

### 1. MATERIAL AND METHODS

The diagnosis of the tumor was determined by clinical examination corroborated when possible with otoscopic examination.

At clinical examination we found pedunculated tumors with different sizes which were in the vertical external ear canal with characteristic aspect of ceruminous gland carcinoma (fig. 1).



Fig. 1. Tumor in the vertical ear canal

Before the surgery we have taken discharge from the ear canal to carry pathogen susceptibility because due to long-term evolution of the pathological process in the ear a septic process was present.

The animals were anesthetized by neuroleptic analgesia with xylazine (1 mg/kg) and ketamine (10-15mg/kg) and after that we made a proper cleaning of the ear concha, the ear canal and the periauricular area to prevent further contamination of surgical wound. To remove the exudate and debris from the ear canal depth we performed a lavage of the ear canal with sterile saline using a syringe to which we attached a thin catheter.

To increase the operator field and identify the implantation of tumor we have made a vertical incision of 2-3 cm from the pretragic incisure that interest the skin on both sides of the ear cartilage and the auricular cartilage itself (fig. 2).

The tumor excision was performed at their base with unaffected peritumoral skin within a radius of 2-4 cm (fig. 3). Hemostasis was done by pressure and after its completion we performed a new ear lavage to remove blood clots from the deep ear canal.

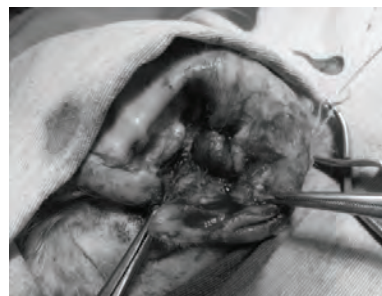


Fig. 2. Aspect of the incision from the pretragic incisure

Incision made to identify the base of tumor was sutured in separate points and in the ear canal we applied a cotton swab on which was previously applied ointment.



**Fig. 3** Tumor excision

Surgical wound was topic treated and the cotton swab from the ear canal was changed daily for 4 days. To prevent scratching in each animal we applied an Elizabethan collar that was maintained for 7 days. Anti-infective therapy was performed for 5 days according to

antibiogram results.

## **2.RESULTS AND DISCUSSION**

Surgical excision of the ear tumoral formation is not difficult but requires following some operating steps, which facilitates on the one hand the surgery on the other hand positively influence further postoperative evolution of the patients.

Because ear canal has a small diameter that does not allow smooth removal of tumor we increased the operative field through the incision of the vertically ear canal side wall starting from the pretragic incisure. Making this incision we managed to identify in all cases the neoplastic formation. Bleeding resulting from the ear canal incision was one of low intensity and easy to control by presure.

The tumor ablation was performed in good condition in all cases and bleeding was one of low intensity. All tumors were peduculated and did not affect in any case the ear cartilage because were limited to the ear skin.

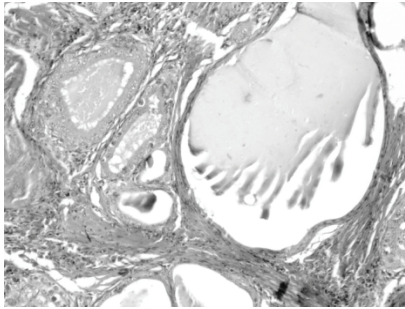
Because we could not make a suture where the tumor was excised we preferred to apply a cotton swab on which we put ointment. By using this type of swab we offered protection to the denude area while preventing his adherence in that area. Protecting the wound for 4 days allowed complete healing; epithelization was finished between 18 and 25 days depending on the wound size resulting after surgery.

Four weeks after surgery all animals were declared clinically healthy fact characterized by full epithelization of the wound.

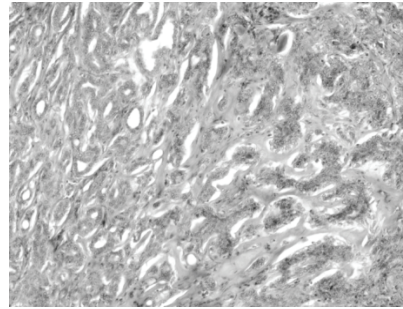


Pathology examination confirmed that the tumor excised were complex and mixed ceruminous gland carcinomas which had in their structure chistic glands (fig.4.) and a tendency to ossification of the connective tissue (fig.5).

In one animal there was a tumor recurrence which required a second surgery more radical.



**Fig. 4.** Chistic gland in the tumor structure



**Fig. 5.** Connective tissue that presents tendency to ossification

### 3.CONCLUSIONS

3.1. To perform surgery is required to increase the operative field by making a vertical incision in lateral wall of the the ear canal.

3.2. Ablation of tumor should be performed together with adjacent healthy tissue to prevent recurrence.

3.3. Protect the wound with a cotton swab premits epithelization of the denude area in a reasonable time preventing the occurrence of septic processes.

3.4. Surgical ablation of ceruminous gland carcinoma is an effective method of treatment of this condition and is easily achieved if the surgent complies with the steps described above.

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## RESEARCH ON AVIAN RESPIRATORY BACTERIOSIS

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**Key words:** colibacillosis, E.coli, intensive, laying hens

### SUMMARY

Avian respiratory bacterioses represent a serious problem for the poultry industry. Avian colibacillosis, respiratory bacterioses frequently found are diseases that cause economic losses in the affected flocks. Pathogenic strains of *Escherichia coli* in poultry can generate airsacculitis, polyserositis, omphalitis, peritonitis, salpingitis, synovitis, septicemia, cellulitis, necrotic dermatitis and granulomatosis syndrome in poultry. The disease was studied in a broiler flock of 190,000 poultry, and losses were significant due to the high rate of mortality and morbidity.

Strains of pathogenic *E. coli* in poultry are responsible for extra intestinal, systemic infections, due to their invasive properties. Clinical manifestations vary depending on the age of the host. Prevailing infections caused by *E. coli* in poultry, both in the broiler as well as in adult hens are those with respiratory symptoms and can be transmitted both vertically and horizontally. Initiating factors can be a viral infection (infectious bronchitis, Newcastle disease), a mycoplasma infection, or some predisposing physical factors (excessive dust, cold, high concentrations of ammonia).

### 1. MATERIAL AND METHODS

Epidemiological investigations have been conducted during the period February 2011-March 2011, on a flock of 190,000 of broiler of the race Ross 308 reared on the ground in 10 halls with a capacity of 19,000 poultry in each hall.

In the halls of broiler poultry mortality rate was high in the first 7-10 days (table 1), reaching at a rate of 5.24% and the morbidity rate was 40%.

From the clinical perspective the poultry presented adynamia, anorexia, there were analysed the lesions in all necropsied poultry. The presence of lesions of vitelline omphalitis as well as non resorption of the vitelline sac

leads to the conclusion that infection has occurred since hatching. There were taken samples in order to specify the diagnosis and to set an optimal treatment. There were made 250 of bacteriological, serologic and metabolic examinations.

Table 1

*Mortality rate on a flock of 190,000 of broiler Ross 308*

<b>Poultry age</b>	<b>No. of dead poultry</b>	<b>Total no of dead poultry</b>	<b>Mortality percent (%)</b>
<b>1 day</b>	500	500	0.26
<b>2 days</b>	750	1250	0.92
<b>3 days</b>	2000	3250	1.71
<b>4 days</b>	2100	5350	2.81
<b>5 days</b>	1950	7300	3.84
<b>6 days</b>	1500	8800	4.63
<b>7 days</b>	500	9300	4.89
<b>8 days</b>	350	9650	5.07
<b>9 days</b>	200	9850	5.18
<b>10 days</b>	115	9965	5.24

The methods of analysis used were based on the isolation and identification of strains on broth and agar. So as a result of growing in broth intense turbidity is observed, with a ring on the surface, and on the agar there were developed opaque, non - pigmented colonies. There have been used also on the specific culture media such as: Levin, Drigalski, and Mc Conkey (SF Altekruse et. al., 2002).

The identification of bacterial strains was performed also by morphological tests confirmed by biochemical examinations: indole reactions, hydrogen sulphide, hydrolysis of urea, and the examination of the reaction to glucose, lactose, sucrose, mannitol, associated with the reaction of agglutination with a polyvalent anti colibacillar serum (Victoria Martin, 2001).

Testing the sensitivity of *E. coli* strains was achieved by the method of diffusimetric antibiograms (Bauer - Kirby) semi quantitative method, which utilizes biodiscs impregnated with different quantities (mg-micrograms) of active substance. (JJ Maurer et al.,2002).

The necropsy examination carried out on a number of 6700 of poultry revealed lesions of airsacculitis and sero - fibrinous perihepatitis (76.9%), lesions of sero - fibrinous pericarditis (89.2%), hemorrhagic-necrotic hepatitis (15.3%), fibrinous salpingitis (23%), fibrinopurulent peritonitis (53.8 %). (Table 2)

Table 2

*Type and frequency of anatomo- pathological changes*

No. of dead poultry	Airsacculitis		Pericarditis		Hepatitis		Peritonitis	
	No.	%	No.	%	No.	%	No.	%
1290	888	68.9	1111	86.2	199	15.5	110	8.6
3845	3591	93.4	3637	94.6	907	23.6	2956	76.9
1565	1389	88.8	934	59.7	-	-	233	14.9

The isolated *E. coli* strains were found to be sensitive to Spectinomycin, Colistin sulfate; moderately sensitive to Neomycin, Enrofloxacin, Lincomycin, Trimethoprim; resistant to Tetracycline, Streptomycin, Amoxicillin, Oxytetracycline.

## 2.RESULTS AND DISCUSSIONS

Respiratory contamination especially by the dust or the fluff contaminated with pathogenic serotypes of *Escherichia coli* is supposed to have been made since the eclosion moment.

In chickens infected, the vitellin changes its normal appearance to become viscous, yellowish-green with caseous or watery aspect, yellow-brown. The vitelline sac is congested and edematic.

There were present also omphalitis and vitelline sacculitis, congestions, serous or sero-fibrinous exudates of the serous and especially of the pericardium. (fig.1) The liver is congestive-dystrophic, wrapped in a fibrinous pellicle. The spleen is increased in volume and has the hue of rotten sour cherry (fig. 2).

In the necropsied poultry, at the level of air sacs, deposits of fibrin of fibro-cazeous form, like scrambled eggs have been revealed. The lungs were hiperemiated and increased in volume, with lesions of catarrhal pneumonia.

It was also highlighted lesions of serous pericarditis and sero- fibrinous pericarditis (fig. 3).



**Fig.1** Omphalitis and vitelline omphalitis in



**Fig. 2** Fibrin deposits in the liver and pericarditis in poultry cord in poultry of 5 days



**Fig. 3** Fibrinous infected with *E. Coli*

For the reduction of losses through mortality, a medicinal treatment was applied for five days with Spectinomycin - 200 ml/100 l in the drinking water. After 2-3 days of the administration the mortality rate began to decrease. (Table 3).

Table 3

Mortality rate after treatment with antibiotics

Duration of treatment	No. of dead poultry	Mortality percent
1 day	510	0.5 %
2 days	200	0.9 %
3 days	280	1.27%
4 days	150	0.53 %
5 days	100	0.45

### 3.CONCLUSIONS

The research on avian colibacillosis in intensive farming system conditions led to the following conclusions:

3.1. Diseases were found in a flock of 190,000 broiler raised in intensive system, starting with the age of 1 day.

3.2. The disease has manifested by a mortality rate of 5.24%.

3.3. Inseminations carried out on usual nutritional environments (agar and broth) and on special environments (Levin) have enabled the isolation and identification of 4 strains of *Escherichia coli*.

3.4. The isolated *E. coli* strains were found to be sensitive to Spectinomycin, Colistin sulfate; moderately sensitive to Neomycin, Enrofloxacin, Lincomycin, Trimethoprim; resistant to Tetracycline, Streptomycin, Amoxicillin, Oxytetracycline.

3.5. Medicinal treatment for five days with Spectinomycin - 200 ml/100 l in drinking water, has resulted in the decrease of mortality in the flock of poultry.

3.6. The necropsy examination carried out on a number of 6700 of poultry has revealed lesions of airsacculitis and sero - fibrinous perihepatitis (76.9%), lesions of sero - fibrinous pericarditis (89.2%), hemorrhagic-necrotic hepatitis (15.3%), fibrinous salpingitis (23%), fibrinopurulent peritonitis (53.8 %).

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## RECONSTRUCTION TECHNIQUES IN THE ODONTAL INJURIES IN DOGS

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**Key words:** *dentistry, composite, caries*

### SUMMARY

The goal of this paper is to introduce on a widescale the most effective methods of restoration of coronary morphology with composite biomaterials (Bellows, 2004)

Currently practicing veterinarian needs to know and to choose the method of restoration in dogs and cats according to each case to get the maximum benefits of these techniques (Gorel Cecilia, 2004).

Veterinary Dentistry is an unexplored area, still in the pioneering stage in Romania, but with a wide field of action, depending on the intended purpose and the individual equipment (Rândașu and Rândașu, 2001).

The veterinary dentistry is a newly approached field in Romania. The veterinarian practitioner has a deficiency in this new area of research and practice. In this paper we studied the practical implications of composite biomaterials that might be used in the morphological restoration of the dental crown (Holmstrom *et al.*, 2004)

### 1. MATERIAL AND METHODS

The study was performed on dogs with lesions of the enamel and dentine.

After clinical diagnosis set by traditional methods, corroborated with special methods with revealing substances, we carried on with the morphological reconstruction treatment with a composite hybrid material.

#### *Surgical stages*

The animals were under neuroleptanesthesia (NLA).

By removing the affected enamel and dentine tissues we obtained an excavation of variable size and depth, depending of the anatomo-clinical

shape of the destructive process. This excavation represents an open wound which must be restored with new generation biomaterials, leading to the morphological reconstruction of the tooth.

Depending on the location of the lesion, there are five types of cavities:

- 1<sup>st</sup> class cavities resulting from the lesions of the occlusal grooves and cusps of premolars and molars;
- 2<sup>nd</sup> class cavities resulting from the treatment of the interproximal sides of premolars and molars;
- 3<sup>rd</sup> class cavities resulting from the treatment of the interproximal sides of the frontal teeth intact incisal angle;
- 4<sup>th</sup> class cavities resulting from the treatment of the interproximal sides of the frontal teeth damaged incisal angle;
- 5<sup>th</sup> class cavities resulting from the treatment of the cervical surface lesions.

During the preparation of the cavities we went through the following stages:

- the opening and widening of the process;
- excision of the damaged enamel and dentine;
- preventive extension
- retention of the cavity;
- control of the cavity walls resistance;
- finishing the enamel angles.

The opening of the cavity represents the approach path and allows the evaluation of the lesion.

We continued with the excision of the altered tissues until the appearance of healthy tissue, fundamental rule in the treatment of simple dental lesions. The excision was done with special rotative instruments.

In the preventive extension stage we continued the excision to prevent the repulse. In frontal teeth where the thickness is small this stage is skipped.

The newly created cavity must have a shape that allows the stability and resistance of the reconstruction material.

The finishing of the enamel angles has the purpose of making the edges resistant to the masticatory forces and a better transition to the composite material.

The treatment of the dentinary wound depends of the depth of the lesions, and it is obtained using calcium hydroxide products.

After the cleaning, disinfection and drying of the cavities, we carried on with the demineralisation of the enamel (30 sec) and dentine (15 sec) with a buffed phosphoric acid. Next the acid is removed with water, the cavity is dried and an adhesive agent is applied to increase the adhesion of the composite material.

The composite is a paste that we applied in layers of 2mm to obtain a low contraction and expansion coefficient.

After the thoroughly preparation of the composite surface, we polymerized it with a blue light of 460 to 470 nm wavelength for 40 sec.

## **2. RESULTS AND DISCUSSION**

We restored the dental morphology of the tooth to reestablish the function, to prevent the spread of the lesions and to redress the aesthetic aspect.

The materials used proved to be resistant, rigid and with a good viscosity.

Modern composite materials are mixture of resins and additional particles. The materials we used had colloid particles of silica dioxide, 0,04 mm in size. The easiest to use was the hybrid composite.

The thickness of the layers must be approximately 2mm because the photopolymerisation is activated by a amine-ketone-blue light complex, the light penetrates the material approximately 2 to 3 mm.

The composite materials have different pigments and stabilisation agents so that the color of the material is similar with the color of the tooth.

The material made a solid bond with the enamel and dentine.

## **3. CONCLUSIONS**

After the analysis of the data from this study we concluded that:

3.1. We tried and proved that these types of new biomaterials, composit resins and glass ionomers, can be successfully be used to restore the coronary morphology, in all types of cavities.

3.2. At the 7 and 30 days recheck we found very good material adhesion.

### Advantages

3.3. Because of the nature of the materials (solid, semisolid) the application of the material is relatively easy.

3.4. We found no evidence to support the hypothesis that these materials have negative effects on the test subjects.

3.5. The temperature inside the composite mass reached 40°C, measured with a digital thermometer, and it is not affecting the dental pulp.

3.6. The finishing was done with diamond cutters and discs covered with aluminium oxide.

### Disadvantages

3.7. It is difficult to obtain perfect contact points with the artificial cavity

3.8. The materials have a small coefficient of shrinkage during polymerisation

3.9. The polymerisation depth is limited, creating problems with deep cavities.

3.10. The cost of these materials is high, increasing the cost of the intervention.

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## **PREVALENCE AND EPIDEMIOLOGY OF *NEOSPORA CANINUM* — LIKE AND OTHER INTESTINAL PARASITE INFECTIONS IN DOGS FROM SOUTHERN ROMANIA**

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**Key words:** prevalence, *Neospora caninum*, dog, intestinal parasite

### **SUMMARY**

*Neospora caninum* is a protozoan recognized as a major abortifacient in cattle, for which the only source of postnatal infection are oocysts eliminated in dog faeces. During October 2010 - October 2011 a total of 267 dog faecal samples were collected from 7 different urban and rural sites in southern Romania, and were analyzed using a flotation technique. Parasites were found with an overall prevalence of 68.9%, with slight differences within categories of dogs: 65% in farm dogs, 58.9% in guard dogs (80% in rural and 32% in urban), and 72.4% in stray dogs. *N. caninum*-like oocysts were found with a total prevalence of 5.2%, but the within-dog category prevalence was different: 5% in farm dogs, 13.3% in rural guard dogs, 0% in urban guard dogs, and 4.7% in stray dogs. *N. caninum*-like oocysts were more common in adult dogs, females, in spring and autumn seasons, in most cases in association with other parasites. Other eleven species of intestinal parasites were identified, with predominance of helminthes over protozoan species. The most prevalent was *Ancylostoma caninum* (40.1%), followed by *Trichocephalus vulpis* (31.5%), *Uncinaria stenocephala* (19.9%), *Toxocara canis* (8.2%), *Cystoisospora* spp. (6.7%), *Dipylidium caninum* (3%), *Toxascaris leonina* (1.5%), *Eucoleus* spp. (1.5%), *Taenia* spp. (0.75%), *Strongyloides stercoralis* (0.4%), and *Sarcocystis* spp. (0.4%). In conclusion, the prevalence of intestinal parasites was high, and this lead to a potential risk for losses in rent animals and zoonoses in humans.

Dogs act as definitive host for many intestinal parasites, some of which are responsible for diseases as neosporosis, recognized as a major cause of abortion in cattle. Although the number of oocysts shed by dogs is usually low (Dubey and Schares, 2011), dogs living in the close proximity of farms can be responsible for *N. caninum* infection in cattle (VanLeeuwen *et al.*, 2010). The ingestion of sporulated *N. caninum* oocysts from the environment is the only demonstrated natural mode of infection in cattle after birth (McCann *et al.*, 2007).

In addition, intestinal parasites are recognized as one of the most common pathogenic agents in dogs, some of them with zoonotic transmission potential (Robertson and Thompson, 2002), as *Toxocara canis* (Good *et al.*, 2004), *Ancylostoma caninum* (Croese *et al.* 1994, Bowman *et al.*, 2010), *Taenia* spp./*Echinococcus* spp. (Robertson and Thompson, 2002), and even *T. vulpis* has been reported to cause human infections but these are in general considered not scientifically sound and have been questioned (Traversa, 2011).

Given the close contact between dogs, cattle and humans, the present survey was undertaken to assess the epidemiology and prevalence of *Neospora caninum*-like oocysts and other intestinal parasite infections in dogs in close contact with dairy cattle and dogs from urban areas (stray dogs and urban guard dogs).

## 1. MATERIAL AND METHODS

The present research was carried out from October 2010 to October 2011 in different urban and rural sites in southern Romania (cities of Bucharest, Pantelimon, Magurele, Giurgiu, Popesti-Leordeni, villages and farms from Ilfov, Giurgiu, Teleorman, Dambovita, and Calarasi counties). A total of 267 faecal samples were taken from randomly selected dogs, females and males, most of them of common breed. Stray dog samples were taken during the sterilization of animals in 2 shelters and one veterinary clinic from urban areas. Fecal samples were collected from rectal level and kept in cold conditions until analyzed.

Samples were examined visually for identification of adult parasites, then processed by flotation technique with saturated sodium chloride solution and examined under a microscope. Samples were classified as positive if at least one parasitic element was present. Each parasitic element was identified by the morphological characteristics as described by Mitrea (2002).

## 2. RESULTS AND DISCUSSION

From 267 dogs examined, the overall prevalence of infection with intestinal parasites was 68.9% (184/267) and *N. caninum*-like oocysts were microscopically detected in 5.2% (14/267) of faecal samples. The within-dog category prevalence was different, as shown in Table 1.

