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SUMMARY

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THERAPEUTIC EFFICACY AND SAFETY EVALUATION OF ERYTHROCYTE CONCENTRATE USED IN DOGS

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Abstract

We studied the clinical and hematological changes of 18 dogs from admission day (T0) until 5 days post-transfusion (5 days after the last administered transfusion), as well as hematocrit changes 6 hours post-transfusion therapy with erythrocyte concentrate. This research took place in a period of 6 months in 2014 in a small animal clinic from Germany. Most of the patients have been diagnosed with immune-mediated hemolytic anemia (n=11), 2 with rodenticide poisoning, 1 with babesiosis, 1 with hemangiosarcoma, 1 with septic peritonitis, 1 with idiopathic hepathopathy and 1 with hypothyroidism. The 18 patients received a total of 30 transfusions with erythrocyte concentrate in a mean dose of 11.46 ml/kg. Of the 30 transfusions, 6 reached the calculated hematocrit rise 6 hours post-transfusion, 2 had a higher than expected value and 22 did not reach the expected value. The hematocrit value 6 hours post-transfusion was statistically extremely significant (p<0.0001). We have observed positive changes in all hematological parameters 5 days after the transfusion therapy of which 3 have been statistically significant. The red blood cell count underwent a statistically very significant (p=0.0052) change, as has the hemoglobin level (p=0.0085). The hematocrit level had a statistically extremely significant change (p=0.0002) from the admission day until day 5 post-transfusion.

Key words: *dogs, erythrocyte concentrate, hematocrit, immune-mediated hemolytic anemia, transfusion therapy.*

INTRODUCTION

The purpose of this study has been the evaluation of the immediate and long term therapeutical efficacy of erythrocyte concentrate in dogs (Kisielewicz et al 2014) with different types of anemia. These objectives have been pursued by measuring the hematocrit level 6 hours after the transfusion therapy has been discontinued and by performing a complete blood count 5 days post-transfusion therapy. We also clinically monitored the patients for any signs of transfusion related adverse reactions.

MATERIALS AND METHODS

This research took place in the Small Animal Clinic of Internal Medicine Department of Justus-Liebing University in Giessen, Germany. The study lasted for 6 months, between February and August of 2014. During this time 18 dogs received transfusion therapy with erythrocyte concentrate. From this patient pool 44.44% (*n=8*) were females and 55.55% (*n=10*) males. Mean age of the patients has been 6.5 years, with the youngest patient being 1 year old and the oldest 11 years old. The patients have been of quite varied breeds: 3 common breeds, 2 Cane Corso Italiano, 2 Labrador Retrievers, 1 Border Collie, 1 Shetland Shepherd, 1 Bearded Collie, 1 Miniature Pinscher, 1 Spitz, 1 Havanese Bichon, 1 Australian Shepherd, 1 Doberman Pinscher, 1 Dachshund, 1 Cocker Spaniel and 1 Belgian Shepherd.

Blood tests have been made with ADIVA hematological analyzer; the 6 hour post-transfusion hematocrit has been determined by performing a micro hematocrit.

Every patient taken into this study has been transferred to the clinics Intensive Care Unit where they were permanently monitored.

On admission every patient received a routine blood test that included 40 hematological parameters and 21 biochemical parameters. In addition, a detailed examination of the blood smears has been also performed by the ADIVA hematological analyzer with 26 parameters that mostly referred to red blood cell and white blood cell morphology.

All erythrocyte concentrate units have been prepared in the clinic, with the help of a special centrifuge designed for blood bags and a plasma separator. This way the obtained erythrocyte concentrate units have been quite similar, with a hematocrit level of about 70%. All units have been stored in a refrigerator used only for blood product storage that has been monitored daily by a technician for the adequate temperature. None of the blood units have been stored for longer than 6 days.

Every patient has been blood typed for DEA 1.1. blood type, using the RapidVet quick test kit. Dogs received only type specific blood and to limit transfusion reaction occurrences, in addition, a crossmatch test has been performed before every transfusion. This crossmatch test served another purpose as well, besides verifying patient-donor compatibility; a positive auto-agglutination test provided an additional proof for the immune-mediated hemolytic anemia diagnosis. The 18 dogs taken into this study received a total of 30 blood transfusions (a *mean of 1.66*). The most transfusions given to one patient has been 4, received in the first 5 days after admission.