Table 1

Prevalence of *N. caninum*-like oocysts compared with total prevalence of intestinal parasites in stray, guard and farm dogs

Category of dogs	No. of dogs	Positive samples		%	
		<i>N. caninum</i> -like oocysts	Total intestinal parasites	<i>N. caninum</i> -like oocysts	Total intestinal parasites
Stray	192	9	139	4.7	72.4
Guard	Urban	25	8	-	32
	Rural	30	24	13.3	80
	Total	55	32	7.3	58.2
Farm	20	1	13	5	65
TOTAL	267	14	184	5.2	68.9

Nearly a similar prevalence rate of total intestinal parasite infections was reported recently by Mircean *et al.* (2011) who found a value of 62.2% in different counties from Transylvania. In southern Romania other studies also reported similar prevalence values: 57.29% in one public place, with access for stray and pet dogs, from Bucharest (Tudor, 2008) and 66.07 % in public places and stray dogs from Bucharest and Valcea County (Costin *et al.*, 2011)

In other geographical regions of the world prevalence values have varied widely, from 99% in dogs from a tea-growing community in India (Traub *et al.*, 2005), 71.33% in stray and housed dogs from Spain (Martinez-Moreno *et al.*, 2007), to 17.6% in urban, rural and shelter dogs from Czech Republic (Dubna *et al.*, 2007). These differences in parasitic prevalence in dog faeces may be due to differences in dog category taken into study, modality of collecting samples, socio-economical differences, and structure of canine populations.

*N. caninum*-like oocysts were found in a surprisingly high prevalence, compared with other similar studies: 0.3% in northern Belgium kennel dogs (Claerebout *et al.*, 2009), 3% in owned dogs from Argentina (Fontanarrosa *et al.*, 2006), and 0.5% in urban, rural and shelter dogs from Czech Republic (Dubna *et al.*, 2007).

*N. caninum* oocysts morphologically resemble oocysts of three other coccidian parasites (*Hammondia heydorni*, *Hammondia hammondi*, and *Toxoplasma gondii*) that might be present in canine faeces. Although *H. hammondi* and *T. gondii* recognizes cat as definitive host, these oocysts have been observed in canine faeces as a result of coprophagia (Schaes *et al.*, 2005).

In positive faecal samples of the present study *N. caninum*-like oocysts density was low (up to 10 oocysts per microscopic slide). *N. caninum* oocysts have been identified in only a few dogs worldwide, and the number of oocysts shed by dog is usually low (Dubey and Schaes, 2011). The difficulty in finding *N. caninum* oocysts in faeces from naturally infected dogs was confirmed in a number of studies. Palavicini *et al.* (2007) found *N. caninum* DNA in four of 34 dog faecal samples, but these samples were negative by direct microscopical examination and by bioassay. Razmi (2009) detected microscopically 4 *Hammondia Neospora*-like oocysts in 174 faecal samples (2.2%), 2 confirmed by PCR. Paradies *et al.* (2007) did not detect any oocysts of *N. caninum* in 230 dog faecal samples, including 160 farm dogs. Regidor-Cerrillo *et al.* (2010) microscopically observed *N. caninum*-like oocysts in the faeces from one farm dog, but the number of oocysts was very low, and the aetiology could not be confirmed.

The highest prevalence of intestinal parasites in dogs was obtained in guard rural dogs, probably because in rural area dog is not receiving the proper attention and antihelminthic treatments are rare or absent. The same situation is encountered when assess the *N. caninum*-like prevalence (highest in guard rural dogs). Rural dogs are more exposed to consume waste of animal carcasses declined from human consumption. Cavalcante *et al.* (2011) showed that dogs can be infected with *N. caninum* and shed oocysts after feeding different tissues of infected cattle (masseter, heart, liver, and brain).

Over the farm dogs *N. caninum*-like oocysts were identified in a single dog, but near by a dairy farm with a high seroprevalence of *N. caninum* infection in cattle (80%) we found a group of dogs with a coprorevalence of 6.1% of *N. caninum* oocysts, and an overall parasite infections prevalence of 90.9%. In dairy cattle from South of Romania seroprevalence of *N. caninum* infection was 41.73% (Mitrea *et al.*, 2011).

*N. caninum*-like predominated in adult dogs (12/14, 85.7%), females (11/14, 78.6%), and prevalence was higher in spring (6/14, 42.9%) and



autumn (6/14, 42.9%). In the most positive samples *N. caninum*-like was observed in mixed infection with other intestinal parasites (12/14, 85.7%).

Overall, infection with a single parasite species and mixed infections with two or more parasite species were found in almost equal proportions. By category, farm and guard rural dogs were more likely to be poliparasitized, while urban dogs often developed single parasitic infections (Table 2). Stray dogs also had a high percentage of mixed infections, as others mentioned before (Olteanu, 2000).

Table 2

Type of parasitic infections in dog populations

Type of parasitic infection	Prevalence (%)					
	Total	Stray	Guard			Farm
			Urban	Rural	Total	
Single species	35.6	39.1	24	30	27.3	25
Mixed species	33.3	33.3	8	50	30.9	40

Following influential factors, intestinal parasites had a higher overall prevalence in dogs less than 1 year old than those over 12 months of age, but there were only slight differences in the prevalence between male and female; infection levels tended to be higher during autumn and less common in winter (Table 3), confirming other investigations (Dubna *et al.*, 2007; Andresiuk *et al.*, 2007).

Table 3

Total prevalence of intestinal parasites by age, sex and season

	Age		Sex		Season			
	<1year	≥1 year	Male	Female	Spring	Summer	Autumn	Winter
No. positive	38	143	48	135	51	44	92	7
No. negative	10	76	25	58	18	20	34	11
%	79.2	65.3	66.2	69.9	66.2	68.8	73	33.9

Enteric parasites identified in this study, others than *N. caninum*-like, are recorded in Table 4, in order of their prevalence.

Table 4

Prevalence of intestinal parasites in dog populations

Parasites	Prevalence (%)					
	Total	Stray	Guard			Farm
			Urban	Rural	Total	
<i>Ancylostoma</i> spp.	40.1	44.8	12	60	38.2	45
<i>Trichocephalus vulpis</i>	31.5	36.5	16	30	23.6	5
<i>Uncinaria stenocephala</i>	19.9	22.9	4	16.7	10.9	15
<i>Toxocara canis</i>	8.2	9.4	-	6.7	3.6	10
<i>Cystoisospora</i> spp.	6.7	4.7	8	13.3	10.9	15
<i>Dipylidium caninum</i>	3.0	3.6	-	3.3	1.8	-
<i>Toxascaris leonina</i>	1.5	0.5	-	-	-	15
<i>Eucoleus</i> spp.	1.5	1.7	-	3.3	1.8	-
<i>Taenia</i> spp	0.7	1.0	-	-	-	-
<i>Strongyloides stercoralis</i>	0.4	-	-	-	-	5
<i>Sarcocystis</i> spp.	0.4	-	-	3.3	1.8	-

Helminthes generally had higher prevalence values than protozoan. Overall, the most prevalent was the nematode *A. caninum* (Table 4), both in young and adult dogs, and also predominated in all dog categories, except for guard urban dogs. The other hookworm, *U. stenocephala*, also had a high prevalence and in most cases was identified in mixed infections with *A. caninum*. *A. caninum* has been referred to as one of the most commonly found parasite of dog in Europe (Bridger and Whitney, 2009) and other parts of the world (Katagiri and Oliveira-Sequeira, 2008; Fontanarroza *et al.*, 2006).

In young dogs prevalence of *T. canis* (20.8%, 10/48), *Cystoisospora* spp. (16.7% 8/48) and *T. leonina* (6.3%, 3/48) had significantly higher values, compared with adult dogs (respectively 5.5%, 4.6%, and 0.45%), as also described by others. In Albania *T. canis* was significantly higher in young dogs (Xhaxhiu *et al.*, 2011). Transplacental and transmammmary transmission of this parasite plays an important role in increased prevalence observed in younger than in older dogs. Also *T. leonina* and *Cystoisospora* spp. were reported to be more prevalent in young dogs than in older ones in other

studies too (Martinez-Moreno et al, 2007, Fontanarrossa *et al.*, 2006). In other studies *T. canis* was reported as the most prevalent or the second more prevalent parasite of dog (Xhaxhiu *et al.*, 2011), but in the present study prevalence was lower. This may be due to dogs submitted to test, randomly selected, not by presence of any clinical sign. Also we have to consider that some dogs might be received some antihelminthic treatment at a time.

The high prevalence of *T. vulpis* (31.5%) observed in the present study is higher than the prevalence found by Mircean *et al.* (2011) in other regions from our country. In other countries too the prevalence of *T. vulpis* was lower: 21.6% in Albania (Xhaxhiu *et al.*, 2011), 7.1% in Brazil (Katagiri *et al.*, 2008). This may be due to resistance of this parasite to commonly used substances for antihelminthic treatment.

### 3. CONCLUSIONS

3.1. The overall prevalence of canine intestinal parasites found in this study (68.9%) revealed a high level of infection that requires an effective anti-parasite control programme.

3.2. The highest prevalence was obtained in guard rural dogs (80%), followed by stray dogs (72.4%).

3.3. *N. caninum*-like oocysts are present in dog faeces with a prevalence of 5.2%, showing that dogs from South of Romania may be exposed to this parasite.

3.4. *N. caninum*-like had a higher prevalence in agricultural locations (13.3% in guard rural dogs and 5% in farm dogs), representing a possible risk for *N. caninum* horizontal infection in cattle.

3.5. *N. caninum*-like was found in association with other parasites in most positive samples.

3.6. Parasites with potential of zoonotic transmission were identified in high prevalence values, so that the risk of human infections cannot be ruled out.

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## CLINICAL, PARACLINICAL AND MORPHOPATHOLOGICAL ASPECTS OF DIGESTIVE PARASITOSEs IN PIGEONS

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**Keywords:** racing pigeons, digestive infestation, *Trichomonas*, *Eimeria*, *Ascaridia*, *Capillaria*.

### SUMMARY

The researches were held in January 2008 - May 2009, on a flock of 464 racing pigeons from private farms, in order to study clinical, paraclinical and morphopathological aspects of the digestive parasitoses. Clinical examination was performed periodically by general inspection of the flock and also by individual examination. Pathological material samples (faeces and scrape of fibrinous deposits) collected in sterile vials were examined specifically by direct and qualitative (Willis) or quantitative (Mc.Master) flotation coproscopic methods, appreciating the extensivity (E%) and parasitic infestation intensity (OPG). Results showed evolutive morbid states caused by *Trichomonas*, *Eimeria*, *Ascaridia* and *Capillaria* genus. In adult pigeons, the *Ascaridia* and *Capillaria* infestation intensity was weak (OPG: 0-200), with subclinical evolution, ensuring the porting of parasites and parasitic space pollution phenomenon. In squeakers and young pigeons, the intensity of infestation with *Eimeria* was average (OPG: 500 - 1000), leading to clinical evolution with losses through morbidity and mortality. In the analysed samples, the extensivity of the parasitic elements was variable, respectively, 32.9% in *Trichomonas*, 16% in *Eimeria*, 12.76% in *Ascaridia* and 8.51% in *Capillaria* genus. Morphopathological was found the fibrinous inflammation of the pharyngo-esophageal area and catarrhal enteritis.

Racing pigeons parasitoses, along with other morbid entities contribute to disruption of vital functions and reduced competition activity, hampering the efforts of passionate breeders (Bilius and Vergilia Bacalu, 2005, Severeanu at al, 1998.). Pigeons endoparasitoses are produced by many kinds of parasitic genus belonging to flagellates (*Trichomonas*, *Hexamita*) sporozoas (*Eimeria*, *Toxoplasma*, *Haemoproteus*), trematodes (*Echinostoma*), cestodes (*Railletina*, *Hymenolepis*), nematodes (*Capillaria*, *Ascaridia*, *Ornithostrongylus*, *Tetrameres*, *Syngamus*), amounting to an impressive concentrated aggression on the parasitized organisms. The digestive system represents an ideal multidimensional niche for many species of parasites that are localized from the mouth to the cloaca, inducing morbid states that are generating great losses in flocks of pigeons. (Severeanu and Ivana, 1991, Olimpia Iacob, 2010).

Investigations were undertaken in order to study the clinical, paraclinical and morphopathological aspects of some digestive parasitoses in racing pigeons and their consequences on health and sports performance.

### **1. MATERIAL AND METHODS**

The research was conducted in January 2008 - May 2009, on a flock of 464 racing pigeons, from six breeders, (4 in area B and two in area I), private properties. The flock was examined clinically, paraclinically and morphopatological for detection of digestive parasitoses. Clinical examination was done by general and individual inspection. From the studied flock, 180 pigeons showed polymorphic morbid conditions including symptoms related to digestive disorders. Youth aged 10-90 days was most affected. Fecal samples (36) collected in sterile containers were analyzed by qualitative (method modified Willis) and quantitative (Mc.Master) coproscopic methods. Pathological material samples (24 samples of fibrinous deposits) from the oral cavity were analyzed by direct method with addition of slightly warmed saline solution and methylene blue. Parasitic elements were examined and photographed at Motic microscope with oc.10, ob. 10, 20. Species identification and taxonomic classification have been made based on morphological characters compared with the specialized literature. Morphopathological, 12 corpses were necropsied observing the digestive system macroscopic changes. Photographic images were taken with a digital camera.

### **2. RESULTS AND DISCUSSION**

The results of the flock clinical investigations from six racing pigeons breeders shows a sporadic evolution of digestive parasitoses, conditioned by specific factors for each flock from every farm. Monthly incidence of disease was different (Table 1), affecting racing pigeons of all ages and both sexes.

Table 1.

The incidence of clinical cases in examined pigeons in 2008-2009

Farm	Sick examined pigeons										
	2008							2009			
	Mar	May	Jun	Jul	Sep	Oct	Nov	Jan	Feb	Apr	May
<b>F1.</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>5</b>	<b>0</b>	<b>5</b>
<b>F2.</b>	<b>0</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>11</b>	<b>0</b>	<b>0</b>	<b>9</b>	<b>0</b>
<b>F3.</b>	<b>0</b>	<b>0</b>	<b>8</b>	<b>0</b>	<b>14</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>F4.</b>	<b>0</b>	<b>0</b>	<b>12</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>7</b>
<b>F5.</b>	<b>0</b>	<b>6</b>	<b>0</b>	<b>0</b>	<b>9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>13</b>	<b>0</b>
<b>F6.</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>12</b>	<b>0</b>
<i>Monthly total</i>	<b>6</b>	<b>10</b>	<b>20</b>	<b>0</b>	<b>20</b>	<b>6</b>	<b>11</b>	<b>0</b>	<b>5</b>	<b>34</b>	<b>12</b>

Monthly dynamics of digestive morbid conditions in racing pigeons, from the six studied farms, is shown in Fig. 1.

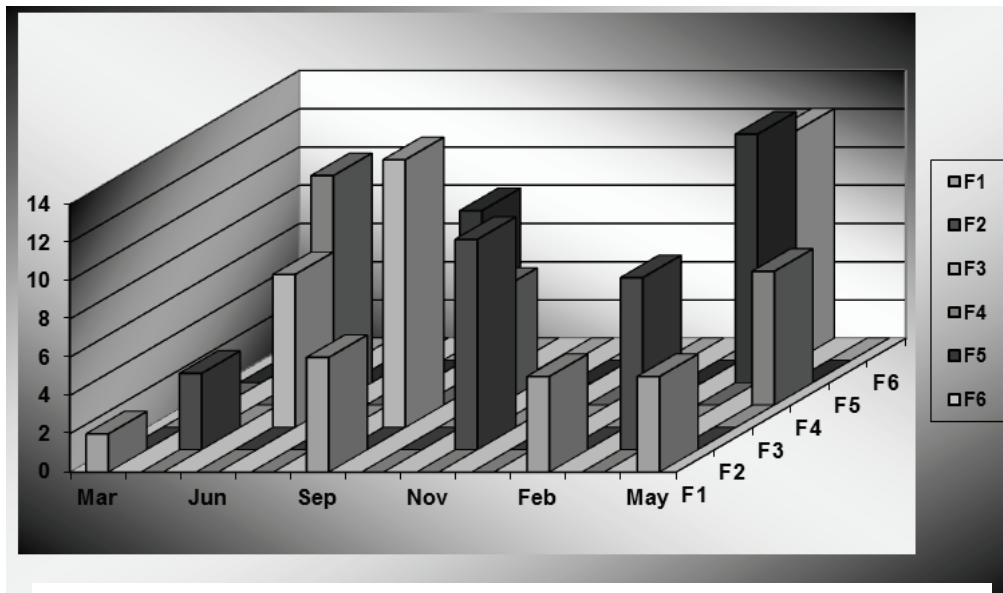
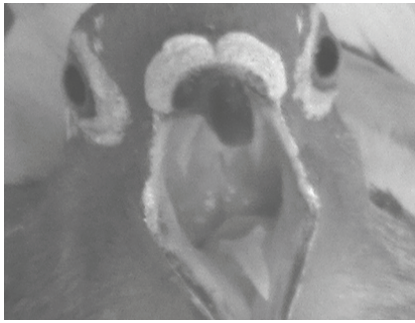


Fig. 1. The monthly disease dynamics in pigeons in January 2008-May 2009 period



The clinical picture was more suggestive in young pigeons than in adults, where symptoms were less significant or poorly expressed. Adult pigeons had modified general state with apathy, loss of appetite and muscle fatigue manifested during flight. In squeakers older than 10 days and in young pigeons up to four months, the clinical picture was characterized by capricious appetite, beak valves partially opened (Fig. 2), head shaking, the presence of whitish deposits in the mouth (Fig. 3), dysphagia, diarrhea alternating with normal stools, changed general condition (Fig. 4), muscle wasting, state of weakness, pale mucous membranes, deviation, horioplumation (Fig. 5), prostration and sterno-abdominal decubitus followed by fatal outcomes.



**Fig.2.** Early pharyngeal trichomonosis in a squeaker of 24 days.



**Fig.3.** Oral trichomonosis: little whitish deposits on oral mucous



**Fig.4.** Eimeriosis. Squeaker with changed general condition and diarrhoea



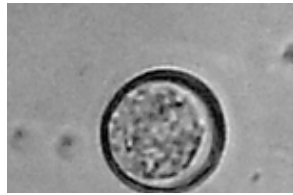
**Fig.5.** Eimeriosis. Pigeon with horioplumation, appetite loss

The evolution of trichomonosis in the flock was due to several factor interactions: high density of of pigeons in farms, manure accumulation in shelters, weather conditions were favorable to parasitic development (heat and humidity), the diffusibility of the parasite, sports context and the absence of specific prophylaxis.

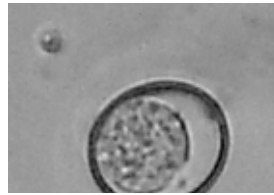
Trichomonosis particularly affected the offspring with growth deficiencies and the offspring resulted from incestuous mates (father - daughter, mother - son, brothers), or between related offspring, who reported a decrease in the general body resistance, favorable to the occurrence of disease. Intestinal eimeriosis was clinically expressed in squeakers and young pigeons through digestive syndrome, losses through mortality being recorded. Ascariidiosis and Capillariosis evolved only sporadically, being observed in pigeons that have been missing for a period of time following their first accommodation flight or in pigeons that have strayed and have been forced to occasionally feed on the ground, to drink from water ponds, associated with additional stress of the days when they have searched their way home. Also, following racing season, many pigeons that no longer recover, are suffering from other diseases with insidious character, which weakens the body resistance to new stress conditions and sustained physical effort.

Paraclinically, coproscopic tests revealed pigeons infestation with parasitic elements of the *Eimeria* genus, species *Eimeria labeana* (Fig. 6), *Eimeria columbae* (Fig. 7), *Eimeria columbarum* (Fig. 8) with average intensivitate (OPG: 1000-2000); *Ascaridia* genus, species *Ascaridia columbae* (Fig. 9), *Capillaria* genus, species *Capillaria columbae* (Fig. 10.), with a low infestation intensivity (OPG: 0-200). In the samples collected from the oral cavity were identified flagellates from *Trichomonas* genus.

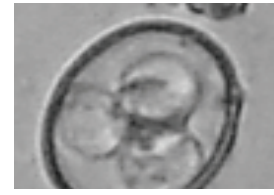
Parasitic elements were morphologically identified compared with specialized literature (Dulceanu, 1980, Severeanu, 1991, Olimpia Iacob, 2010). The extensivity (E%) of the digestive parasitic elements studied in racing pigeons during the investigations period, in the six farms, is shown in Table 2.



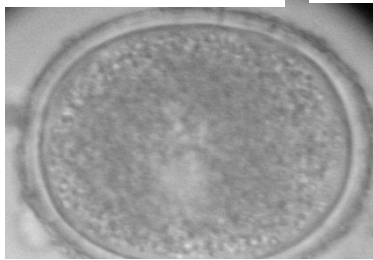
**Fig.6. *Eimeria labeana*:**  
immature oocyst  
(Willis 10x40)



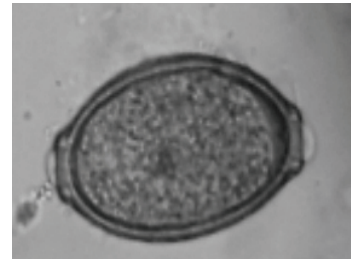
**Fig.7. *E. columbae*:**  
Immature oocyst  
(Willis 10x40)



**Fig.8. *E. columbarum*:**  
Sporulated oocyst  
(Willis 10x40)



**Fig. 9. *Ascaridia Columbae*:** egg  
(Willis 10x40)



**Fig.10. *Capillaria columbae*:** egg  
(Willis 10x40)

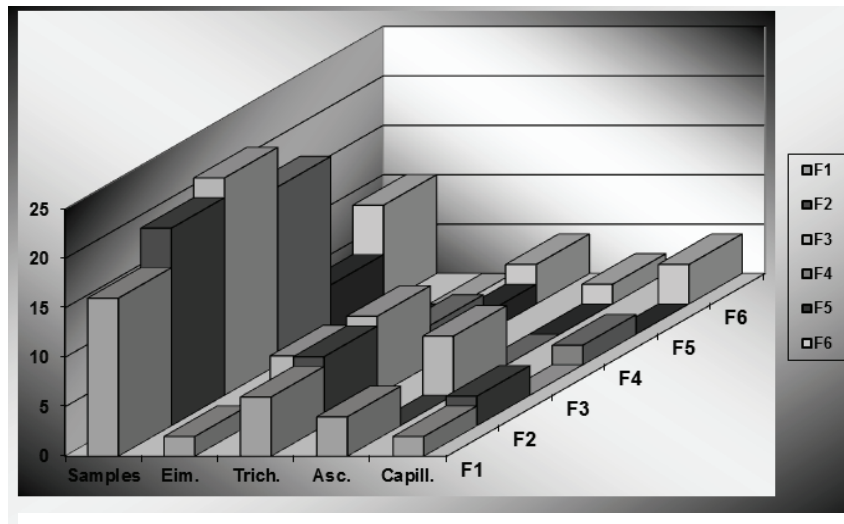
*Table 2.*

The coproparasitological extensivity (E%) of the digestive parasitoses in racing pigeons in March 2008-April 2009 period

Farm	Samples (n)	<i>Eimeria</i>		<i>Trichomonas</i>		<i>Ascaridia</i>		<i>Capillaria</i>	
		n	%	n	%	n	%	n	%
F1.	16	2	12,5	6	37,5	4	25,0	2	12,5
F2.	20	3	13,63	7	35,0	0	0	3	15,0
F3.	22	4	18,2	8	36,3	6	27,2	0	0
F4.	18	3	11,11	4	22,2	0	0	2	11,11
F5.	8	1	12,5	2	25,0	0	0	0	0
F6.	10	2	20,0	4	40,0	2	20,0	1	20,0
<b>Total</b>	<b>94</b>	<b>15</b>	<b>16,0</b>	<b>31</b>	<b>32,9</b>	<b>12</b>	<b>12,76</b>	<b>8</b>	<b>8,51</b>

The analysis of digestive parasitoses extensivity within the studied flocks of pigeons shows different results for each genre of parasite identified by invasive elements. Of the studied samples (14.55%, respectively, 94 samples), 16% (15 samples) have contained invasive elements of the genre *Eimeria*, 32.9% (31 samples) were positive for flaggelates from *Trichomonas*

genus, 12.76% (12 samples) contained invasive elements of the genus *Ascaridia* and 8.51% (8 samples) were positive for species of the *Capillaria* genus. The dynamics of the digestive parasitic elements identified in the studied flocks of pigeons is shown in Fig. 11.



**Fig. 11.** The dynamics of the digestive parasitoses extensivity in racing pigeons in January 2008 –May 2009 period

Morphopatological, the parasitic aggression was expressed by changes of the digestive system mucosa not only in squeakers in the first days of life (10 days of age) but also in young and mature pigeons. In squeakers prevailed the oral cavity fibrinous deposits, followed by changes of the intestinal loops volume and changes of their contents. The described changes are consistent with the specialized literature for trichomonosis and eimeriosis (Dulceanu, 1980, Paul, 2001).



**Fig.12.** Squeaker of 21 days of age with eimeriosis: turgescence intestinal loops, filled with gas.



**Fig.11.** Squeaker of 10 days of age with trichomonosis: fibrinous deposits in pharyngo-esophageal area.

The carried out investigations observed the clinical and subclinical evolution of the most common digestive parasitoses in racing pigeons, generating economic and emotional losses. Due to the sensitivity of the species and the particular way of the farmers involvement, medical investigations in pigeon farms face difficulties and are regarded with reserve.

A real collaboration between owners and veterinary health services, leads to the correct epidemiological surveillance of the racing pigeons flocks and is limiting the parasitoses expansion.

## 2. CONCLUSIONS

3.1. The studied flocks of racing pigeons were affected by trichomonosis, eimeriosis, ascaridiosis and capillariosis.

3.2. The dynamics of digestive diseases reveals the reduced evolution during January-March low, and an increase from April to August, seen as an upward curve with a peak in June.

3.3. Paraclinically were morphologically identified the invasive elements belonging to the *Trichomonas*, *Eimeria*, *Ascaridia* and *Capillaria* genus.

3.4. The digestive parasitoses have induced economic losses in investigated farms and also the retiring of the affected pigeons from sports competitions.

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## **CANINE ECHINOCOCCOSIS ASSESSED BY COPROANTIGEN ELISA AND COPROSCOPICAL EXAMINATION, IN URBAN AND RURAL AREAS FROM SOUTHERN ROMANIA**

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**Key words:** *Echinococcus granulosus*, dog, coproantigens, ELISA, Romania

### **SUMMARY**

*Echinococcus granulosus* infection is an important zoonotic disease transmitted by dogs worldwide. In the recent years, immunodiagnostic techniques are used for diagnosis of infection in dogs. The aim of this study was to assess the overall prevalence of *E. granulosus* infection in dogs from southern Romania, in urban (Bucharest) and rural (Valcea County) areas, by coproscopical examination and copro-ELISA test. A number of 132 individual dog fecal samples were collected and analyzed by flotation technique, followed by detection of coproantigens using the commercial kit CHEKIT- Echinotest. The coproscopical examination revealed taeniid eggs in four (3.03%) of the examined samples, while the coproantigens were found in others 11 of the fecal supernatants analyzed, with an 8.33% overall prevalence. It was not found similarity within positive coproELISA and coproscopical tests. There is no statistical significant differences between the prevalence of echinococcosis in dogs from rural areas (8.69%) and that from urban areas (8.13%) revealed by coproantigen detection.

Parasitism is the most encountered disease of dogs all over the world (Zelon, 2003). Cystic echinococcosis is an important zoonotic helminthosis caused by the tapeworm *Echinococcus granulosus*. The parasite's life cycle is maintained through dogs (which are definitive hosts) and a number of domestic livestock as intermediate hosts.

Due to the potential ability of dogs to contaminate the environment, detection of infection in the definitive host has a great importance for epidemiological and ecological studies (Dalimi et al., 2002). It is important to know that *E. granulosus* infection in canids cannot be diagnosed by microscopic eggs detection in fecal samples. Routine coprological techniques cannot differentiate the eggs of *Echinococcus* from other *Taenia* spp. due to extreme morphologic similarity (Dinkel et al., 1998).

The development of sensitive and specific ante-mortem diagnostic methods for the detection of canine echinococcosis is important for epidemiological baseline data and for surveillance of hydatid control programmes.

For many years two major diagnostic methods have been used in dogs: purgation with arecoline compounds and necropsy of the small intestine, but these methods are laborious, expensive and with biological risks (Schantz et al., 1995).

Detection of parasite antigens in faeces has become an important alternative laboratory based method for diagnosis of intestinal infections caused by helminthes (Frase and Craig, 1997). Coproantigen ELISA assays have been developed for diagnosis of canine echinococcosis incorporating polyclonal antibodies against somatic or excretory/secretory antigens of adult *E. granulosus* (Allan et al., 1992; Deplazes et al., 1992).

Coproantigen specificity was initially reported to be high (96%) (Allan et al., 1992), with good sensitivity (77–88%) based on confirmation by arecoline purge (Craig et al., 1995). Recently, a meta-analysis of several published coproantigen ELISA studies indicated an overall sensitivity of 83% versus necropsy and 76% versus purgation, and a specificity range of 88–96% (Allan and Craig, 2006).

Detection of coproantigens in feces of infected dogs by ELISA is suitable for detecting patent and prepatent infections. Studies have demonstrated that coproantigens can be detected in feces within 10- 20 days after infection, and about 1- 4 weeks prior to eggs appearing in the feces (Allan et al., 1992; Jenkins et al., 2000). After a successful treatment, coproantigen levels drop rapidly and become negative within 1-5 days (Allan and Craig, 2006). This technique is confirmed by WHO as the standard protocol for the diagnosis of echinococcosis infection in dogs (Allan et al., 1992). The detection of parasite-specific antigens in fecal samples is perhaps one of the most useful ways for collecting prevalence data in large surveys.

In Romania, cystic echinococcosis is endemic and affects besides humans, various animal species including sheep, cattle, and swine (Mitrea, 1998; Bart et al., 2005). There is urgent need to implement surveillance systems to monitor the prevalence of echinococcosis in humans and in animals in Romania. CE is an important zoonotic disease in Romania.



The aim of this study was to assess the overall prevalence of *E. granulosus* infection in dogs from southern Romania, in urban (Bucharest) and rural (Valcea County) areas, by coproscopical examination and copro-ELISA technique.

## 1. MATERIALS AND METHODS

The study was conducted during the period of October-November 2011. A number of 132 individual dog fecal samples were collected from some areas in southern Romania, as follows: 86 (65.15%) from urban areas (Bucharest) and 46 (34.84%) from rural areas (Valcea County). In urban areas, the samples were collected from a dog shelter (42/86) and public places (parks) (44/86), while, in rural areas, the samples were collected from places near a slaughterhouse from Bujoreni village (27/46) and from Stoenesti village (19/46).

Fecal samples were collected in sterile plastic recipients from the rectal level or from ground and recorded. For safety reasons, the feces were stored at

-20°C for at least 14 days before being further processed.

### • Coproscopical examination

Fecal samples were examined using flotation technique with saturated sodium chloride solution, followed by microscopical examination of the slides. It was recorded the number of samples which contained at least one Taeniid egg. Additionally, all parasitic elements from the slides were registered.

### • Coproantigen ELISA

The fecal samples were further processed and examined for detection of coproantigens, using the commercial kit CHEKIT- Echinotest, produced by IDEXX Laboratories, Switzerland. The test was performed according to the manufacturer's instructions, using 1g of fecal sample diluted in a 1:4 ratio with the kit's sample diluent. The fecal suspensions were centrifuged at 3000 x g for 10 minutes, and the supernatants were further used for ELISA. The optical density of each sample was read using a photometer at a wavelength of 450 nm. At the base of the formula provided by the manufacturer in the instructions for calculating the results of the test is the parameter called the *net extinction* (NE). The NE of the controls and

samples was determined by subtracting the corresponding OD obtained in the wells coated with control antibodies from the values obtained in wells coated with anti *Echinococcus* antibodies. The results of this formula are expressed in percentage value (%). All the samples which showed the NE values bigger than 40% were considered positives, those with the NE between 30- 40%, ambiguous, and those with values under 30% were considered negative.

## 2. RESULTS AND DISCUSSIONS

### • Coproscopical examination

Parasitic elements (eggs or oocysts) were identified in 108 of the examined samples, with an overall prevalence of 81.81%. The results regarding the prevalence of intestinal parasites in dogs from areas taken in study are shown in Table 1 in decreasing order: hookworms (*Ancylostoma*, *Uncinaria*) in 81 samples (61.36%), *Trichocephalus vulpis* in 45 samples (34.09%), *Toxocara canis* in 24 samples (18.18%), *Isospora* spp. in 19 samples (14.39%), *Dipylidium caninum* in 17 samples (12.87%), *Taenia* spp. and *Eimeria* spp. each one being found in equal proportion, in 4 samples (3.03%). The lowest prevalence (0.75%) was obtained for *Neospora caninum*/*Hammondia heydorni* and *Capillaria aerophila*.

The prevalence of *Echinococcus granulosus* couldn't be established by coproscopical examination, because tapeworms from *Taeniidae* family shed similar eggs. For this reason, all the samples were further analyzed by Coproantigen ELISA.

Table 1

Prevalence of endoparasites, by coproscopical examination, in dogs from southern Romania

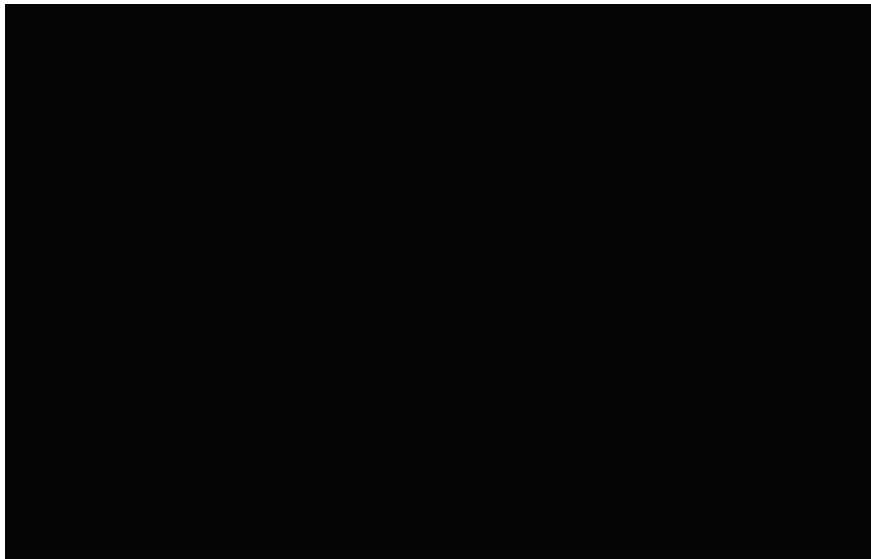
Species	No. of positive samples from urban areas (%)	No. of positive samples from rural areas (%)	Total positive samples no. (%)
Hookworms ( <i>Ancylostoma</i> , <i>Uncinaria</i> )	43 (50)	38 (82.6)	81 (61.36)
<i>Trichocephalus vulpis</i>	35 (40.69)	10 (21.73)	45 (34.09)
<i>Toxocara canis</i>	5 (5.81)	19 (41.3)	24 (18.18)
<i>Isospora</i> spp.	4 (4.65)	15 (32.6)	19 (14.39)

<i>Dipylidium caninum</i>	3 (3.48)	14 (30.43)	17 (12.87)
<i>Taenia</i> spp.	2 (2.32)	2 (4.34)	4 (3.03)
<i>Eimeria</i> spp.	0	4 (8.69)	4 (3.03)
<i>Neospora caninum</i> / <i>Hammondia heydorni</i>	1 (1.16)	0	1 (0.75)
<i>Capillaria aerophila</i>	0	1 (2.17)	1 (0.75)

• **Coproantigen ELISA**

The coproantigen ELISA CHEKIT —Echinotest was positive in 11 samples (8.33%), ambiguous in 8 cases (6.06%) and negative in 113 samples (85.6%) (Fig.1).

The prevalence of *Echinococcus granulosus* coproantigens observed in others similar studies from Romania, ranged between 7.1 and 47.6 % (Iacobiciu et al., 2001; Morariu, 2004; Sereş et al., 2010). Higher prevalence rates of *Echinococcus granulosus* infection in dogs from Romania have been reported by Sereş et al. in the following studies: in Alba County a 28.6% coproantigen prevalence (2006), while in year 2010 found in northwestern Romania a prevalence of 19.2% (Sereş et al., 2010).



**Fig.1.** Diagram of coproantigens ELISA results

Of the positive samples, four were from rural areas, while eight of them were from urban areas. There was no statistical significant differences between the prevalence of echinococcosis in dogs from rural areas (8.69%) and that from urban areas (8.13%) revealed by coproantigen detection.

The dogs positive at copro ELISA test were analyzed according to their age, as follows groups: A) under 1 year, B) between 1 and 5 years, C) between 5 and 10 years and D) over 10 years. The highest prevalence (36.36%) was found in category B of dogs with age ranged between 1 and 5 years (Table 2). The lowest prevalence was registered in categories of age A and D (18.18%).

Regarding to gender of the coproantigen positive dogs, six (54.54%) of them were males and five (45.45%) were females.

Table 2

**The age and origin of the coproantigen- positive dogs**

Dogs age	No. of dogs from urban areas (%)	No. of dogs from rural areas (%)	Total
A) < 1 year	2 (28.57)	0	2 (18.18)
B) 1-5 years	3 (42.85)	1 (25)	4 (36.36)
C) 5-10 years	0	3 (75)	3 (27.27)
D) > 10 years	2(28.57)	0	2 (18.18)
Total	7 (63.63)	4 (36.36)	11 (100)

Comparing the results of the coproscopical prevalence of *Taenia* eggs with the coproantigen ELISA results it was not any correlation between these two tests.

### 3. CONCLUSIONS

3.1. The prevalence of *Echinococcus granulosus* infection in dogs from southern Romania was determined by coproscopical examination and coproantigen ELISA.

3.2. The coproscopical examination (flotation technique) revealed the presence of *Taeniid* eggs in 3.03% (4/132) of the examined samples.

3.3. The coproantigen ELISA test showed an overall prevalence of *Echinococcus granulosus* of 8.33% (11/132). The highest prevalence was found in category of dogs with age ranged between one and five years.

3.4. There were no statistical significant differences between the prevalence of infection in dogs from rural areas (8.69%) and those from urban areas (8.13%), by coproantigen detection.

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## A STUDY REGARDING CANINE BABESIOSIS IN A VETERINARY CLINIC IN TÂRGOVIȘTE- DÂMBOVIȚA

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**Key words:** Babesiosis, dog, prevalence

### SUMMARY

Babesiosis is a parasitic disease encountered in numerous species of animals, both wild and domestic, produced by protozoa of the *Babesia* genus and transmitted through the sting of *Ixodidae* ticks. The clinical symptoms in babesiosis differ according to the species and subspecies of *Babesia*. This study shows epidemiologic and clinical aspects regarding babesiosis in dogs examined inside Agervet-Clinic-Târgoviște, Dâmbovița. During 2010 a number of 197 dogs were diagnosed with babesiosis, belonging to several breeds. 115 were females and 82 were males with ages between 5 months and 14 years. The disease was diagnosed through microscopic evaluation of blood smears, MGG coloring. The prevalence of canine babesiosis in the studied area was 15.07%. From most of the cases (75.64%) tick removal for identification was successful. The clinical evaluation in dogs didn't reveal a homogenous clinical picture, discovering subclinical evolutions (62.44%) and clinical evolutions (37.56%) of the disease. The variation in the clinical picture and the identification of two species of ticks us may lead to suspicion of the presence of two subspecies of *Babesia* (large forms) in the studied area.

Babesiosis is a parasitic disease which can manifest in many domestic and wild species and can be produced by protozoa of the genus *Babesia*. This protozoa was named after the Romanian researcher Victor Babes who set the base to the etiology of hemaglobinury at bovines more than 120 years ago (Uilenberg, 2006, Mihalca *et al.*, 2010). At dogs, the first *Babesia* case was diagnosed in 1885 by Hutcheon, in South Africa (Lobetti, 2002). At present, there are two species of *Babesia* that produce this disease in dogs and that are known world- wide: *B. canis* and *B. gibsoni* (Lobetti, 1998). Historically, the identification of these species has been based on the morphologic aspect of intra-erythrocytic forms (Furlanello *et al.*, 2005), being identified as large and small forms. Thus, *B. canis* is considered to be the large form since the merozoites found in red cells are 3-5 $\mu$ m in size, have got a pyriform shape and there are, usually, more than one merozoite in a single erythrocyte. On the other hand, *B. gibsoni* is considered to be the

small form because the merozoites are 1.5- 2.5  $\mu\text{m}$  in size, they have got a pleomorphic aspect, varying from a round to an oval shape and there is one merozoite in an erythrocyte (Lobetti, 2003; Kjemtrup *et al.*, 2006). According to the genetic data, the specificity of vectors and the variations of pathogenicity, we could establish the fact that the first species includes three sub-species: *Babesia canis canis* caused by *Dermacentor reticulatus*, *Babesia canis vogeli* caused by *Rhipicephalus sanguineus* and *Babesia canis rossi* caused by *Haemaphysalis leachi* (Uilenberg *et al.*, 1989). A fourth type of large *Babesia* was identified and described in North Carolina, U.S.A, without being given a name and without identifying the type of ticks that causes it (Birkenheuer *et al.*, 2004). As far as small *Babesia* is concerned, only one type of *Babesia* has developed in dogs and that is *Babesia gibsoni*. Previous studies proved that there are at least three types of small *Babesia*-like organisms that can infest dogs. The types identified are: *Babesia gibsoni* “Asia genotype” (which is manifest in Asia), *Babesia gibsoni* “California genotype”/*Babesia conradae* (which can be found in North America) and *Theileria annae* (in Europe - Spain, Portugal, Croatia) (Zahler *et al.*, 2000; Camacho *et al.*, 2001; Birkenheuer *et al.*, 2003; Kjemtrup, 2006; Beck *et al.*, 2009; Simoes *et al.*, 2011).

Canine babesiosis can have a different evolution ranging from mild forms to severe, sometimes fatal forms, depending on the pathogenicity of the species or the sub-species involved, the age and the immune system of the host and on the presence/absence of other infections (Shaw *et al.*, 2001; Irwin, 2009). The pathogenicity of *Babesia gibsoni* is high but, in the case of *Babesia canis*, the pathogenicity is differs: *B. c. canis* has a moderate pathogenicity while *B. c. rossi* has a high pathogenicity, and *B. c. vogeli* has a low pathogenicity. The clinical manifestation of canine babesiosis involves a wide range of symptoms like: fever, lethargy, anorexia, pale mucous membranes, anemia, jaundice, hemoglobinuria etc. (Lobetti, 1998; Furlanello *et al.*, 2005).

Canine babesiosis cases were reported in various regions of our country: Mircean, 2006, in Transylvania; Tudor *et al.*, 2008, in Bucharest; Chesler *et al.*, 2008, in Dobrogea; Ilie *et al.*, 2010 in Banat. This study was determined by the increase in the number of babesiosis cases in dogs and it attempts to present the epidemiologic and clinic aspects of canine babesiosis in the area represented by Targoviste and surrounding locations.

## 1. MATERIAL AND METHODS

Throughout 2010, Agervet Clinical Center in Târgoviște has examined 1307 samples of blood taken from dogs registered in our clinic. Each animal has been examined according to standard procedures specified by medical literature (Vlăgioiu and Tudor, 2008) and the results were recorded. Smears were taken out of the analyzed samples and these were MGG highlighted. 197 were positive and *Babesia* was identified. The anamnesis showed that some of the animals had gone hunting while others had been taken for walks in parks and in the neighbouring areas of the town. We or the dogs' owners discovered that the dogs were infested with ticks. The ticks found on dogs were extracted to be identified according to standard procedures described by specialized literature (Didă *et al.*, 2000).