The causes of anemia encountered in this patient group has not been very versatile, mostly because the study has been made in a reference clinic where the most difficult to diagnose and to treat patient are being sent and attended. Most patients (61.11%; *no=11*) have been diagnosed with idiopathic immune-mediated hemolytic anemia. Of these 11 patients only in one could the immune-mediated anemia be linked later to a lymphoma. The rest of the group there have been 2 patients (11.11%) diagnosed with rodenticide poisoning, 1 (5.55%) with babesiosis, 1 with a hemangyosarcoma, 1 with adenocarcinoma and septic peritonitis due to complications that occurred after surgery, 1 idiopathic hepatopathy and 1 with hypothyroidism.

Mean dose of administered erythrocyte concentrate has been 11.46 ml/kg, with a minimal dose of 3.4 ml/kg and a maximum dose of 24.7 ml/kg. Mean transfusion rate has been 21.86 ml/kg/h, with a minimum speed of 6ml/kg/h and a maximum speed of 45 ml/kg/h.

Research concerning the long term therapeutically efficacy (5 days post-transfusion) of erythrocyte concentrate used in transfusion therapy has been conducted upon 13 of the 18 initial patients that were used in the starting phases of the study. No data has been available for 4 of these 5 patients that were excluded from the second phase of the study because they have been released from the clinic in less than 5 days.

The 5th patient suffered from an extremely severe form of immune-mediated hemolytic anemia and needed 4 transfusions in the course of 5 days. Among the patients that have been included into the long term therapeutical efficacy study of erythrocyte concentrate 3 have received 2 transfusions in less than 24 hours apart and the other patients received one transfusion each.

Statistical analysis of the transfusion therapy efficacy has been performed with GraphPadInStat 3.0 statistical program and the graphical depiction in form of a box-plot of the obtained results has been made using the Origin8.5. graphics program.

RESULTS AND DISSCUSIONS

In the present study we have followed-up the evolution of the total white blood cell level, the neutrophil level, lymphocyte numbers, the total red blood cell count, the hematocrit level, platelet number, the total number of reticulocytes and the spherocyte percentage from the day of admission (T0) up to the 5th day (T5) after the last administered transfusion therapy with erythrocyte concentrate. In the case of those patients that needed multiple transfusions, T5 has been considered the 5th day after the last administered transfusion therapy.

The decision of starting a transfusion therapy has been made by the attending physician of each patient. Administered doses had been given according to the results obtained from the following equation:

Transfused volume = (desired Ht-patient Ht/donor Ht) x kg b.w x 90

This equation has been chosen according to the results presented in recent studies in this field conducted upon the efficacy of various equations used to determine the ideal transfusion dose for dogs (Short et al, 2012).

The desired hematocrit level that the patient should have reached after the transfusion therapy has been set by the attending physician of each patient. Alongside the transfusion therapy, the patients have been treated according to the their pathology, but none received any kind of intravenous or other type of treatment as long as the transfusion therapy has been administered.

Of the 18 patients that have been monitored for the hematocrit level changes at 6 hours post-transfusion therapy, only in one we have observed transfusion related adverse reactions represented by vomiting, pyrexia, melena, hemoglobinuria and hemoglobinemie. This patient suffered from a very severe form of immune-mediated hemolytic anemia and presented transfusion related adverse reactions after every transfusion therapy.

There have been administered a total of 30 transfusions to the 18 patients taken into the study of the immediate therapeutic efficacy of erythrocyte concentrate in dogs.

Of these 30 transfusions 6 (20%) reached exactly the desired hematocrit level at 6 hours post-transfusion, used in the above presented equation. In 2 occasions (6.66%), both transfusion given to patients with immune-mediated hemolytic anemia, we have observed a higher than expected level of the hematocrit (of 6% and 12%, respectively) at 6 hours after transfusion. The other 22 administered transfusion therapies (73.33%) did not produce the calculated augmentation upon the hematocrit level at 6 hours after transfusion. Besides the 4 transfusions that have been administered to the patient with the very severe form of immune-mediated hemolytic anemia, none could have been related to a potential transfusion related adverse reaction. We did not find any proof of a potential intravascular or extravascular hemolytic reaction in any other patient. Patient-donor compatibility of the administered blood product has been determined by blood typing of both individuals and a crossmatch test performed before every transfusion therapy. The mean difference observed between the desired hematocrit level used in the equation and the actual hematocrit level reached after 6 hours post-transfusion therapy has been 3.59%, with a minimal difference of 2% and a maximum difference of 12%.