## 2. RESULTS AND DISCUSSION

The present study highlights the fact that out of the total of blood samples examined by the Agervet Clinic, 197 were diagnosed with *Babesia*, the large form, which means a 15.07% prevalence. The disease was diagnosed by microscopically evaluation of the smears which revealed intraerythrocytic parasitic forms, pear-shaped, in pairs and having large dimensions (in comparison with the radius of the erythrocytes).

The positive identified animals were represented by 115 females (58.37%) and 82 males (41.63%) having ages between 5 months and 14 years, belonging to various breeds (Brack - 71, German Shepherd - 39, Rotweiller - 16, Yorkshire terrier - 11, Transylvanian hound —11, Fox terrier - 6, Chow-chow - 3, Lagotto romagnolo - 1, Mixed breeds - 39). Out of these, 34 (17.26%) were younger than one year, 71 (36.04%) were between 1 and 5 years old, 85 (43.15%) had ages between 5 and 10 years old and 7 (3.55%) were older than 10 years.

According to the time of year when the *Babesia* cases were identified the analysis revealed that, in January, we had no case, there were 3 in February, 12 in March, 41 cases in April, 33 in May, 11 cases in June, 2 cases in July, no case in August, 30 in September, 39 in October, 22 in November and 4 cases in December.

The symptoms identified in the sick animals were varied, 62.44% (123/197) of the cases were mild forms while 37.56% (74/197) were a bit more serious. The symptoms included: hyperthermia (93.40%), lethargy



(97.46%), anorexia (82.74%), hemoglobinuria (10.66%), jaundice (7.61%), nervous signs (1.02%).

On 75.63% (149/197) of the dogs infested with babesiosis we could spot ticks, their number varying from one to seven. We identified 12 dogs with one tick, 35 cases with 2 ticks, 56 cases with 3, 16 cases with 4, 13 cases with 5 ticks, 9 cases with 6 ticks and 8 cases with 7 ticks. A total of 489 ticks was extracted and identified using a stereomicroscopic magnifying glass as *Rhipicephalus sanguineus* - 93.87% and *Dermacentor pictus* - 6.13%.

The results of this study reveals a relatively low prevalence (15.07%) in comparison with other studies previously done in our country. Tudor *et al.*, 2009, reported a prevalence of 27.9%, and Anfim *et al.*, 2008, mentioned a prevalence of 31% (93/ 300) - 65% (195/ 300). Chesler *et al.*, 2008, recorded a prevalence of 35.41% (108/ 305) in the Eastern part of the country, and Ilie *et al.*, 2010, shows a prevalence between 6.25% (2/28) and 17.3% (8/59), in the Western part of the country.

By analyzing the results obtained we noticed that the disease appeared both in young and adult animals, belonging to both sexes, having a ratio of 1.4: 1 in favour of females, a fact which is probably due to the fact that there are more females in the canine population in the studied area. Former studies reveal that in the case of canine babesiosis there is no connection or sensibility to sex, age or breed (Dantas-Torres and Figueredo, 2006).

The present study identified a high number of positive cases for Brack breed, a breed that is often used in hunting, a fact which increases the chance to come into contact with *Babesia* vectors. The anamnesis showed that most of the infested dogs took part in hunting sessions and their owners saw ticks on their bodies when coming back home. A rather high percent was also recorded in mixed breeds which were taken for walks in the park or on picnics at week-ends. These aspects show the existence of vectors (ticks) in different areas of Târgoviște (44<sup>o</sup>55'27''N 25<sup>o</sup>27'24''E) and its surroundings.

The obtained results indicate a high prevalence during spring and autumn in comparison with the other periods, a fact which is due to the seasonal activity of the vectors, the data obtained being similar to previously done studies (Tudor *et al.*, 2009). But, some cases also appeared during

winter months, when we do not normally treat babesiosis cases. A possible explanation may be the fact that winters have been warmer lately and they determined a change in the activity of ticks. Studies on the effects of climate changes on the evolution of some species of ticks and the diseases spread by them in Europe pinpointed the fact that the recent climate changes have determined the expansion of ticks' habitat and the dynamic of these species (Gray *et al.*, 2009). All these changes created proper conditions for the appearance of babesiosis cases in the cold season as well.

The clinic image expressed by sick animals was not homogenous since these animals experienced different forms of the disease, from milder forms to moderate ones and, in their cases, the treatment was highly effective. There were also two deaths whose cause was the fact that the owners came late to our clinic. The clinical manifestation of sick animals corresponds with the presence of identified tick species, *Rhipicephalus sanguineus* and *Dermacentor pictus*. Previous studies established the fact that each of the two tick species causes a certain subspecies of *Babesia canis*, *Rh. sanguineus* causes *B. c. vogeli*, and *D. pictus* causes *B. c. canis* (Uilenberg *et al.*, 1989).

It is widely known the fact that that *B. c. vogeli* has a lower pathogenicity causing milder forms of the disease, while *B. c. canis* has a moderate pathogenicity, causing a more evident symptomatology (Lobetti, 1998). The predominance of milder forms of canine babesiosis in the analyzed group of animals is correlated with the high prevalence of the *Rh. sanguineus* species. The variation of symptomatology expressed by animals can be due to the presence of the two subspecies of *Babesia canis*. But the studies have to continue since we cannot establish a real existence of the two subspecies through molecular biology determinations.

### 3. CONCLUSIONS

**3.1.** The study regarding the epidemiology and clinical aspects of canine babesiosis in Târgoviște and its surroundings has revealed the following aspects:

- the prevalence of canine babesiosis in the studied area was 15.07%;
- the disease was diagnosed in all age categories but predominates in adult animals (43.15%);

-the sick animals had, in most cases (62.44%), mild, uncomplicated forms of the disease;

-two tick species have been identified, *Rh. sanguineus* and *D. pictus*, with the predominance of the first species (93.87%).

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## **RETROSPECTIVE STUDY REGARDING PANCREATIC DISORDERS IN DOGS**

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**Key words:** pancreatic disorders, dog, clinical exam

### **SUMMARY**

This paper contains the results of a retrospective study regarding acute and chronic pancreatic disorders in dogs. From October 2010 until September 2011, a number of 89 dogs were diagnosed with pancreatic disorders, inside AGERVET Clinic-Târgoviște. The animals were examined with general methods and laboratory studies, using a dry biochemical analyzer-ARCRAV. After the data analysis, 64 dogs (71.91%) were diagnosed with acute pancreatitis and 25 dogs (28.09%) with chronic pancreatitis. The clinical symptoms in the acute form were represented by anorexia, vomiting, loss of weight, abdominal pain, sometimes jaundice and fever. The animals with chronic forms presented apathy, vomiting, diarrhea, loss of weight with an exaggerated appetite, feces impregnated with fat and repulsive smell, and abdominal pain. The laboratory results have shown an increases in blood biochemical parameters, including increased amylase and lipase.

The pancreas, an important gland of the digestive system, carries out an endocrine function as well as an exocrine function. The endocrine function consists mainly in producing, storing and secretion of digestive enzymes important in ingested protein, lipid and carbohydrate degradation (Mix and Jones, 2006). Lack of enzymes makes digestion and absorption of food that reaches the digestive tract impossible. The pancreatic disorders relate to several issues, the inflammatory processes being the most frequent disorders in dogs. Clinical signs in pancreatitis are nonspecific, which determined along the years a high interest in approaching the subject, a number of studies being carried out in that area (Watson et al., 2007; Xenouli et al., 2008; Shukla, 2010).

The paper shown here presents the results of a retrospective study regarding pancreatic disorders in dogs examined in a private veterinary clinic.

## 1. MATERIAL AND METHOD

Between October 2010 and September 2011, inside AGERVET Clinic-Târgoviște, a number of 3628 new cases of dogs have been registered, of which 89 were diagnosed with pancreatic disorders. The animals were examined through general investigation methods, according to special literature (Vlăgioiu and Tudor, 2008). A number of blood samples were taken to check biochemical parameters, using a dry biochemical analyzer-ARCRA Y.

## 2. RESULTS AND DISCUSSIONS

From the new cases registered inside AGERVET Clinic in the study period, 89 of them were diagnosed with pancreatic disorders with a prevalence of 2.45%. The affected dog population consisted of 51 females (55.06%) and 38 males (44.94%), ages between 8 months and 12 years. Pancreatic disorders were detected in half castes as well as in pure breeds, according to *Table 1*.

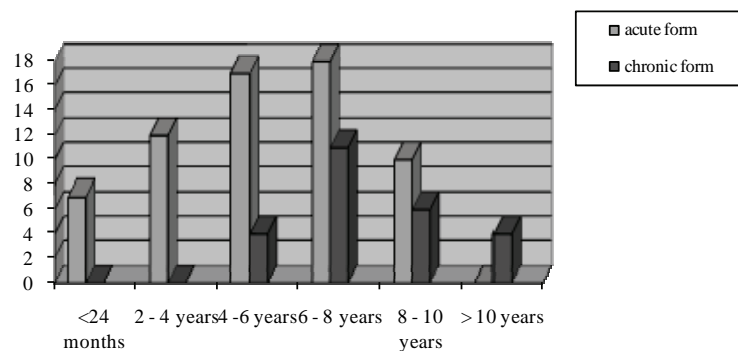
*Table 1*

**Distribution of cases with pancreatic disorders relating to breed and sex**

<b>Nr. Crt.</b>	<b>Breed</b>	<b>No of cases</b>	<b>Females</b>	<b>Males</b>
<b>1.</b>	German Shepherd	15	8	7
<b>2.</b>	Yorkshire Terrier	12	7	5
<b>3.</b>	Bichon	11	6	5
<b>4.</b>	Mixed	11	4	7
<b>5.</b>	Cocker spaniel	8	5	3
<b>6.</b>	Labrador	8	6	2
<b>7.</b>	Chinese Shar-Pei	8	5	3
<b>8.</b>	Shih-Tzu	7	4	3
<b>9.</b>	American Staffordshire Terrier	5	4	1
<b>10.</b>	Siberian Husky	2	1	1
<b>11.</b>	Slovenský copov	2	1	1
<b>No of cases</b>		<b>89</b>	<b>51</b>	<b>38</b>

Following clinical signs analysis of affected animals, it has been established that pancreatic disorders had an acute evolution in 64 dogs (71.91%) and chronic in 25 dogs (28.09%), as shown in *Fig.1*.

Clinical signs were multiforme. From owners case history and what has been noticed inside the clinic, in some situations the illness started out of a sudden, with significant alteration of general status with: anorexia (60/64; 93.75%), vomiting (59/64; 92.19%) sometimes with a sudden debuted, abdominal pain (50/64; 78.13%), loss of weight (20/25; 85%), fever (12/64; 18.75%) and jaundice (8/64; 12.50). On 25 dogs showed a chronic evolution, observing: apathy (23/25; 92%), weakening (20/25; 85%) sometimes associated with exaggerated appetite (18/25; 72%), diarrhea with steathoreea (22/25; 88%) and with very bad smells (20/25; 80%), vomiting (18/25; 72%), abdominal pain (17/25; 68%).



**Fig.1**-Distribution of pancreatic disorders relating to age

Biochemical blood results showed an increase in: total protein (68/89; 76.40%), glycemia (59/89; 66.29%), cholesterol (51/89; 57.30%), bilirubin (47/89; 52.81%), amylase (73/89; 82.02%)(over 2500 UI/L) and lipase (69/89; 77.53%)(up to 759 UI/L).

Pancreatitis is the most common disorder of exocrine pancreas in dogs (Xenoulis et al., 2008; Newman et al., 2004). In veterinary medicine, a standard classification, unanimously accepted regarding pancreatitis cannot be found. Therefore, a number of time classifications from human medicine were followed, although there are disagreements regarding the validity and utility of this system (Xenouli et al., 2008). From a clinical perspective pancreatitis can be broadly categorized as acute, recurrent acute or chronic

(Simpson, 2006). Relating to patient effects all forms can evolve mild or severe, non-fatal or fatal (Simpson, 2006). Few things are known regarding pancreatitis etiology and physiopathology in dogs, most of these being considered idiopathic because the factor that initiated the process cannot be established (Xenouli et al., 2008). Previous studies show a number of causes or a number of risk factors in pancreatitis initiation, such as: obesity, high-fat diets, hyperlipidemia, trauma, severe intestinal disorders, duodenal reflux, systemic infections (e.g., babesiosis), concurrent endocrinopathies (hyperadrenocorticism, diabetes mellitus), pharmaceutical agents, hereditary predisposition, age (Simpson, 2006; Spillmann, 2007; Washabau, 2009; Gal, 2011).

Results from this study show a low prevalence of pancreatic disorders in dogs. In human medicine, it is estimated that a percentage of 90 of pancreatitis cases are left undiagnosed (Newman et al., 2004). In veterinary medicine, a high percentage of pancreatitis are also undiagnosed due to an insidious evolution, especially in mild or subclinical cases. Gal (2011) in USA reveals a 0.8% prevalence gained after a study carried out in a period of ten years. Thompson et al. (2009) reveals a 1.46% prevalence of acute pancreatitis cases, registered between 2001 and 2007. The real prevalence remains unestimated due to diagnostics difficulties before death (Mix and Jones, 2006; Van den Bossche et al., 2010). Retrospective studies based upon after death findings indicate variable values of inflammatory processes at pancreatic level. Ludlow (2009) revealed a 1.5% prevalence in necropsy cases. Watson et al. (2007) identified inflammatory pancreatic lesions in 34% of cases examined after death. Newman et al. (2004) identified histological lesions of pancreatitis in 64% of examined cases.

Pancreatitis can be clinically diagnosed if is supported by appropriate symptoms, a history, physical examination and results of hematological exam, and at the same time to exclude other diseases with similar symptoms (Gal, 2011). The severity of clinical signs depends on the severity and duration of the pancreatic inflammatory process and systemic consequences (Spillmann, 2007). Our results, similar to other studies (Simpson, 2006; Mix and Jones, 2006; Ludlow, 2009), reveal a heterogeneous clinical panel. In the study shown here, acute forms were predominant to chronic forms. Acute form is characterized by sudden onset of manifestations with predominant signs as anorexia, vomiting, fever with



high abdominal sensibility during palpation, symptoms considered suggestive in pancreatitis (Xenouli et al., 2008). Chronic forms were characterized by apathy and loss of weight, regarding that sometimes appetite is high, the animal being in continuous pursuit for food. Clinical data accompanied by medical history and biochemical findings have determined us to establish pancreatitis diagnose. The pancreas still remains the “hidden organ” (Spillmann, 2007) which makes diagnose very difficult.

Regarding age from Fig. 1 it is shown that pancreatitis affects animals of all ages, the highest prevalence being registered in animals with ages between 6 and 8 years, our results being similar to other studies (Simpson, 2006; Xenouli et al., 2008). As animals age the probability of developing illnesses is very high. Otherwise, age is known, in previous other studies (Washabau, 2009), to be a risk factor in developing pancreatitis.

Our results indicate a high prevalence of pancreatitis in females compared to males, with a rate of 1.34 to 1. Results can be influenced by the configuration of canine population in the respective area. It is unclear if there is a sex predisposition (Simpson, 2006; Xenouli et al. 2008). Some studies revealed a high risk in males and sterilized females compared to unsterilized ones (Washabau 2009). Mix and Jones (2006), quote studies that have shown a high risk in developing acute pancreatitis in middle-aged females compared to males.

Pancreatitis was diagnosed in dogs belonging to both pure breeds and mixed breed. From the data of literature is know that the disease can affect both pure breed and mixed breed, but is more common in some breeds. Yorkshire-Terrier, German Shepherds, Bichon and Mixed breeds were the most affected. Previous studies show a high risk in Miniature Schnauzers and Yorkshire Terriers for pancreatitis, especially acute forms (Simpsons, 2006; Washabau, 2009), assuming the existence of a hereditary component.

In most cases, biochemical examination revealed high values of some parameters such as proteins, glucose, cholesterol, bilirubin and most important amylase and lipase and accompanied by clinical symptoms lead to a diagnosis. Results of this study are similar to results of previous studies (Mix and Jones, 2006). Although high levels of amylase and lipase levels have been associated with pancreatitis, a predictive value could not be

demonstrated (Mix and Jones, 2006). Normal values of serum amylase and lipase could not dismiss pancreatitis, many dogs with acute or chronic pancreatitis having normal enzyme levels (Xenouli et al., 2008). Some studies suggest that values over three times the superior limit of the reference interval of serum amylase or lipase can be suggestive to pancreatitis. Careful evaluation of blood biochemical parameters values accompanied by animal symptoms and correct interpretation of physical examination leads to diagnosis of pancreatic disorders in dogs and establishing an appropriate therapeutic conduct.

### 3. CONCLUSIONS

3.1. The study shown here reveals a high prevalence of acute pancreatitis compared to chronic pancreatitis.

3.2. Both forms had a higher prevalence in adult animals, ages between 6 and 8 years, especially in females

3.3. Pancreatitis are characterized by a multisymptomatic picture, non-specific, differential diagnosis with other illnesses that have similar symptomatology has to be carried out carefully.

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## EPIDEMIOLOGICAL DATA ON UROLITHIASIS IN CATS

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**Key words:** urolithiasis, dog, epidemiological data

### SUMMARY

The paper contains data regarding the diagnose of urolithiasis in cats examined at Agervet Clinic-Târgoviște. During the year 2010, a number of 109 cats were diagnosed with urolithiasis, 15 (13.76%) of which were females and 94 (86.24%) were males, with ages between 1 and 14 years. The animals were examined clinically with general and complementary methods. Data were obtained regarding patient history from their owners, for each particular case. Cats that were diagnosed with urolithiasis belong to the following breeds: Persian (3 cases), Russian Blue (5 cases), Birmanese (17 cases), Domestic Short Hair (66 cases), Domestic Long Hair (18 cases). The clinical signs were represented by dysuria, stranguria, hematuria, high volume bladder and sometime systemic signs. The imaging examination revealed calculi inside the urinary bladder in 100% cases, kidneys in 4.58% cases and urethra in 3.67% cases. The microscopic examination of urinary sediment carried out in 52 cats revealed the presence of struvite in 63.46%, calcium oxalate in 32.69% cases, and 3.85% it had a different nature.

Urolithiasis is characterized by the fact that urinary calculi are formed in kidneys, renal pelvis and urinary bladder. Calculi are crystals formations and proteic matrix that appear in the urinary tract when urine becomes oversaturated with crystallogenic substances and these exceed the body's capacity of solidifying them (Polzin, 2007; Appel et al., 2010). Formation of uroliths involves several physiological and pathological processes (Osborne, 1996). In general, the factors that influence the formation of a calculus include urine pH, renal mineral excretion, the presence of promoters, the absence of inhibitors, and the presence of infection or inflammation (Palma et al., 2009). Not only this, but also breed, age, sex and reproductive status are factors that involve risk in developing urolithiasis in cats (Lekcharoensuk et al., 2000). In the world, the appearance of the disease varies from one study to another, from one geographical area to another being determined by type of life and type of food (Cannon et al., 2007; Houston, 2007).

The present paper presents epidemiological data on urolithiasis in the cats examined by the medical clinic AGERVET- Târgoviște.

## 1. MATERIALS AND METHODS

Throughout the year 2010, 109 cats belonging to different breeds were diagnosed with urolithiasis. The animals were clinically examined by using general methods (inspection, palpation, appreciation of body temperature) and by complementary methods (X-ray exam, ultrasonography, sounding, examination of the urinary deposit). Data about the medical record of each patient was obtained from their owners.

## 2. RESULTS AND DISCUSSION

A number of 109 out of a total of 1873 cats that were examined in our clinic during the studied period and were diagnosed with urolithiasis, this representing a prevalence of 5.82%. Out of these 15 (13.76%) were females and 94 (86.24%) males. Their age varied between 1 and 14 years, with an average of 7.9% of the feline population diagnosed with urolithiasis. There were 25 pure breed cats (22.94%) and 84 (77.06%) mixed breed cats: Domestic Short Haired (DSH) (66 cases), Domestic Long Haired (DLH) (18 case). The pure breed cats were represented by Persians (3 cases), Russian Blue (5 cases) and Burmese cats (17 cases).

The clinical picture was represented by different signs of the urinary tract such as: pollakiuria (54.13%; 59/109), dysuria (22.94%; 25/109), enlarged urinary bladder (12.84%; 14/109), stranguria (3.67%; 4/109), but also systemic signs such as: anorexia (22.01%; 24/109), vomition (11.93%; 13/109), hypertermia (33.94%; 37/109), hypotermia (10.09%; 11/109).

The X-ray and ecographic examinations highlighted the presence of calculi in the kidneys (4.58%; 5/109), bladder (100%; 109/109) and urethra (3.67%; 4/ 109). In 2 out of 5 cases of nefrolithiasis, calculi could be clearly seen and for the rest we could notice the presence of urinary „sand”. The bladder calculi had varying dimensions. For 6.42% (7 cases) of cases the lowest dimension was of more than 5 mm, in 28.44% (31 cases) of cases urinary bladder microlithiasis was identified (less than 5 mm), and in 65.17% (71 cases) there was urinary „sand”. In 14 cases we performed

sounding to eliminate urine, in 6 cases we performed surgery to eliminate several calculi from the bladder.

The microscopic examination of the urinary deposit performed on 52 cats highlighted the presence of crystalluria, represented by struvite in 33 cases (63.46%), calcium oxalate in 17 cases (32.69%), and in 2 cases it had a different nature (3.85%).

The formation of calculi in the urinary system represents a frequently encountered problem of feline pathology, urolithiasis being among the main causes of feline lower urinary tract disease (FLUTD) production (Houston, 2007). The predominance of urolithiasis varies from one region to another being influenced by the differences that exist in the characteristics of the studied population, by geographical location, individual data, type of food, way of feeding, water consumption, life style, by the presence or absence of concurrent diseases in the examined individuals, etc. Palama et al. (2009) identified the fact that urolithiasis appeared in cats from 15% to 23%, cats suffering from different conditions of the lower urinary tract. Bente et al. (2010), diagnosed urolithiasis in 11.8% of cats having the above affections.

When it comes to a categorization according sex of urolithiasis, the present study noticed a high predominance in males than females. Sex represents one of the factors that can lead to the development of the disease in cats (Palm and Westropp, 2011). Due to their anatomical configuration, and by this we mean, a narrowing of the penien urethra, males (52.58%) have a higher predisposition to urethral obstructions in comparison with females (47.42%). The differences that appeared were determined by the duration of the period when the estimation was done and also by the feline population that was examined.

Urolithiasis can be found in all age categories (Lekcharoensuk et al., 2000). Our results highlighted an average of 7.9 years, with age limits between 1 and 14 years. Bente et al. (2011), established an average of 5.4 years, and Kyles et al., (2005), obtained an average age of 7 years (with limits between 8 months and 16 years), for cats that were diagnosed with urethral calculi. The age of the animals represents a determining factor in the development of urolithiasis. The study also identified other determining factors such as diet and lifestyle. Cats that live in houses (inside), are highly exposed to suffering from urolithiasis as a consequence of a longer period of

inactivity worsened by a low quantity of water consumption and by long use of the litter tray in view of emptying the urinary bladder (Houston et al., 2003; Hostutler et al., 2005).

Our results indicate a higher predominance of urolithiasis in mixed breed cats and a lower predominance in pure breed cats. Former studies reached a similar conclusion and had similar results. Hostoun and Moore (2009), noticed that most of the examined urolithes came from DSH (68.38%), Domestic Medium Haire and DLM (21.91%), while in the case of Persian and Siamese cats they found lower values, 2.95% and 1.91%.

As far as the location of the calculi is concerned, the result showed that the main place where urolithiasis can be found is the urinary bladder. Our results are according with previous similar studies (Cannon et al., 2007). Lekcharoensuk et al. (2000), highlighted the fact that 92% of the uroliths as calciun oxalate (CaOx) and 96% as magnesium ammonium phosphat (MAP) and that they came from the urinary bladder or from the urethra while 5% of the CaOx urolites and 0.6% of MAP urolites came from the kidneys and ureters. Unlike these results, Kyles et al. (2005) established that 163 of cats presented urethral calculi and that only 9% also had bladder calculi.

Microscopic examination of the urinary deposit represents an important stage in the evaluation of the urinary system. The presence of crystals in the urine can or cannot have a clinical significance. It was proven that in the case of many normal cats can identify crystalluria in different degrees, especially in highly concentrated urine (Laboto, 2001; Houston, 2003). But in the case of an animal having a certain clinical record as far as urolithiasis is concerned, the presence of crystals must be taken into consideration (Archer, 2005). The formation of uroliths depends on the oversaturation of urine in calculogenic minerals (Hostutler et al., 2005). The increase in renal minerals excretion, the presence of crystalization promoters and the absence of natural inhibitors of crystalization, to which the modification of urine pH is added, determines oversaturation of urine with minerals. The result will be represented by the formation of crystals which will come close together and produce urinary calculi. The uroliths can be of different types according to the type of the crystal present in the composition. Likewise, they can vary in size from “sand”- like matter to large stones. In cats, the uroliths formed can be grouped into four main

types of minerals: urate (including ammonium urate, sodium urate and uric acid), cystine, magnesium ammonium phosphate (MAP or struvite) and calcium (calcium oxalate - CaOx and calcium phosphate) (Stevenson, 2002). Previous epidemiological studies done in different countries highlighted the fact that struvite and calcium oxalates are the most frequent types of minerals found in cats, and uroliths formed from urate have a low percentage, approximately 10% (Cannon et al., 2007). The prevalence of one or another of the two types of uroliths diagnosed in cats, varies from one study to another, from one geographical area to another. The result obtained in the current study, on the basis of microscopic examination of the urinary deposit, shows a high prevalence of struvite in comparison with CaOx uroliths. But the exact determination of the type of minerals that forms uroliths and the comparison with other similar studies requires the performance of quantitative analysis of the stones, the only way to establish the type of uroliths exactly. This aspect has great importance in urolithiasis management and in establishing the adequate treatment.

### 3. CONCLUSIONS

3.1. The present study highlights the fact that there is a prevalence of urolithiasis of 5.82% in the examined cats.

3.2. Urolithiasis affects especially males of mixed breed, with an average age of 7.9 years.

3.3. Most cases presented a bladder urolithiasis, and the clinical pattern was represented by disfunctions of the lower urinary tract.

3.4. We recommend that quantitative analysis should be performed on the sampled uroliths to establish the type of mineral and to recommend the adequate therapy.



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## ACTUAL DATA ON ETIOEPIDEMIOLOGY OF CYSTIC ECHINOCOCCOSIS ON LIVESTOCK IN ROMANIA

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**Key words:** *cystic echinococcosis*, epidemiology, livestock, Romania

### SUMMARY

Cystic echinococcosis is a major zoonosis with an important economic and public health impact. For the domestic livestock the economic impact of cystic echinococcosis consists in lowering the productivity and in addition losses from condemnation of the affected organs.

A retrospective study on the prevalence and epidemiology of cystic echinococcosis in livestock (cattle, sheep, and pigs) from different regions of Romania, based on three year period (2008-2011) surveillance, was performed. The highest prevalence of disease was registered in sheep (50.80 %, with limits of variation between 15.44 and 64.09), followed by cattle (41.01%, with limits of variation between 34.57 and 68.73), and the lowest in pigs (5.74%, with limits of variation between 1.96 and 7.85). Pulmonary cysts were predominant in ruminants, while in pigs, hepatic cysts had a biggest prevalence. Very few cysts were found in spleen and kidney. The viability of hydatid cysts, was highest in sheep, more of half of the examined cysts (58.53%) being fertile. A very low viability of cysts was registered in cattle (0.36% from the examined cysts).

The present study is a sign of hyperendemic circulation of *E. granulosus* in Romania, and outlines the severity of the situation from human- and livestock-associated cystic echinococcosis. Therefore, a national strategic program for surveillance and control of echinococcosis/hydatidosis in both, definitive and intermediate hosts, including humans, is desirable and necessary.

Cystic echinococcosis (CE) caused by the larval stages of *Echinococcus granulosus* is one of the most important parasitic infections in livestock in the world, as well as a major zoonosis (Eckert and Deplazes, 2004). Infections with cestodes from genus *Echinococcus* are usually acquired by oral ingestion of eggs, direct contact with carriers, and contaminated food (especially vegetables) or water.

In Romania, CE is endemic and affects besides humans, various animal species including sheep, cattle, and swine. Cystic echinococcosis continues to pose significant problems of public health and cause important economic losses by condemnation of parasitized organs in slaughtered animals.

As a result of the increased number of human and animal cases of CE, Romania was framed in the forefront of the European countries and among the first countries worldwide in 1995 (Mitrea 1998, 2000).

A retrospective study on the prevalence and epidemiology of CE in livestock, based on three year period surveillance, was performed.

## 1. MATERIALS AND METHODS

The study was conducted during of Oct 2008 - Oct 2011 period in different slaughterhouses from three main areas (N-E, N-V and S-E) of the country. More than 62000 animals were examined, including: 17943 cattle, 23448 sheep, and 21347 pigs (Table 1).

*Table 1*

Number of the animals examined in slaughterhouses from three different areas (North-East, North-West, and South-East) of Romania

<b>County</b>	<b>Cattle</b>	<b>Sheep</b>	<b>Swine</b>	<b>TOTAL</b>
<b>SV</b>	2337	3906	2447	8690
<b>NT</b>	1412	1018	1073	3503
<b>MM</b>	2800	2714	3725	9239
<b>BN</b>	2092	1846	1134	5072
<b>AB</b>	3117	1274	1922	6313
<b>CT</b>	1378	7519	3260	12157
<b>TR</b>	2791	1958	1864	6613
<b>VL</b>	2016	3213	5922	11151
<b>TOTAL</b>	<b>17943</b>	<b>23448</b>	<b>21347</b>	<b>62738</b>

The presence of hydatid cysts has been detected post-mortem by routine examination (inspection, palpation and section) of the viscera. Location, number, size, and other particular characteristics of the hydatid cysts were recorded.

A number of 477 cysts from different viscera were randomly collected for advanced morphological analyses in the laboratory, including:

- the external appearance, macroscopically characteristics of the cyst wall, germinal membrane and hydatid fluid (general aspects), color and transparency;

- the fertility of the cysts (presence of the protoscoleces) by microscopic examination of the germinative layer and/or hydatid sand from hydatid cysts with normal aspect.

## 2. RESULTS AND DISCUSSIONS

The prevalence of cystic echinococcosis (CE) in the examined slaughtered animals is presented in table 2.

Table 2

Synoptic data on prevalence of CE in livestock from three areas of Romania, examined during of the 2008 –2011 period

County	CATTLE			SHEEP			PIGS		
	Examined animals	Infected animals		Examined animals	Infected animals		Examined animals	Infected animals	
	No.	No.	%	No.	No.	%	No.	No.	%
<i>N - E of Romania</i>									
SV	2337	451	<b>19.30</b>	3906	1500	<b>38.40</b>	2447	62	<b>2.53</b>
NT	1412	218	<b>15.44</b>	1018	352	<b>34.57</b>	1073	21	<b>1.96</b>
<i>N - V of Romania</i>									
MM	2800	760	<b>27.14</b>	2714	1172	<b>43.18</b>	3725	147	<b>3.94</b>
BN	2092	622	<b>29.73</b>	1846	956	<b>51.78</b>	1134	342	<b>3.01</b>
AB	3117	1851	<b>59.38</b>	1274	814	<b>63.89</b>	1922	151	<b>7.85</b>
<i>S - E of Romania</i>									
CT	1378	600	<b>43.54</b>	7519	3820	<b>50.80</b>	3260	118	<b>3.61</b>
TR	2791	1789	<b>64.09</b>	1951	1341	<b>68.73</b>	1864	127	<b>6.81</b>
VL	2016	1068	<b>52.97</b>	3213	1958	<b>60.93</b>	5922	259	<b>4.37</b>
<b>Total</b>	<b>17943</b>	<b>7359</b>	<b>41.01</b>	<b>23448</b>	<b>11913</b>	<b>50.80</b>	<b>21347</b>	<b>1227</b>	<b>5.74</b>

The highest prevalence of disease was registered in sheep (50.80 %, with limits of variation between 15.44 and 64.09), followed by cattle

(41.01%, with limits of variation between 34.57 and 68.73), and the lowest in pigs (5.74%, with limits of variation between 1.96 and 7.85).

Livestock involved in the transmission pattern of CE in Romania includes sheep (*Ovis aries*), cattle (*Bos taurus*), and swine (*Sus scrofa*). A survey performed throughout Romania during a 15 years period, reported in 1997, revealed prevalence of CE ranging between 33 and 40% in both sheep and cattle (Olteanu et al., 1999).

Recent studies conducted during of two years (2009, 2010) revealed a high prevalence of CE in sheep and cattle, ranging from 25.4 to 97.1% in sheep and 27.2 to 64.6% in cattle, with a fertility of 58.53% and 0.36%, respectively. In pigs, the prevalence of CE ranged from 0.69% to 5.05% (Mitreă et al., 2010; Ionita et al., 2011).

The most authors reported the biggest value of prevalence of CE on sheep, compared with other species as intermediated host, the disease being found especially in areas where pastoral activities are predominant (Euzebuy, 1971, 1991; Mitreă et al., 2008, 2009; Scala and Mazzette, 2009; Rinaldi et al., 2008). This is supported by the fact that sheep are recognized as the most receptive host. On the other hand, there is also known that this species uses the pasture the longest period of year, this behavior facilitating the contact with the definitive host of the parasite —dogs or with the parasitic infecting elements (Euzebuy, 1991; Mitreă et al., 2008; Scala and Mazzette, 2009).

An other study performed on field data collection in the west area of Romania (Timis district), from 1998 to 2003, confirmed active transmission of CE in animals. For sheep, 674 carcasses out of the 11543 that were checked were positive (5.83%). For cattle, 8783 carcasses out of 39272 were positive (22.36%). For pigs, 93276 carcasses out of 2157600 were positive (4.32%) (Morariu et al., 2011).

Analyzing the registered data revealed some differences between different areas. It seems that the biggest infestation is in the S-E area, followed by N-V area, and N-E (Table 2).

A large variation was registered between different animal lots in the same area, county, or even slaughterhouse. This aspect is emphasized by some selected data in table 3. In many situations, all animals from a lot were infected.

*Table 3*

The variability of prevalence of CE registered in different lots of animal slaughtered  
in the same unit

Period of examination	CATTLE		SHEEP		PIGS	
	Examined animals <i>No.</i>	Infected animals <i>No. (%)</i>	Examined animals <i>No.</i>	Examined animals <i>No. (%)</i>	Infected animals <i>No.</i>	Examined animals <i>No. (%)</i>
<b>- Slaughterhouse C -</b>						
<b>January</b>	625	207 (33.1)	35	21 (60.00)	423	14 (0.33)
<b>June</b>	271	181 (66.79)	78	78 (100)	345	21 (6,09)
<b>October</b>	418	330 (78.95)	153	25 (16.34)	374	8 (2,14)
<b>- Slaughterhouse G -</b>						
<b>September</b>	47	20 (42.55)	143	143 (100)	377	18 (4.77)
<b>October</b>	46	38 (82.61)	120	12 (10)	509	10 (1.96)
<b>- Slaughterhouse M -</b>						
<b>April</b>	569	526 (92.44)	215	19 (8,83)	103	0
<b>September</b>	27	14 (51.85)	154	123 (79.87)	210	16 (7.62)

These results could be explained based on the animal husbandry system. A previous study emphasized significant differences of CE in animals, according to their provenience (Mitrea et al., 2010). This fact was obviously in cattle, where the prevalence of infected animals was double (88.23%) in the animals from household areas than those provided from specialized farms (40.34%); in sheep, the percent was triple.

This situation is quite very common for Romania, where usually many animal species have access to the same pasture, and the most important thing the access of dogs, as well. Though, it is obviously that these animals are exposed to a higher pressure of infection comparing to those raised in specialized farms, where the circulation of dogs is controlled. Of course, human populations from that area are exposed to the same high risk of infection.

In terms of location of the hydatid cysts, pulmonary cysts were predominant in ruminants (63.96% in cattle; 62.19% in sheep), while in

pigs, hepatic cysts had a biggest prevalence (69.04%). Very few cysts were found in spleen and kidney.

Hydatid cysts examined presented different aspects, regarding the sizes, their stage of development and evolution. Parasitized organs were enlarger in size, with irregular surface, showing cysts of various shapes and sizes. Small (< 2 cm) and medium (2 —5 cm) cyst were predominant, while bigger cysts (> 5 cm) were fewer. The presence in large number of hydatid cyst determined sclerosis phenomena in the affected organs, caused by atrophic and compressive actions of cyst. Hydatid cysts located in the deep of organs, were sectioned, and after this the parenchyma had cavernous aspect.

Regarding of the aspect of hydatid cysts, different types were registered, being found normal, calcified and caseating cysts.

The viability of hydatid cysts was determined in the laboratory by presence of protoscolecies in microscopic examination of the germinative layer and hydatid sand from hydatid cysts with normal aspect. The highest viability of cysts was registered in sheep, where more of half of the examined cysts from this species were fertile (58.53%) (table 4). A very low viability of cysts was registered in cattle (0.36% from the examined cysts).

Table 4

Viability of the hydatid cysts in ruminants and pigs

Host species	No. of hydatid cysts examined	Type of hydatid cysts			
		Fertile (%)	Sterile (%)	Calcified (%)	Caseificated (%)
Cattle	271	1 (0.36)	80 (29.52)	112 (41.32)	78 (28.78)
Sheep	164	96 (58.53)	0	42 (25.6)	26 (15.85)
Pigs	42	0	9 (21.42)	16 (38.09)	17 (40.47)

These results are in according to other studies in which the fertile cysts were prevalence in sheep, while in cattle the sterile cysts were predominant (Kebede et. al, 2009; Banks et al., 2006; Rinaldi et al., 2008; Yildiz and Tuncer, 2005). For this reason, even the cattle are often infected, their role in the epidemiology of hydatidosis is lower (Euzaby, 1991; Romig et al., 2005).

In conclusion, these data confirm the hyperendemic circulation of *E. granulosus* and outlines the severity of the situation from human- and livestock-associated cystic echinococcosis in Romania.

Consequently, the economic losses are expected to increase as well (Torgerson and Dowling, 2001; Butke et al., 2006). After an evaluation of the economic losses during of 1994-2004 period, was noticed that important organs condemnations were made because of cystic echinococcosis, totalizing 80.5 billion lei (Iacobiciu et al., 2001). Therefore, during of 1994-2004, in Mehedinti county, 59.9 ton of viscera with hydatidosis from cattle, sheep and pigs were confiscated, the annual condemnations varying between 5.9 and 13.6 ton. In Dolj county, during of the 1992-1995 period, 653 ton of infested viscera from cattle, sheep and pigs were confiscated, with an annual variation from 122 to 220 ton (Siko and Bokor, 1991).

Therefore, a national strategic program for surveillance and control of echinococcosis/hydatidosis in both, definitive and intermediate hosts, is desirable and necessary.

In fact, in this year, after confirmation for the first time in Romania of the presence of *Echinococcus multilocularis* (Siko, 2011), Romania has been put on the map of the European Disease Surveillance System. This supposes encompassing efforts of both human and veterinary medicine, with the support of the administrative and other authorities, for monitoring of echinococcosis.

### 3. CONCLUSIONS

3.1. A retrospective study on the prevalence and epidemiology of cystic echinococcosis in livestock (cattle, sheep, and pigs) from different regions of Romania, based on three year period (2008-2011) surveillance, was performed.

3.2. The prevalence of hydatid cysts was highest in sheep (50.80 %, with limits of variation between 15.44 and 64.09), followed by cattle (41.01%, with limits of variation between 34.57 and 68.73), and the lowest in pigs (5.74%, with limits of variation between 1.96 and 7.85).

3.3. Pulmonary cysts were predominant in ruminants (63.96% in cattle; 62.19% in sheep), while in pigs, hepatic cysts had a biggest prevalence (69.04%). Very few cysts were found in spleen and kidney. Small (< 2 cm) and medium (2 –5 cm) cyst were predominant.



3.4. The viability of hydatid cysts, determined by presence of protoscolexes in microscopic examination of the germinative layer and hydatid sand, was highest in sheep, where more of half of the examined cysts (58.53%) were fertile. A very low viability of cysts was registered in cattle (0.36% from the examined cysts).

3.5. The present study is a sign of hyperendemic circulation of *E. granulosus* in Romania, and outlines the severity of the situation from human- and livestock-associated cystic echinococcosis.

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## **REHABILITATION OF DOGS WITH MEDULLAR COMPRESSION SYNDROME USING DIFFERENT METHODS OF PHYSIOTHERAPY**

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**Keywords:** cord compression syndrome, physiotherapy, rehabilitation.

### **SUMMARY**

Spinal cord injury (SCI) is one of the most catastrophic damage to the nervous system leading to permanent neurological deficits. Electrical stimulation in the form of functional electrical stimulation (FES) can help facilitate and improve upper/lower limb mobility along with other body functions lost due to injury e.g. respiratory, sexual, bladder or bowel functions by applying a controlled electrical stimulus to generate contractions and functional movement in the paralyzed muscles (3).

The study was conducted in the Surgery Clinic of the Veterinary Medicine Faculty from Cluj-Napoca, during 2009-2011, on a number of 6 animals of canine species, which presented for consultation with various disorders of the spine.

After establishing the certainty diagnosis by clinical and paraclinical exam, a therapeutic protocol was made for each individual in part, which general includes: surgical intervention for decompressing the spinal cord, postoperative drugs treatment and a physiotherapy program for functional recovery.

Most patients responded well to the established treatment program, recovery being good, with temporary relief of symptoms (after application of physiotherapy treatment for 1-3 hours) during the first week, and permanent at the end of the third week.

Structural discontinuity in the spinal cord after injury results in a disruption in the impulse conduction resulting in loss of various bodily functions depending upon the level of injury. (3).

Although complete recovery of function in an injured spinal cord is still not possible in clinical setting, numerous research efforts are being conducted to promote regeneration and repair the interruption of nerve impulses as seen in SCI (1). Currently there are three main research avenues under progress:

1. The first approach aims to minimize the extent of initial and secondary injury in the spinal cord and attempts to limit or even reverse the physiological conduction blockade by preserving the surviving viable non-functioning white matter with the help of various pharmacological compounds.

2. The second approach concentrates on ways to regenerate and reconnect the injured axons within the spinal cord by modifying the various factors responsible for the antagonistic CNS environment and make it more receptive towards regeneration with various kinds of cellular transplants.

3. The third approach is directed towards regaining the functional recovery, regardless of the anatomical connections within the spinal cord. This involves the use of electrical stimulation through neural prosthetic devices for partially restoring the lost functions. Electrical stimulation can overcome the deficit produced by lesions in the spinal cord and maintain the physical integrity of the various neuromuscular functions (3).

The purpose of this study was to implement methods of physiotherapy, electrotherapy especially, in spine disease recovery in dogs and establishment of treatment protocols, depending on the obtained effects.

## 1. MATERIALS AND METHODS

The study was conducted in the Surgery Clinic of the Veterinary Medicine Faculty from Cluj-Napoca, during 2009-2011, on a number of 6 animals of canine species, which were presented for consultation with various disorders of the spine (tabel 1).

*Tabel no. 1.*

Casuistry taken in study.