The statistical analysis revealed an extremely significant ($p<0.0001$) rise of the hematocrit level changes observed at 6 hours after transfusion. Of the initial 18 patients we could use 13 patients for our study of the long term therapeutic efficacy of erythrocyte concentrate in dogs. Each of these 13 patients survived until discharge.

This study focused upon hematological changes observed between admission day (T0) and the 5^{th} day after the transfusion therapy with erythrocyte concentrate has been discontinued (T5). Table 1 depicts the hematological parameters on admission day that we followed through this study. We have observed major changes in the evolution of every hematological parameter that we followed. The statistical analysis conducted on the obtained results reveal that the total red blood cell count underwent very significant changes ($p=0.0052$); the hemoglobin suffered as well a very significant change ($p=0.0085$). Of all the hematological parameters that we studied, the one with the most significant evolution has been the hematocrit. This suffered an extremely significant ($p=0.0002$) statistical evolution. As far as the other hematological parameters are concerned, none underwent statistically significant evolutions from T0 until T5. After they have been discharged all patients have had outpatient treatments continued at home. In addition to the outpatient treatments, all 13 patients have been called in weekly for the first month after discharge for follow-up examinations and blood tests performed by the same veterinary physician that they were assigned to on admission day. Patients, who could not come to the clinic for the scheduled follow-up examinations, performed the necessary check-ups and blood tests at a local veterinarian office that kept in touch and consulted the next treatment steps with the cases initial physician from the clinic.

According to the patient's evolution, these follow-up examinations developed from weekly to monthly visits. Every case has been followed through until the treatment could be discontinued.

From these periodically performed not only clinical but hematological and biochemical reexaminations, we have learned that none of

the patients included in this study manifested any delayed transfusion related reactions.

Only one of the 18 initial patients suffered a relapse of a severe immune-mediated hemolytic anemia and came back to the clinic in a critical state.

The owners refused treatment and the patient has been consequently euthanized.

Transfusion therapy using erythrocyte concentrate has been proven to be well tolerated by dogs. The safety of this blood product in therapy, even in dogs in critical state has been demonstrated by the few transfusion related adverse reactions we have observed that have been in fact induced by the patients primary pathological process.

Table.1 Hematological parametres on admission day (T0)

Nr	WBC 10^9 /l	N 10^9/l	L 10^9 /l	PLT 10^9 /l	RBC 10^{12}/l	Ht l/l	Hb mmol/l	Reti 10^9/l	Spher %
1.	42.0	7.25	29.67	958	1.33	0.14	3.4	668.5	3
2.	17.08	10.90	4.82	183	1.80	0.14	3.9	82.90	2
3.	73.04	38.53	26.75	359	1.25	0.13	3.2	605.5	2
4.	26.59	18.28	6.51	563	1.57	0.18	2.8	530.2	3
5.	18.03	11.03	1.98	573	4.99	0.26	8.3	156.7	1
6.	8.40	5.57	2.19	16	3.48	0.23	4.7	5.10	3
7.	34.63	26.82	5.32	75	2.11	0.15	3.0	231.1	2
8.	19.53	9.53	8.19	174	1.35	0.10	2.1	85.70	3
9.	17.24	14.38	1.14	70	2.52	0.15	5.2	126.3	2
10.	12.96	7.91	3.51	185	1.96	0.15	4.7	79.2	3
11.	6.44	2.65	3.19	440	1.85	0.12	2.8	10.0	0
12.	8.62	6.48	1.40	81	2.22	0.17	2.9	438.3	1
13.	32.3	19.92	6.38	156	2.03	0.15	3.1	171.1	2
Mean	24.37	13.7	7.77	294.8	2.18	0.15	3.85	245.4	2.1
StD	18.2	9.98	9.33	273.1	1.02	0.04	1.61	232.2	0.95
Min.	6.44	2.65	1.14	16.0	1.25	0.10	2.1	5.1	0
Max.	73.04	38.53	29.67	958	4.99	0.13	8.3	668.5	3
Ref. val.	6.0 -17.0	2.78 -8.73	0.72 –4.71	150 – 500	5.5 – 8.5	0.39 – 0.56	8.06-12.21	0 – 60	<4%