<b>Crt.</b>	<b>Rasă</b>	<b>Sex</b>	<b>Vârsta</b>	<b>Simptome</b>	<b>Diagnostic</b>
1.	Teckel	♂	7 ani	Paralysis of the hindquarters Urinary incontinence	Cord compression syndrome
2.	Common breed	♀	5,5 ani	Paralysis of the hindquarters Urinary and fesses incontinence	Luxation of the spine with spinal section
3.	Copoi	♂	2,5 ani	Paralysis of the hindquarters Urinary incontinence	Luxation of the spine with CCS
4.	American	♂	7 ani	Paralysis of the	CCS with slow

	Cocker				hindquarters Urinary incontinence	evolution
5.	Metis collie	Border	♀	8 luni	Paralysis of the hindquarters Urinary incontinence	Dislocation / fracture of the spine
6.	Westie		♂	2 ani	Failure to carry out support on the hindquarters Urinary incontinence	CCS with slow evolution

General therapeutic protocol includes several steps, namely:

1. Clinical and paraclinical patient exam for establishing the certainty diagnosis. In general, patients in the study were presented for consultation with similar symptoms: failure to carry out support on the hindquarters to paralysis (Fig. 1), urinary or fesses incontinence. To establish the site of the compression we performed X-rays.



**Fig. 1.** Clinical exam of animals



**Fig. 2.** Laminectomy intervention

2. Surgery and drug treatment setting. After determining the severity and the compression site, we performed laminectomy (Fig. 2) for decompressing the spinal cord. To prevent infection, were administered antibiotics for 5 days i.m.

3. The physiotherapy program establishment and its modulation for each patient in part.

Recovery program began in the third postoperative day, and aimed to train rear motor function recovery and to prevent the muscle atrophy installation, consecutive to the spinal cord compression. This was achieved by several methods of physiotherapy related, namely: electrotherapy, functional exercise, therapeutic massage.

Electrotherapy sessions were conducted with interferential current devices with vacuum electrodes Interferenz System Med-mode 3, brand BOSCH (Fig. 3), and vacuum applicator BTL vac (Fig. 4). If the vacuum is not formed for various reasons, we used a neutral gel that is applied to the skin to facilitate the electrodes adhesion to the skin.



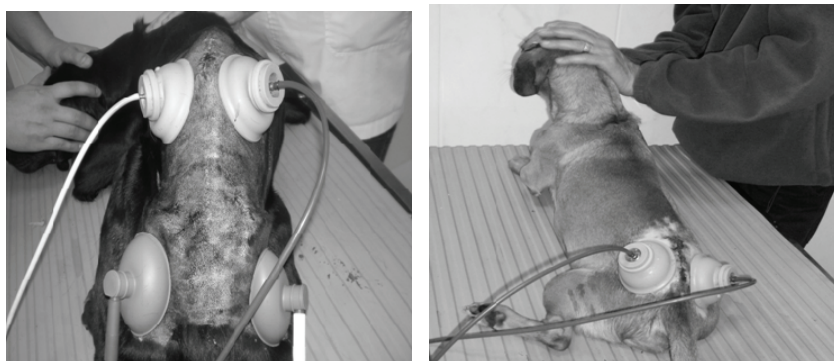
**Fig.3.** Interferenz System Med-mode 3, brand BOSCH



**Fig. 4.** Vacuum applicator BTL vac

Interferential current parameters used were as follows:

- intensity 6-8 mA;
- frequency 90-100 Hz;
- pairs of electrodes: can be used one or two pairs of electrodes (Fig. 5), depending on area size and degree of muscular atrophy;
- time: one session of 10-15 minutes, depending on the severity and evolution of each patient;
- duration and frequency of treatment: initially a session each day within 2 weeks and in the third week a session in two days.



**Fig. 5.** Electrodes application on the skin.

To strengthen the effects of electrotherapy and to prevent complications such as muscle atrophy, joint stiffness and loss of function, we used other auxiliary physiotherapy methods such as massage and functional gym (4,5) (Fig. 6).



Fig. 6. Massage and functional gym.

## 2. RESULTS AND DISCUSSIONS

To assess the effects of the physiotherapy program in healing lesions of the spinal cord, as well as accurate interpretation of these effects, during treatment were monitored following:

1. the animal's reaction to the interferential currents contact and its behavior during the electrotherapy session;
2. currents parameters influence on the recovery;
3. hindquarters sensitivity evolution during treatment;
4. hindquarters motor compound evolution during treatment.

In most cases, the animals reacted with a short-term discomfort to the starting current, they getting used very quickly with the current application,

the period of adjustment being somewhere around 30 seconds. After the adjustment period the animals showed a state of physical comfort, even pleasure manifested by peaceful, voluntary adoption of sterno-abdominal decumbent, in some cases the animals becoming very sleepy.

The state of physical comfort during electrotherapy sessions is explained by the massage sensation produced by the vacuum electrodes. This feeling of massage is given by the suction action of the electrodes, which leads to a profound and systematic massage.

To avoid burns and unpleasant local sensations at the start of electric current application, it is necessary to gradually increase the intensity of current, up to a maximum intensity of 10mA. An increase in current intensity too quickly led to a feeling of discomfort, and a threshold of 10 mA resulted in congestion and local burns.

In terms of intensity, is known (from previous research cited in the literature) that the intensity under 8mA is used for sensory stimulation, and the over 8mA for motor stimulation (2).

In terms of frequency, to achieve the desired beneficial effects, we always started with lower frequencies, 10-25Hz, and only in the 3<sup>rd</sup> or 4<sup>th</sup> meeting we have reached the maximum frequency of 80-120Hz. We used this gradual increase in frequency to accommodate the body and to prevent the occurrence of electrical current applied to burns and local intravascular coagulation (4).

Sensitive component of the nervous system was tested by animal response to different painful stimuli (needle pricking, pinching with forceps or compressions in different regions). Hindquarters sensitivity, respective the hind limbs sensitivity was checked daily, measuring the intensity with which the animal reacts or not to the applied stimuli. If initially we started on day 1 of treatment with a very low sensitivity, even absent, the animals not reacting/or reacting very poorly to the applied stimuli, after about a week of treatment we began to observe notable changes in this respect.

The motor nervous system was monitored by the patient's ability to resume normal urinary and defecation function, to make voluntary movements with the hind legs, to carry out support on them to make them and to adopt the standing up attitude (5).

Recovery of motor function was gradually, patients being gradually helped during treatment to regain their own confidence (4). Thus, initially



were helped to make their own movements by pinching interdigital hind legs, then during the evolution in treatment have been helped to lift the hindquarters and make support on it. After successfully conducting the train back support, slowly were made the first steps, one by one, and only after a few days to try walking, feeding and watering in standing up position (Fig. 7).



**Fig. 7.** First steps after the operation and feeding in standing up position.

### 3. CONCLUSIONS

After conducting this study on the use of electrotherapy in the treatment of spinal disorders in dogs, we reached several important conclusions, namely:

3.1. Electrotherapy is a safe and simple procedure, non-invasive and without side effects, through which electric currents are applied to different types of tissue treatment areas stimulating them and causing a quickening of the healing process.

3.2. Animal patients respond well to interferential current application with vacuum electrodes due to the massage sensation produced by them. Besides the feeling of comfort afforded by the vacuum electrodes during treatment, they also have the advantage of greatly reduced skin resistance to electric currents.

3.3. Electrotherapy can not be used as unique therapy because it can not correct etiological links, but can improve and control the clinical,

symptomatic and functional elements such as pain, inflammation, swelling, muscle tension, joint stiffness, etc.

3.4. Physical therapy is an adjunctive therapy in spinal cord compression syndrome, imperative because without it the chances of recovery are minimal due to complications such as muscle atrophy, joint stiffness, and internal disorders consecutive to the extended decubitus.

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## THE INCIDENCE OF DEGENERATIVE MITRAL VALVE DISEASE IN DOGS

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**Key words:** valvular diseases, radiographic examination, mitral valve, heart murmur, animal gender.

### SUMMARY

1389 dogs with cardiac and extracardiac diseases were examined in the Internal Disease Clinic —Cardiology Department from the Faculty of Veterinary Medicine Bucharest. The examinations included a clinical examination, electrocardiographic, echocardiographic and radiographic examination, blood pressure determination and laboratory evaluations. Only animals with III —IV degree heart murmur and specific clinical signs at the echocardiographic examination have been included in the study.

A population of 1972 dogs, 50.50% males and 49.50% females from different breeds was examined. 263 dogs (13.33%) were diagnosed with valvular disease. 96.57% (254 dogs) have been diagnosed with mitral valvular disease. Dogs diagnosed with MVD (murmur on the mitral area, III - IV murmur intensity, increase of ventricular cavities, mitral dystrophy) were small breed dogs (73.18%) and small to medium breed dogs (26.82%). Regarding sex predisposition, males seem to be much more predisposed to MVD. It has been observed that: a) valvular disease was diagnosed in 68.45% males and 31.55% females; b) MVD was diagnosed in 68.33% males and 31.67% females; c) valvular (non-mitral) disease was diagnosed in 71.43% males and 28.57% females. In small breed dogs the disease was diagnosed in 67.42% males and 32.58% females, and in small to medium breed dogs in 70.83% males and 29.17% females. The examinations revealed male dogs are much more predisposed to valvular diseases. A 2:1 ratio —males/females- has been described in this study.

Degenerative Mitral Valve Disease (DMVD, also known as endocardiosis or myxomatous valve degeneration) is a condition frequently met in medical practice, with mitral valvulopathies being the most significant in this category of diseases (3, 4, 7).

Degenerative mitral valve disease is the most common acquired cardiac disease in dogs and a frequent cause. It results in early mitral insufficiency, which in turn leads to the appearance of left apical systolic murmur. The progression of Degenerative mitral valve disease includes left

atrial and left ventricular dilatations, modifications of P wave on ECG, and at a later stage pulmonary edema, myocardial failure and pulmonary hypertension. The prevalence of Degenerative mitral valve disease is known to increase with age; prevalence and progression are believed to be higher and faster in males than females (5, 7, 9). This paper is part of a wider study which deals with the diagnosis and treatment of cardiopathies with regard to the animal welfare (6).

The aim of this research paper is to show that cardiac diseases, in this case DMVD, is much more frequent in males. In human medicine, the low incidence of cardiopathies in women is considered to be due to the protective role of feminine hormones on the heart.

## **1. MATERIALS AND METHODS**

This research paper includes a statistical study regarding the incidence of DMVD, and more specifically of mitral myxomatous valve degenerative in dog.

The research has 2 phases:

- the first phase was realized in a 12 month period;
- in the second phase, the research has been extended over a period of 16 and a half months.

Only the patients with B and C degree valvular disease have been considered in this study (1, 2, 5).

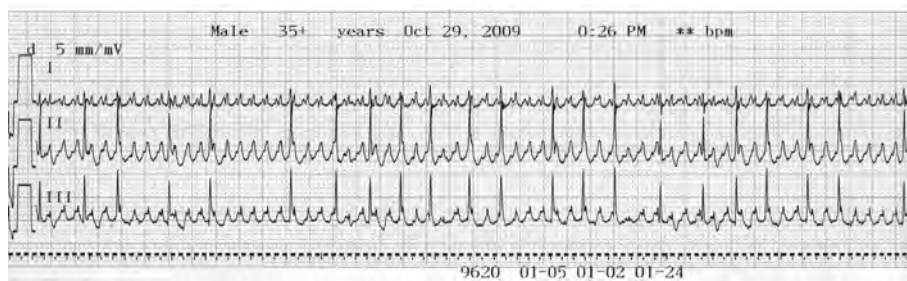
### **DMVD DIAGNOSIS**

Patients included in the study presented:

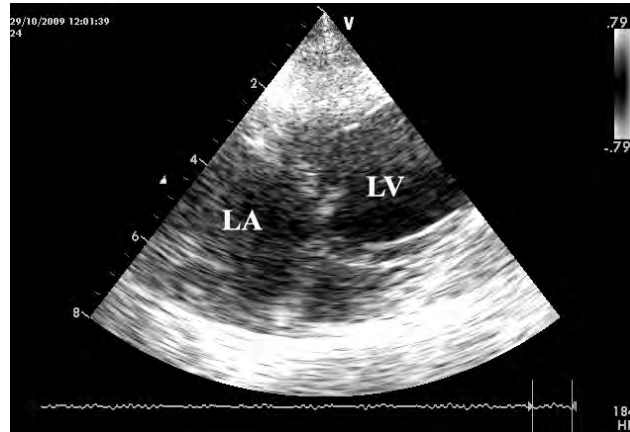
1. Symptoms of cardiac decompensation (3<sup>rd</sup> and 4<sup>th</sup> degree modified NYHA classification, 2009. (5, 10).
2. Functional symptoms (3<sup>rd</sup> and 4<sup>th</sup> degree heart murmurs).
3. Physical symptoms detected by echography, radiography and electrocardiography (fig. 1,2,3,4) (8).



**Figure 1.** Dachshund, female, 14 years.  
Degenerative mitral valve disease —Radiographic aspect.



**Figure 2.** Dachshund, female, 14 years.  
Degenerative mitral valve disease. Atrial flutter



**Fig. 3.** Degenerative mitral valve disease.

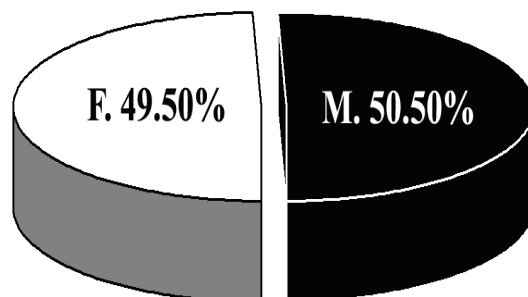


**Fig. 4.** Mitral valve prolaps

Patients with 1st and 2nd degree heart murmur and patients with mitral thickness and reduced atrio- ventricular regurgitation observed at the echographic examination have not been included in the study.

## **2. RESULTS AND DISCUSSIONS**

A number of 1972 dogs from different breeds have been examined in the Internal Disease Department of the Faculty of Veterinary Medicine Bucharest, during a period of 1 and a half years (November 2008 - May, 2010) (fig. 5). There were clinically healthy dogs, with cardiac or non-cardiac diseases.



**Figure 5.** Total number of examined animals (1972 animals; left = females, right = male)

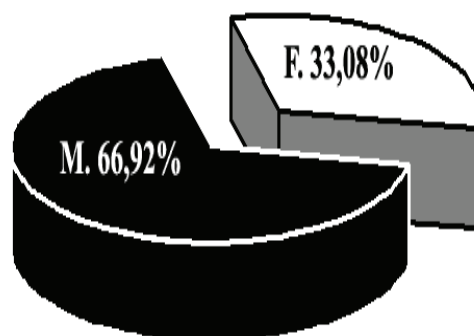
From the total number of dogs, 49.50% were females and 50.50% were males. Sex ratio was therefore almost equal.

Following clinical examinations, 263 dogs have been diagnosed with cronical valvulopathies (13.33%). From the 263 patients, 254 showed mitral valve disease (96.57%). The results are comparable with data from literature - 10% (1, 8, 9).

CVHD has been diagnosed in small breed dogs, small to medium and medium breed dogs.

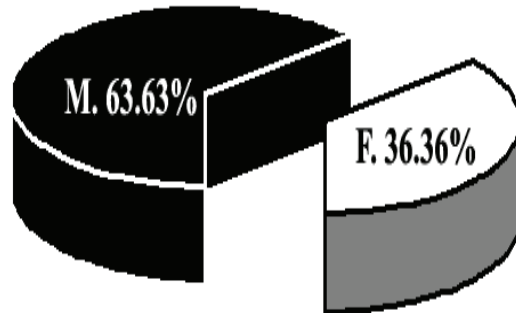
The incidence of chronic valvulopathies was much higher in males than in females, with a ratio of 2:1.

A) Valvulopathies (all included): females 33.08% - males 66.92% (fig. 6).



**Fig. 6.** The incidence of valvulopathies/mitral Valvulopathies depending on animal gender (left = male, right = female)

- B) Mitral valvulopathies: females 33.08%, males 66.92%.  
C) Non-mitral valvulopathies: females 36.36%, males 63.63% (fig. 7).



**Figure 6.** The incidence of non - mitral valvulopathies depending on animal gender (left = male, right = female)

If we compare the values from the 12 month period with those obtained in the 16 month period (table 1), we can conclude that the ratio 2:1 (males: females) didn't change.

A research has shown that 26 (60.47%) from the 43 females diagnosed with mitral valvulopathies have been neutered several years ago (7 - 8).

### 3. CONCLUSIONS

1. DMVD has been diagnosed in 13.33% from the 1972 dogs evaluated in a 18 months period.

2. 96.57% from the examined animals have been diagnosed with mitral valve disease.

3. Even though the ratio males/females has been almost equal in the population evaluated, males have been much more affected than females. Ratio males/females was 2: 1.

4. Neutered females are much more prone to develop mitral valvulopathies (60.47%), compared to intact females (39.53%).

5. Statistical researches show that females are less affected by cardiac diseases than males (the same situation has been observed in dilatative cardiomyopathy). Therefore, it could be inferred that sexual hormones have a protective role on the heart.



## ACKNOWLEDGMENTS

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## **STUDY REGARDING THE RELEVANCE OF THE ULTRASOUND EXAMINATION IN UTERINE DISEASES ASSOCIATED WITH POLYURIA-POLYDIPSIA SYNDROME IN BITCH**

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**Key words:** ultrasonography, uterine, carnivores, polyuria-polydipsia

### **SUMMARY**

The clinical and therapeutic approach polyuria syndrome/polydipsia, of paramount importance is the establishment of primary etiology and pathogenesis complex responsible for its induction.

All data included in the diagnostic certainty conclude that the diagnosis of the syndrome of polyuria/polydipsia is made by corroborating elements of clinical and laboratory data (the latter defining the final diagnosis).

From the ultrasound investigated cases with pyometra (n = 21), 85.71% had polyuria-polydipsia syndrome, and from the total number 71.43% were with opened and 28.57% with closed cervix.

Ultrasound diagnosis in cases of pyometra (polyuria-polydipsia syndrome associated) is easily achieved with high degree of accuracy (100% positive) being considered a non-aggressive technique for the patient and the examiner too.

The first intention therapeutically measure is represented by ovary-hysterectomy (especially if the cervix closed pyometra). The young females and the high reproductive value can try conservative therapy, resorting to systemic antibiotic therapy supported with PGF2 $\alpha$  type uterine stimulants, followed by ultrasound examination — for confirming the curative efficiency.

Atypical or unusual urination are concerning and represent one of the most common causes of patient presentation to the veterinarian, as determined by the removal of large quantities of urine, frequent urination, loss of urine apparent, inappropriate urination, or less, due excessive consumption of water.

Formation and excretion polyuria means large amounts of urine (> 50 ml/kg/24h), usually with a low specific gravity. Polydipsia can be defined as

increased thirst, resulting in increased volumes of fluid consumption (> 100 ml/kg/24h).

Depending on water content of its diet, a healthy dog drinks an average of 50-60 ml/kg/24h. Normal urinary flow rate varies between 20-40 ml/kg/24h. Exceeding these values lead to polyuria - polydipsia syndrome. Fluid consumption and urine production are controlled by complex interactions between: plasma osmolarity, volume of fluid in the vascular bed, thirst center, kidney, and pituitary - hypothalamus. The two main mechanisms responsible for maintaining fluid balance are: thirst mechanism and renal concentrating mechanisms.

Any interference with patho-physiological order affecting one of the components involved can lead to polyuria and polydipsia default. In most cases compensate for polyuria polydipsia, polyuria ie is the main issue and results in secondary or compensatory polydipsia. This is true for all diseases that are associated with primary polydipsia or psychogenic polydipsia except where polyuria is secondary and occurs in response to the amount of water in excess.

Antidiuretic hormone (ADH/vasopressin) play crucial role in: control of tubular re-absorption of water, urine production, urine concentration and fluid balance ensuring the body. ADH acts on epithelial cells of distal convoluted tubules and collecting tubules - which increase the hydro-osmotic permeability of these cells.

Primary urine (diluted) of the tube passes into the interstitial (concentrated) in renal medulla. In the presence of ADH's fluid volume decreases the nephron, thereby conserving body fluids. In the absence of ADH's (central diabetes insipidus) or if its action strength tubular structures (nephrogenic diabetes insipidus) - no longer made and water diffusion in the interstitial fluid (renal medulla) - hypotonic glomerular ultrafiltrate passes unchanged in tubules distal tubules and collecting.

## 1. MATERIAL AND METHODS

The study was performed on 21 bitches, of different breeds, age or body conditions, diagnosed with polyuria-polydipsia, secondary to pyometra.

Were clinical and ultrasonographic examined 21 cases with pyometra (n = 21). From these, in 18 bitches was registered as an important and constant clinical sign, the polyuria-polydipsia syndrome (85.71%).

Clinical examination revealed that in 15 cases (71.43%) were with diagnosed with opened cervix, the other 6 females were diagnosed with closed cervix (in 28.57%).

The ultrasound examinations were performed with usual portable ultrasound (Aquila and Mylab 30 - Esaote Pie Medical machines) with convex probes with different frequencies between 5 and 8 MHz.

In such cases for evaluating the uterus, as a cavitary organ, for ensuring a maximum accuracy of the ultrasound investigations we've followed and respected strictly the steps for obtaining the most relevant images, without or with minimum artifacts images, in order to enhance the relevance of the obtained ultrasound images.

In most cases we've tried to perform the examinations with filled urinary bladder (actioning as a veritable acoustic window for the uterus).

The conduct of the two different symptoms, so there is polyuria with polydipsia may offset or primary polydipsia (psychogenic). Polyuria with compensatory polydipsia- caused by osmotic diuresis: diabetes mellitus, primary renal glucosuria/Fanconi syndrome, renal failure, polyuria; or interference with the release of ADH and/or response to ADH: chronic renal failure, nephritic syndrome, pyelonephritis, pyometra, Cushing syndrome, chronic liver failure, hypercalcemia, central diabetes insipidus, nephrogenic diabetes insipidus, hyperthyroidism, iatrogenic causes.

Pyometra or cystic endometrial hyperplasia is considered the most important disease in dogs and cats affections. It is considered that the basis of this condition be a progressive endocrine disturbance acting against a particular susceptibility of the uterus to progesterone. Elevated estrogen for short periods followed by long periods of increased levels of progesterone cause cystic endometrial hyperplasia, followed by uterine gland hyper secretion and accumulation of uterine secretions (Carmel and Peterson, 2004; Codreanu and Diaconescu, 2003).

Obvious symptoms and occurs during the luteal phase, usually 4-10 weeks after estrus or after exogenous progestative substances.

The clinical picture is varied as evolutionary (with closed or open cervix). The general registered symptoms were: depression, dehydration, anorexia, polydipsia and polyuria. Sometimes vomiting and rarely diarrhea was registered. Rectal temperature was mostly normal or close to/or hyperthermia.

The local clinical signs of the pyometra were dominated by abdominal distension (enlargement of the abdomen), sometimes pendulous appearance (these cases the abdomen is painful and prevent animal movement). The abdomen may feel a pasty mass occupying much of it.

Shape of the uterus in pyometra is determined by the content of uterine horns. Usually, uterine horns are uniformly dilated. Can be registered situations where uterine horns are unequal or similar to nodular aspect (similar to gestation of 30-35 days), situations avoided by examining in different incidences.

When is missing the vaginal discharge the uterine collection in about a third of cases of pyometra is sterile. The other cases are secondary infections (with *E. coli*, staphylococci, streptococci, etc.)

Endotoxins from *Escherichia coli* (most commonly associated with pyometra) prevent ADH's recognition in the kidney structure (renal tubules), practically a form of reversible nephrogenic diabetes insipidus (Codreanu *et al.*, 2011).

## 2. RESULTS AND DISCUSSION

The most important objective of our study it was to achieve and to correlate the clinical expression — dominated by polyuria/polydipsia syndrome in cases of pyometra in bitches, in order to confirm the ultrasound diagnosis.

The uterus horns and body can be very difficult to identify and to appreciate in normal females (Codreanu and Diaconescu, 2003).

In terms of ultrasound, pyometra can provide a variety of issues rather than depending on the form of development, age, etc. (Codreanu and Diaconescu, 2003; Han *et al.*, 1994; Penninck, 1995).

In case of closed cervix pyometra we have registered the most characteristic aspects: area round/ovoid shaped or oblong, large (3.5-10 cm) containing anechoic (with artifactual distal enhancement) and thin-wall - without parietal thickening (Figure 1 and Figure 2).



**Fig. 1.** The uterus is distended by anechoic content (with distal enhancement), without severe alteration of the uterine wall.



**Fig. 2.** The uterine horns became ultrasonographic visible, as rounded/ovoid shaped structures, because of the fluid accumulation (with corpuscular elements). The uterine wall is not visible affected.

In some cases, because of folding uterine horns, the cavity layout is unique or poly-compartment, with thinned uterine wall (Bîrțoiu and Seiciu, 2006, Crivineanu and Codreanu, 2008).

Uterine contents may sometimes particulate matter, which in light of the abdominal wall movements baling are triggered and generates characteristic appearance of "snow storm".

Sometimes within the uterine cavity, relaxed purulent content, you can see areas of varying sizes echoic, adherent to the uterine wall (polyps or tumors of the uterine wall), or type hypo/anechoic (glandular-cystic endometrial degeneration), quite common in animals (Fig. 3 and Figure 4).

The forms of development with open cervix, the image obtained is totally different. The uterus contains significantly lower quantities of pus, therefore will be lower uterine distension. Instead a parietal hypertrophy appears obvious uterine wall appeared thickened and hyperechoic.

Often, the appearance of the uterus in pyometra the cervix is opening nodular, the dilation is of round/ovoid shape aspect, which can be confused with early pregnancy (Codreanu and Diaconescu, 2003).

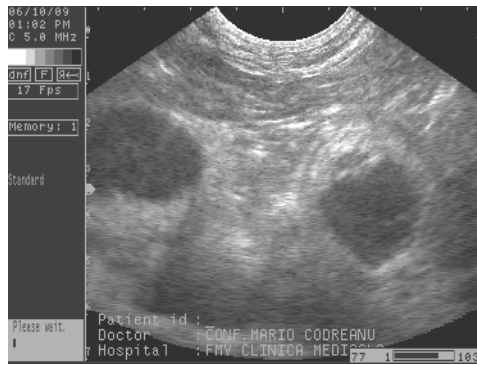
Ovario-hysterectomy is the treatment of choice (Bîrțoiu and Seiciu, 2006, Crivineanu and Codreanu, 2008). If it is aimed at saving the female reproductive capacity should be considered medical management. In this respect, it should start the fluid therapy and antibiotics - using broad-spectrum substances (intravenously).

Correction is done as soon as possible fluid imbalances, electrolyte or acid-base, before the ovario-hysterectomy. Bacterial infection is responsible for the bad condition of the dam and will not settle until it is removed uterine exudates. Typically, oral antibiotics should be continued for 7-10 days after surgery.

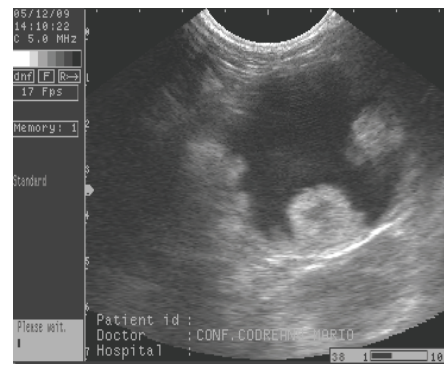
Conservative therapy, prostaglandin (PGF $2\alpha$ ) can be used to stimulate uterine contractions and uterine mucus default (Crivineanu and Codreanu, 2008).

Particular caution is advised if the cervix pyometra closed (because of the risk of uterine rupture).

Natural prostaglandins are recommended (0.25 mg / kg, 5 days), synthetic analogues (cloprostenol, fluprostenol) are more intense as the action, but side effects are incompletely studied until now (in carnivores).



**Fig. 3.** The uterine horns contents particular elements (pus), with affection on different levels of the mucosa, but without losing the parietal architecture/or reduced endometrial involvement.



**Fig. 4.** Chronic case of open cervix pyometra, with endometrial mucosa reaction (hypertrophic changes) — medium/high echogenicity proliferations and hypo/anechoic content (with specific distal enhancement).

Animals should be ultrasound reevaluated at 2 weeks after medical therapy for identifying and appreciating the presence or absence of the uterine content (Codreanu and Diaconescu, 2003).

If a leak is still presented bloody or mucous or purulent or if the uterus is enlarged, PGF $2\alpha$  therapy may be repeated using the same protocol, the prognosis becomes reserved.

### 3. CONCLUSIONS

Synthetic analysis of the results of investigations, allows detachment following conclusions:

3.1. Polyuria and polydipsia is a symptomatic association, rarely manifesting unilaterally, which met and demonstrated with the other syndrome conditions dominated or accompanied by polyuria/polydipsia (diabetes insipidus, diabetes mellitus, Cushing's syndrome, renal failure).

3.2. The clinical and therapeutic approach of the polyuria syndrome/polydipsia, of major importance is the establishment of primary etiopathogenetic complex, responsible for its induction.

3.3. All data included in the diagnostic certainty conclude that the diagnosis of the Polyuria-Polydipsia syndrome is made by corroborating elements of clinical and paraclinical —additional data (the latter defining the final diagnosis).

3.4. From the ultrasound investigated cases with pyometra (n = 21), 18 bitches had polyuria-polydipsia syndrome (85.71%) and from these, 15 (71.43%) were with opened and 6 with closed cervix (28.57%).

3.5. Ultrasound diagnosis in cases of pyometra (polyuria-polydipsia syndrome associated) is easily achieved with high degree of accuracy (100% positive) and non-aggressive for the patient and the examiner, dominated by the distensions of uterine horns by hypoechoic/anechoic content, without or with minimum wall thickening.

3.6. The first intention therapeutically measure is represented by ovary-hysterectomy (especially if the cervix closed pyometra), but in young females or of high reproductive value, can try conservative therapy, resorting to systemic antibiotic therapy supported with PGF $2\alpha$  type uterine stimulants, followed for controlling by ultrasound examination —for confirming the curative efficiency.

3.7. In addition and in close connection with the management complexity of all etiopathogenetic and it must be done with maximum care, curative effectiveness is closely linked to precocity, accuracy and consistency of application of measures to be taken.



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## **THE CHARACTERIZATION OF MIDDLE LATENCY AUDITORY RESPONSES RECORDED WITH SURFACE ELECTRODES ON DOGS**

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**Key words:** MLR, dog, surface electrodes

### **SUMMARY**

In current veterinary practice auditory evoked potential with medium latency (MLR) isn't used currently. MLR offers data regarding the integrity of auditory pathways from caudal colliculi to auditory cortex. Until now different methods were reported in order to obtain better results for MLR. The audiological test was made with the Neuropack S, MEB 9400K Electrodiagnostic system (NIHON KOHDEN) in the ABR program. Our study describes and analyzes MLR recorded with surface electrodes in dogs. Statistic analysis of the results showed a high correlation ( $r > 0.9$ ,  $p < 0.05$ ) between the waves with the same polarity.

Comparing with human medicine, in current veterinary practice, the recording of auditory evoked potential with middle latency (MLR) is not usually performed, being used only for research (Woods, Alain et al. 1995; Baez-Martin and Cabrera-Abreu 2000; Murrell, de Groot et al. 2004). Different recording methods in which needle subcutaneous electrodes were used were reported until now (Barth and Di 1991). In this study we describe MLR recording technique performed with surface electrodes and we will analyze the morphology of recorded waves.

### **1. MATERIAL AND METHODS**

This study was approved by the Council of Ethics of the University of Agricultural Sciences and Veterinary Medicine from Iași (11332/20.07.2009). It was performed on 6 dogs (4 cross breed, 1 Fox Terrier, 1 Husky), age average  $4 \pm 1.5$  years). The animals were included in the study only if their history and clinical examination wouldn't have revealed any neurological or hearing disorders. After anesthetizing them with medetomidine hydrochloride (Domitor, Pfizer), 0.05 mg/kg inj. I.M.,

the audiological testing was performed with the Neuropack S, MEB 9400K Electrodiagnostic System (Nihon Kohden) in the ABR program. The waves were recorded with surface electrodes placed as follows: the active electrode on the vertex, reference electrodes at the base of each ear and the grounding electrode on the median line, retrooccipitally. The area on which the electrodes were placed was trimmed, degreased with alcohol and Skin Pure (Nihon Kohden), and covered with special adhesive EEG paste (Elefix, Nihon Kohden).

An impedance check was performed before each test and it was shown to be lower than  $5\Omega$ . Alternating click stimuli of 0.1 ms were applied through earphones inserted into the auditory canal. We performed individual tests on each ear, as well as binaural stimulation, with a stimulus with standard intensity of 80 dB SPL. Evoked potentials were recorded also when the intensity of the stimulus decreased from 80 to 40 dB SPL, by steps of 10 dB SPL (the non-tested ear was masked with white noise of an intensity 40 dB lower than that one used on the tested ear). Each waveform was the average of 500 stimulations, using a High-cut filter of 20 Hz and a Low-cut filter of 1000 Hz. Artifactual data were automatically rejected; when rejected waveforms have represented more than 5% of the average, the tests were repeated. The waves were manually labeled by the same examiner with  $N_0$ ,  $P_0$ ,  $N_a$ ,  $P_a$  and when was possible with  $N_b$ .

## 2. RESULTS AND DISCUSSION

For all tested dogs it was possible to record and mark electrical traces corresponding to the electrical response generated by passing of the auditory impulse along the nuclear structure and auditory cortical area (fig. 1).

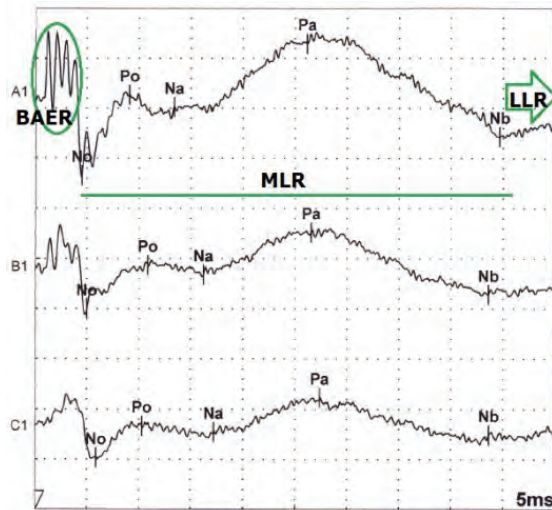


Figura 2.1 —MLR trace obtained after binaural stimulation at 80, 70 and 60 dB SPL in a cross breed dog. Waves  $N_0$ ,  $P_0$ ,  $N_a$ ,  $P_a$  and  $N_b$  are marked. The  $N_0$  wave is preceded by the waves of brain (BAER). After  $N_b$  evoked potential with long latency is starting (LLR/SVR). The successions of the waves reflect the conduction of auditory stimulus from the caudal colliculi to auditory cortex. We observe that the amplitude of the waves decrease with the intensity of stimulus.

The obtained MLR latencies values were:  $N_0 = 4,83 \pm 0,53$  ms,  $P_0 = 9,01 \pm 0,1$  ms,  $N_a = 14,38 \pm 2,52$  ms,  $P_a = 26,2 \pm 1,12$ ,  $N_b = 42,93 \pm 3,96$  and  $P_b = 58,46 \pm 11,07$  ms. Statistical analyses of the above data proved that a high correlation degree exists ( $r > 0,9$ ;  $p < 0,05$ ) between the pair of waves that presented the same polarity  $N_0N_a$  (0,97),  $N_0N_b$  (-0,982),  $N_aN_b$  (-0,99)  $P_0P_b$  (-0,99) and for pairs  $N_aP_a$  (-0,92),  $P_aN_b$  (0,91) and a high correlation degree ( $r = 0,7-0,9$  to  $p < 0,05$ ) for  $P_0P_a$  (-0,74) and  $P_aP_b$  (0,78).

We consider that the MLR recording technique with surface electrodes provides an accurate morphology of the waves, comparing to needle electrodes technique. The application of stimuli allows the recording of all morphological structures of the waves and offers reliable information about the passing of auditory stimulus along the nuclear structures placed rostrally from caudal colliculi.

The obtained MLR latencies are smaller than those reported previously only for  $N_0$  and  $P_0$ , without difference for the others. The best stimulus intensity for recording and analyzing the waves is 80 dB SPL. In the cases where the intensity was lower, the interpretation has become subjective or the flexions positive / negative were absent.

We can conclude that by analyzing of the MLR morphology and latencies with surface electrodes the interpretation of the bioelectric cerebral activity can be assessed.

### 3. CONCLUSION

MLR recording technique with surface electrodes provides an accurate morphology of the waves, comparing to needle electrodes technique

By analyzing of the MLR morphology and latencies with surface electrodes the interpretation of the bioelectric cerebral activity can be asses.

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## **STUDY CONCERNING THE PREVALENCE OF THE PULMONARY METASTASES AND THE CLINICAL ASPECTS IN THE BITCHES WITH MAMMARY TUMORS**

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**Key words:** bitches, mammary tumors, pulmonary metastasis, radiological examination.

### **SUMMARY**

This paper has analysed the identification of the pulmonary metastases generated by the mammary tumors, the prevalence related to the breed, age and clinical manifestations. The research has been performed in the clinics of the Veterinary Medicine Faculty in Bucharest on a number of 41 bitches, with mammary tumoral lesions. The animals were subjected to the clinical examination, completed with a pulmonary X-ray examination and with the anatomico-pathological examination.

The clinical and X-ray examination has highlighted the presence of the pulmonary metastasis at 21.95% (9 bitches) out of the investigated cases, the highest prevalence being found at the Caniche breed, namely 25%. From the radiological point of view, out of the 9 cases with pulmonary metastasis, 8 had multiple nodular groups and a case with a sole tumoral pulmonary form. The predominant clinical signs were the cough and the dyspnoea.

The pulmonary metastasis lesions were diagnosed at the animals older than 10 years of age. At the analysed cases, different morphological types of carcinoma were diagnosed.

The mammary tumors are the most frequent neoplastic illness in bitches. Moreover, these tumors can disseminate by means of the lymphatic and blood vessels, having the route at the level of the regional lymph nodes and at the level of the lungs (Harvey, 1998; Hedlund, 2007; Robbins, 2003).

The mammary tumors form metastases especially at the lungs (60 - 80% of the studied metastases), but also to other organs: lymph nodes, suprarenal glands, kidneys, heart, bones, liver, brains, eyes, nose, spleen, uterus (Fontbonne et al., 2007; Lagadic and Cohn-Bendit, 1995; Muller and Guaguère, 2006; Sorenmo, 2003). Occasionally, the skin can be as well a place of metastases for the canine mammary tumors, causing the cutaneous metastases (Muller and Guaguère, 2006). Therefore, the early and correct diagnosis of the pulmonary metastases has a significant importance in establishing the therapeutical actions (Hedlund, 2007).

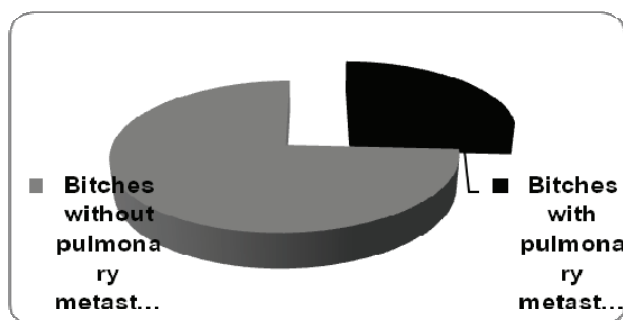
Radiologically, the pulmonary metastases can be in a form of well-defined nodules or with weak delimitation, with/without pleura effusion, but without a clear evidence of pulmonary aggression (Robbins, 2003). This research has aimed at the identification of the pulmonary metastases generated by the mammary tumors, the prevalence related to the breed, age and clinical manifestations.

## **1. MATERIAL AND METHODS**

The investigations were performed between October 2010 and August 2011 at the Veterinary Faculty in Bucharest, where a number of 41 bitches of different breeds were examined, aged between 7 and 16, which had mammary tumor lesions. The animals were subjected to the clinical examination, completed with pulmonary X-ray examination, achieving two radiographic thoracic images, (perpendicular one on the other), followed by anatomico-pathological examination (cytopathological and histopathological), to a part of the mammary tumor lesions, after the performance of mastectomy.

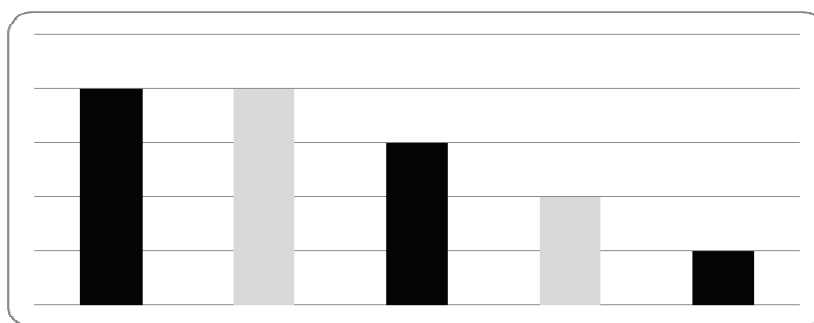
## **2. RESULTS AND DISCUSSION,**

Following the analysis of the obtained data it has been found out that the Caniche breed is on the first place concerning the presence of the pulmonary metastases, with a prevalence of 25% (2 cases), being followed by the other breeds: German Shepherd, Boxer, Cocker, Doberman, half-breed, Rottweiler, Pekingese, with a prevalence of 12.5% each. As far as the age is concerned, it fluctuates between 10 and 16 years. The pulmonary radiographic examination has confirmed the presence of the pulmonary metastases in 9 bitches, which represents 21.95% out of the studied cases (Fig. no. 1). It must be mentioned that the average age of the bitches having mammary tumors was of 11.59 years, while the average age of the bitches with pulmonary metastases was of 12.89 years.



**Fig. 1:** Percentual distribution of the bitches with and without pulmonary metastases

Following the clinical and paraclinical examination of the bitches diagnosed with pulmonary metastases, it has been found out that the main symptoms were the *cough* and the *dyspnoea* (fig. 2)



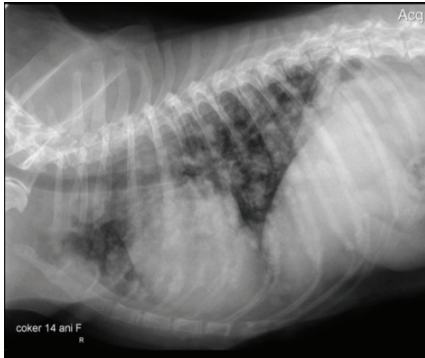
**Fig. 2:** Graphic distribution of the clinical signs in the bitches with pulmonary metastases

Concerning the type of the pulmonary metastases (9 cases) it has been noticed that they were both single (1 case), and multiple (8 cases), with very different dimensions and pulmonary localizations (Fig. 3 and 4). In a number of 32 females with mammary tumors were not noticed detectable lesions by the digital radiographic technique, being included in the I.T.N. category (normal thoracic image).

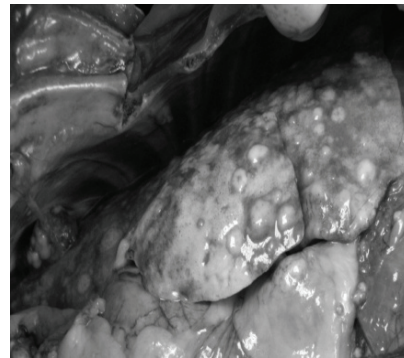
Related to the extrapulmonary metastases, it has been found out that 3 bitches (out of the 9 with pulmonary metastases), had metastases at the following levels: heart, liver, hepatic, pancreatic splenic and renal, and the balance of 5 cases had only metastases at the pulmonary level. Certain studies reveal the fact that the main place of metastases at the level of the mammary tumor processes is the lungs (about 60-80% of the studied metastases), followed by the bones, liver and brains, but the skin must also



be mentioned (cutaneous carcinomatosis), (Alhaidari and Magnol, 1986; Ginel et al., 2000; Nafe et al., 1979; Pin, 1999; Poujol et al., 2005; White et al. 1985).

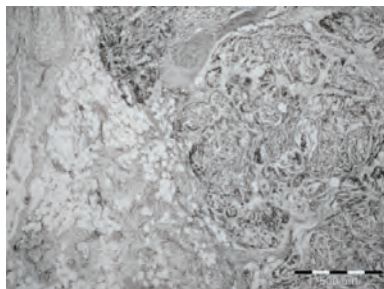


**Fig. 3:** Cocker, age: 14, F.  
Lateral-lateral radiography, multiple  
of areas of pulmonary radioabsorption

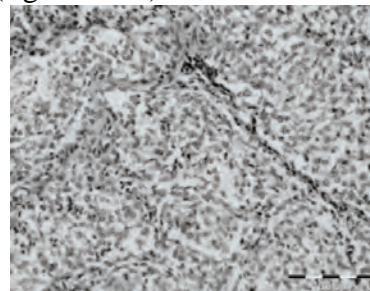


**Fig. 4:** Cocker, age: 14, F.  
Necropsic examination, presence  
multiple pulmonary metastases

All the studied cases have been diagnosed with different morphological types of carcinoma. Out of the studied tumors, one had osteo-cartilaginous component, being included in the diagnosis of carcinoma on a frame of benign mixed tumor. The analysis of the metastases process has revealed that 3 of the investigated carcinoma have developed lymphonodal metastases (2 cases had popliteal adenopathy, and only one case had axillary adenopathy) (fig. 5 and 6).



**Fig. 5:** Cocker, age: 14, F.  
Mammary carcinoma, compact type -  
malignant mammary tumor,  
characterized by the arrangement  
of cells in the form , or compact  
agglomerations ( Ob.  $\times 4$  ).



**Fig. 6:** Cocker, age: 14, F.  
Pulmonary metastasis —mammary  
carcinoma, compact type, malignant  
epithelial cells with anisocariosis  
and anisocytosis (Ob.  $\times 20$ ).

### 3. CONCLUSIONS

3.1. The breed with the highest prevalence concerning the pulmonary metastases was the Caniche, with a percentage of 25%.

3.2. The pulmonary radiographic examination of 41 bitches with mammary tumors has highlighted the presence of the pulmonary metastases in 9 of them, which represents 21.95%.

3.3. The clinical signs of the sick animals was dominated by cough, dyspnoea and lymphadenopathy.

3.4. The pulmonary methastases lesions have been diagnosed at animals older than 10 years of age, the multiple pulmonary metastases are predominant.

3.5. In the analysed cases, different morphological types of carcinoma have been diagnosed.

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## **RELATIONSHIPS BETWEEN THE EVOLUTION OF PLASMATIC CORTISOL LEVELS AND SOME BLOOD MORPHOLOGICAL PARAMETERS IN PREGNANT DOE, FROM MATING TO DELIVERY**

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**Key words:** cortisol level, blood parameters, pregnant doe.

### **SUMMARY**

Blood morphological and biochemical parameters were monitored in pregnant doe vs no pregnant. In the same time the evolution of cortisol level was monitored. Monitoring stretched between the mating and delivery. Cortisol level was found higher in pregnant does beginning from the day 11<sup>th</sup> of pregnancy and then the level rose constantly along the pregnancy period up to the 28 day of pregnancy when a peak was found. The maximal values of blood cortisol level were found in the day before delivery. Some blood parameters followed the serum cortisol levels modifications, among them increased WBC and RBC, lymphopenia and granulocytosis.

Despite of a large number of researches, the particularities concerning the evolution of the cortisol level along pregnancy in different species and of the relationships between the cortisol level evolution and main metabolic processes and/or blood parameters show enough unknowns (Dury, 1956; Hennessy *et al.*, 1982; Ikegami *et al.*, 1996). This works analyses the evolution of the cortisol level in pregnant domestic rabbits and the relationships between the cortisol level and some blood morphological parameters.

### **1. MATERIAL AND METHODS**

One group of six month age ten does was constitutes from animals in heat physiological state (estrous period). The does were inseminated in the same day by natural mating. A control group was constituted from ten does of the same age, but in diestrous physiological state. The two groups of does was fed *ad libitum* and monitored from health and obstetrical points of view. Experimental monitoring stretched form the day of mating to the day before

delivery. Blood was sampled three or four days intervals from the beginning of the monitoring to the day before delivery. The samples were stored at 2 — 4°C up to the processing moments, but no more than 24 hours from the moment of sampling.

There were monitored white blood cell number (WBC), red blood cell number (RBC), platelets number, hemoglobin, hematocrit percent, neutrophil, lymphocyte, eosinophil and basophil percents. The number of erythrocytes and leucocytes was determined using capillary blood samples, according to Manta *et al.*, 1976. Plasma cortisol level was determined using an immunoassay method. The data were statistically processed and expressed as mean  $\pm$  standard error of mean ( $X \pm s_x$ ). The differences between pregnant and no pregnant doe groups were characterized using the Student *t* test, according to Tacu, 1968.

## 2. RESULTS AND DISCUSSION

Table 1 and Table 2 show the evolution of the cortisol level in the two groups of does: pregnant and no pregnant (control). In the group of no pregnant does, the level of cortisol oscillated between 5.15 ng/mL (minimal value) and 5.73ng/mL (maximal value) while in the group of pregnant does the plasmatic level of cortisol oscillated between 5.60 ng/mL of plasma (the day of mating) and 8.48 ng/mL of plasma (the day before delivery). Maximal differences between the two groups ( $P < 0.01$ ) were found in the 30<sup>th</sup> day of pregnancy, when the cortisol level in the group of non pregnant does was  $5.60 \pm 1.54$  ng/mL and the cortisol level in the group of pregnant does was  $8.48 \pm 2.87$  mg/mL of plasma (fig. 1). The higher level of cortisol before delivery could be due to an increase secretion by the mother adrenal glands as an anticipation of the delivery stress, but in the same time to a double secretion, by the fetal adrenal glands (Hennessy *et al.*, 1982).

According to some data, pregnancies are characterized by increased maternal cortisol during this period were more likely to result in spontaneous abortion. This evidence links increased levels in this stress marker with a higher risk of early pregnancy loss in humans (Nepomnaschy *et al.*, 2006; Reisman and Matheny, 1968).

Wood (1991, 1998) showed that cortisol rise during pregnancy is connected with fetal maturation. Together with the increase of insulin

resistance it might have a role in allocation of nutrients between mother and fetus. Cortisol and insulin secretion during pregnancy seems to be induced by fluctuations in basal metabolism rate. The rise of maternal serum cortisol and insulin suggests the activation of hypothalamo-pituitary–adrenal axis (Wood, 1991, 1998). The same authors showed that in both, ovine and human pregnancy, maternal plasma cortisol concentrations increase. Neither the mechanism nor the physiological significance of this chronic increase in maternal plasma cortisol concentration is enough understood (Wood, 1991).

*Table 1*

The evolution of the plasmatic levels of cortisol in six barren does, six months aged, as a control group for 30 days between 02 September 2009 and 01 Oct. 2009

No.	Identity number	Timetable of blood sampling / the obtained values (in ng/mL of plasma)							
		02 Sept.	07 Sept.	11 Sept.	15 Sept.	21 Sept.	25 Sept.	28 Sept.	01 Oct.
1	No mark	5.32	5.45	6.65	6.34	5.44	6.89	7.56	8.51
2	No mark	4.44	5.87	6.45	6.66	7.45	7.87	7.31	7.98
3	593	6.21	6.07	6.55	7.54	6.50	7.77	7.86	8.54
4	574	6.89	6.65	6.55	6.34	6.43	7.07	7.00	8.54
5	389	4.39	4.76	6.44	4.50	5.00	5.32	5.11	7.82
6	504	5.94	5.89	6.90	5.43	6.02	6.30	7.20	8.44
7	439	6.04	6.56	6.45	6.78	7.02	7.43	7.32	7.89
8	$\bar{X} \pm s_{\bar{x}}$	5.60± 1.43	5.89± 2.21	6.52± 1.87	6.22± 1.56	6.26± 3.32	6.95 ±2.75	7.05± 2.43	8.48± 2.87
9	Min. value	4.39	4.76	4.44	4.5	5.00	5.32	5.11	6.32
10	Max. value	6.89	6.65	7.00	7.54	7.45	7.87	7.86	8.54

*Table 2*

The evolution of the plasmatic levels of cortisone in seven pregnant does, six months aged, as an experimental group for 30 days between 05 September 2009 (day of mating) and 01 October 2009 (day before delivery)

No.	Identity number	Timetable of blood sampling / the obtained values (in ng/mL of plasma)							
		02 Sept.	07 Sept.	11 Sept.	15 Sept.	21 Sept.	25 Sept.	28 Sept.	01 Oct.
1	439	5.43	5.35	6.34	5.50	5.34	4.89	5.21	5.40
2	464	5.40	6.34	6.66	6.54	6.06	6.35	6.50	6.32
3	382	5.24	5.54	5.48	5.40	4.98	4.67	4.40	4.90
4	265	4.68	4.65	5.55	5.45	5.65	5.90	5.32	5.40
5	390	3.89	4.30	3.76	3.50	4.02	5.03	4.30	5.30

6	432	6.29	6.43	6.60	6.32	6.34	5.98	6.05	6.30
7	$\bar{X} \pm s_{\bar{x}}$	5.15± 2.01	5.43 ±2.2 2	5.73± 1.54	5.45± 1.76	5.39± 2.43	5.47± 1.79	5.29± 1.60*	5.60±1. 54**
8	Min. values	3.89	4.3	3.76	3.5	4.02	4.67	4.3	4.9
9	Max. values	6.29	6.43	6.66	6.54	6.34	6.35	6.5	6.32

\*P<0.05

\*\*P<0.01 (statistically processed by comparing to the control doe in the same day of blood sampling).

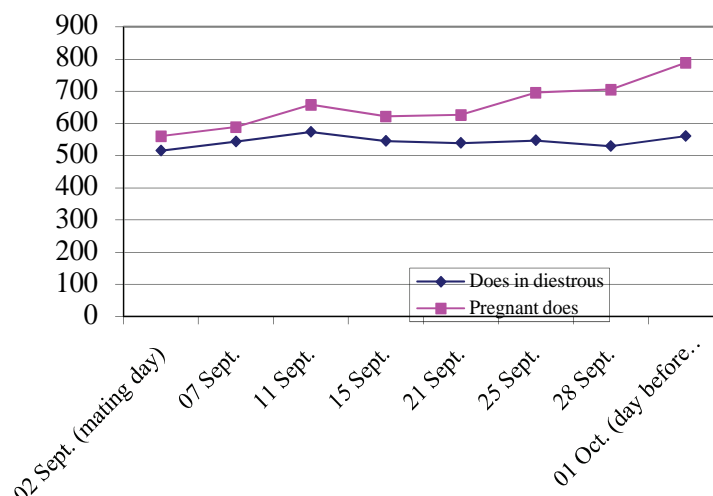


Fig. 1. The evolution of the plasmatic level of cortisol in blood of pregnant does from mating to delivery vs nonpregnant does

Table 3 presents the evolution of the main blood morphological parameters in the group of pregnant does along of the 30 days of pregnancy vs in diestrous non pregnant does, in the same period. According to data of table 3 it was found an increase of the erythrocytes number, from 4.65 to 5.90x10<sup>6</sup>/mmc of blood, while in the control non pregnant does the red blood cells number remained relatively constant during the same period of time. Parallel, the number of leukocytes registered similarly evolution: an increase from 9.7 to 12.6x10<sup>3</sup>/mmc of blood, while in control does the evolution of the number of leukocytes remained relatively unmodified, like in the case of erythrocytes.

Hematocrit percent had an increase in the last week of pregnancy, when it increased from 40.0±22.9% to 43.0±15.4% in the day before delivery.

Table 3

The evolution of some blood morphological parameters in pregnant does vs no pregnant does along the pregnancy

Item	Normal values		The data of blood sampling/values					
			02 Sept.	09 Sept.	15 Sept.	21 Sept.	25 Sept.	01 Oct.
Leucocytes - $10^3/\text{mm}^3$	6-10*	C	8.5±0.4	8.7±1.9	9.1±0.9	8.4±3.3	8.8±2.9	8.3±2.7
	6-13** 6-12***	P	9.7±2.1	9.9±3.2	12.1±4.4	10.2±3.3	12.4±4.2	12.6±1.9
Erythrocytes ( $10^6/\text{mmc}$ )	5.8*	C	4.6±2.1	3.8±2.0	4.0±0.6	4.3±1.5	4.9±1.3	4.6±1.4
	4-6***	P	4.65±1.45	4.44±1.65	5.61±2.21	5.55±2.87	5.43±2.90	5.90±3.71
Hemoglobin (g/dL)	12***	C	10.4±2.1	10.0±3.9	9.6±3.0	9.9±1.9	9.5±2.0	10.1±1.9
		P	8.9±1.3	8.7±3.1	8.0±3.0	8.6±4.6	8.9±2.0	9.0±2.5
Hematocrit (%)	41.5***	C	42.1±8.9	40.4±9.6	39.9±6.5	42.1±11.7	40.8±12.6	40.7±9.0
		P	39.4±12.4	30.8±20.0	40.4±19.0	40.0±22.9	41.4±18.5	43.0±15.4
Platelets — ( $10^3/\text{mm}^3$ )	125-250*	C	411.0±61.0	394.3±95.9	366.5±110.5	390.0±209.5	420.5±142.7	411.9±94.0
		P	396.8±187.5	432.2±211.9	454.7±169.0	440.3±252.2	390.6±94.8	412.9±175.5
Lymphocytes (%)	39* 40-60** 35-45***	C	56.8±21.5	52.1±9.4	50.4±22.1	52.9±19.5	49.5±20.5	50.6±10.0
		P	56.6±21.9	54.5±32.0	48.0±25.0	43.0±19.5	46.9±15.9	38.2±17.7
Monocytes (%)	4-12* 2-6**	C	3.5±0.6	5.3±1.1	3.3±2.0	3.5±2.1	2.9±1.9	6.4±0.9
		P	2.2±0.5	3.2±0.9	4.2±1.7	4.4±1.0	5.3±2.6	5.0±2.9
Neutrophils (%)	40* 35-55** 45-55***	C	40.4±8.8	42.0±12.0	39.1±8.5	40.9±8.5	39.0±12.0	41.9±4.9
		P	39.0±9.8	38.6±9.5	43.5±16.5	47.2±21.0	43.9±19.4	53.8±23.0
Eosinophils	4* 1-3**	C	3.0±1.1	3.3±1.4	4.0±2.0	3.9±1.5	3.2±1.0	3.6±1.6
		P	4.1±0.8	4.0±1.9	3.2±0.7	3.0±1.7	2.9±1.1	2.5±0.8
Basophils	2-7* 1-5***	C	2.7±0.4	1.6±0.5	1.5±1.0	2.3±1.2	2.7±1.3	1.9±0.4
		P	0.0±0.0	1.7±1.3	1.9±1.6	2.0±1.5	1.0±0.7	0.5±0.4

Legend:

C = control does

P = pregnant does

Results are expressed as  $X \pm s_x$  = mean ± standard error of mean

\* Normal values in adult rabbit according to Pintea et al, 1982.

\*\* Normal values in rabbit according to Reece, 1996.

\*\*\* Normal values in adult rabbit according to Kolb, 1974.

Leukocyte formula (percentage distribution of leukocytes) presented a lymphocytopenia in the group of pregnant does, more elevated in the last week of pregnancy, when registered a value of 38.2±17.7%, compared to 56.6±21.9%, the value of the day before mating. On the other hand, the



percent of neutrophils increased along the same period in the group of the pregnant does from  $39.0 \pm 9.8\%$  to  $53.8 \pm 23.0\%$  while in the control group no significant modifications were found.

High number of RBC and WBC and limphopenia were reported in high cortisol level syndrome, such in Cushing syndrom (Dury, 1956; Hennessy *et al.*, 1982). A strong correlation was revealed between variations in cortisol concentration and leukocyte count by some authors, such as Tolchanov *et al.*, 2005. They found differences in the hormonal regulation of individual cell populations in the peripheral blood.

### 3. CONCLUSIONS

Our results on the relationships between the evolution of the cortisol level and some blood morphological parameters in pregnant does from mating to delivery followed, in main, the same schema which is found in other species. There is the increase of cortisol level along the pregnancy in does, with a pick at the end of the period. This increase is followed by significant modifications of some morphological blood parameters, among them increased WBC and RBC, lymphopenia and granulocytosis.

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**PROJECT HRDSOP 86/1.2./S/63654 “HIGHER EDUCATION AT  
EUROPEAN LEVEL IN THE FIELD OF VETERINARY MEDICINE”**

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**SUMMARY**

Paper presents the progress of the project HIGHER EDUCATION AT EUROPEAN LEVEL IN THE FIELD OF VETERINARY MEDICINE, co-financed by European Social Fund through the Human Resources Development Sectoral Operational Programme 2007-2013, implemented by University of Agronomical Sciences and Veterinary Medicine Bucharest, University of Agricultural Sciences and Veterinary Medicine “Ion Ionescu de la Brad” Iasi, University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, University of Agricultural Sciences and Veterinary Medicine Timisoara and Shotron Association.

The project aims to increase the relevance and compatibility of undergraduate curricula in relation to labor market demands and to the changes induced by the knowledge society through developing new curricula for a number of 2500 students and improving the internal quality assurance in higher education institutions of veterinary medicine at the sectorial level. Relevant information about the project can be found at the address: [www.eduvet.fmvb.ro](http://www.eduvet.fmvb.ro).

The initial higher education of human resources is the precondition for performing a competitive labor market. A highly skilled workforce is essential to a knowledge-based competitive and sustainable economy. (National Development Programme 2007 — 2013, Lisabona Strategy, National Reference Strategic Framework 2007 — 2013, Study regarding the implementation of Bologna higher education process 2009). The objectives of veterinary medical education institutions are the provision of adequate veterinary training, ethics and science-based, which allows graduates to practice veterinary profession in all recognized areas of veterinary medicine.

The specific objectives are:

□ develop / implement a modern curriculum, which meets the CNCSIS methodology/ European requirements in veterinary care and a system of e-

learning - to modernize the teaching learning process for 2500 students enrolled in veterinary medical education system;

- flexible learning opportunities to the students in the undergraduate program of study by developing an online learning community to provide resources and materials to those interested in innovative digital veterinary medicine;

- develop and implement an integrated quality management system to support quality assurance at institutional level, as required by EU veterinary medical education system;( Standards and Coordinates for Quality Insurance in the European Higher Education Area; Standards of Quality Assurance in Higher Education, Helsinki, 2005)

- strengthen professional skills of 80 people, teaching staff involved in developing modern curricula and adapted to European veterinary medical education system;

- strengthen professional skills of 20 people, teaching staff involved in quality assurance at higher education institution in the field of veterinary medicine through peer learning activities with colleagues from other universities in the field.

## **1.MATERIAL AND METHODS**

Project aims to improve the extension of e-learning curriculum offers to allow a better preparation of students through access to virtual information resources and interactive communication, and flexible learning opportunities and to attract a more diverse student population, the promotion of best practices both among students and between staff involved in the development of university curricula and digital resources by establishing a virtual community that allows saving time and resources allocated for initial training; the strengthening of the university autonomy, coupled with the introduction of a national quality assurance system, assessing both external and internal, as well as measures of institutional quality management - prerequisite for professional employment and improve European competitiveness in the field of veterinary medicine.

A team of very experienced experts from the fourth veterinary faculties wrote new modular curricula in relation to labor market demands and to the changes induced by the knowledge society. The new curricula is structured in four modules:

- Veterinary medical deontology and professional ethics
- Development of professional abilities and career guidance
- Quality Management of the veterinary medical act
- Quality Management in veterinary medical higher education

These four modules were introduced in the students' mandatory curricula and they were transformed in digital course and uploaded in the e-learning informatics system. The informatics system will include working tools and digital materials, which will allow creating a flexible and permissive academic environment, which promotes student centred learning. Also, the computer system allows access to a library and a virtual community, which hosts resources, communication facilities and flexible learning in order to form key competencies needed to provide a quality education in higher education.

## **2. RESULTS AND DISCUSSION**

- 80 university teachers from the fourth universities partners in the projects, participants at the course “ Improvement of didactic skills in elaborating and developing modern curricula, according to the European requests in the field of veterinary medicine”

- 20 members of the quality commissions from the fourth universities partners in the projects, participants in the peer —learning activities for the improvement of the quality management systems at university level.

- A new curricula implemented in the fourth universities partners in the projects

- 1 workshop “Best practices exchange between universities. Effective methods of dialogue and partnership for the academic performance supported by European Social Fund”

- 1 seminary “ European higher education for a sustainable future in the field of veterinary medicine”

## **4. CONCLUSIONS**

3.1. e-learning curriculum will allow a better preparation of students through access to virtual information resources and interactive

communication, and flexible learning opportunities and to attract a more diverse student population

3.2. The informatics quality management system will strengthen the university autonomy, coupled with the introduction of a national quality assurance system, assessing both external and internal, as well as measures of institutional quality management - prerequisite for professional employment and improve European competitiveness in the field of veterinary medicine.

3.3. The course “Improvement of didactic skills in elaborating and developing modern curricula, according to the European requests in the field of veterinary medicine” will strengthen the professional skills of personnel involved in the development of university curricula, the development of modern curricula and tailored European veterinary medical education system using digital resources.

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The content of this paper does not necessarily represent the official position of European Union or the Romanian Government.

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