Table.2 Hematological parameters on the 5th day post-transfusion

Nr	WBC 10^9/l	N 10^9/l	L 10^9/l	PLT 10^9/l	RBC 10^{12}/l	Ht l/l	Hb mmol/l	Reti 10^9/l	Spher %
1.	19.59	7.61	10.30	901	3.81	0.34	6.3	724.4	10
2.	35.68	27.48	4.39	18.51	2.37	0.18	5.5	206.50	0
3.	43.9	26.43	12.21	538	3.17	0.29	4.8	974.5	20
4.	30.33	23.53	3.06	905	2.69	0.26	4.5	440.7	3
5.	29.86	27.57	1.17	832	4.26	0.25	4.8	53.5	1
6.	10.22	7.34	2.34	19	4.41	0.30	6.2	7.10	1
7.	10.56	6.92	2.90	129	7.00	0.50	10.1	507.10	10
8.	10.90	3.45	6.54	292	4.01	0.29	5.5	363.90	5
9.	12.02	8.45	2.37	136	4.60	0.33	6.3	227.80	4
10.	10.05	7.75	1.69	464	3.08	0.25	4.4	108.0	7
11.	7.53	3.93	2.80	301	3.39	0.24	4.8	13.70	0
12.	2.76	1.18	1.02	207	3.47	0.26	4.8	42.20	0
13.	76.95	39.11	26.86	110	1.18	0.12	4.9	275.70	7
Mean	23.1	14.67	5.97	373.2	3.64	0.27	5.6	303.4	5.7
StD	20.4	12.3	7.17	327.0	1.37	0.08	1.5	295.5	5.23
Min.	2.76	1.18	1.02	18.51	1.18	0.12	4.4	7.1	0
Max.	76.95	39.11	26.86	905	7.00	0.22	10.1	974.5	20
Ref. val.	6.0 -17.0	2.78 - 8.73	0.72 – 4.71	150 – 500	5.5 – 8.5	0.39 – 0.56	8.06-12.21	0 – 60	<4%

Research conducted upon the hematocrit level changes pre- and 6 hours post-transfusion, revealed a small percentage of cases in which the desired hematocrit rise has been achieved using the blood product dose obtained using the equation. Our study showed a mean difference between the desired hematocrit level used in the equation and the obtained one at 6 hours after transfusion to be 3.59%. This small difference still allows us to stipulate the efficacy of the transfusion therapy. However, a revision of the used equation is needed since in most of the administered transfusions there has been an absence of any transfusion related adverse reactions, but the desires hematocrit level has not been achieved. Also an important fact that must be taken into account is that the transfusion treatment has been initiated shortly after the patient has been admitted. Most of the patients that have been taken into this study were diagnosed with an immune –mediated pathology in which the body destroys its own red blood cells. The treatment for the immune-mediated hemolytic anemia has been started in the same day as has the transfusion therapy. The failure to achieve the calculated heamtocrit rise at 6 hours post-transfusion involves the pathological process from which suffered the patients as well. Taking into account the hematocrit level evolution from the day of admission and 5 days after the transfusion therapy has been discontinued, it can be stated that this therapy has reached the desired effect. In the 5th day after transfusion a rise in the hematocrit level can be observed (Fig.1.).

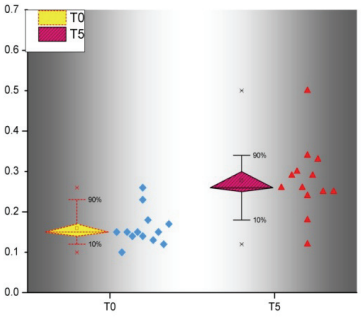

Figure 1. Hematocrit level evolution between day of admission and the 5th day post-transfusion

The same positive effect can be seen in the total number of red blood cells. We could observe a rise of this parameter in each of the 13 patients from the study (Fig. 2.).

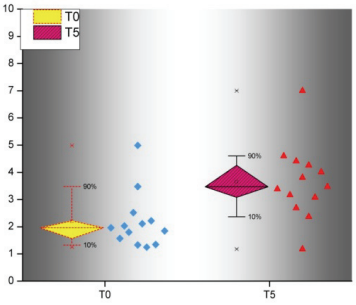

Figure 2. Red blood cell count from admission day and the 5th day post-transfusion

Similar data was found by Gibson and his collaborators (2007), as well as Helm and his collaborators (2010), which made some indications on dosage and good practice protocols following their research. Also Ognean and collaborators (2015) have reached similar conclusions in a study focused on transfused dogsNext to the hematocrit level and the red blood cell count, hemoglobin is the third hematological parameter used to evaluate the severity of the anemia and bloods oxygen carrying capacity (Callan et al, 2010).
Hemoglobin levels have risen in every patient taken into study in the 5 days after the transfusion therapy has been discontinued (Fig.3).

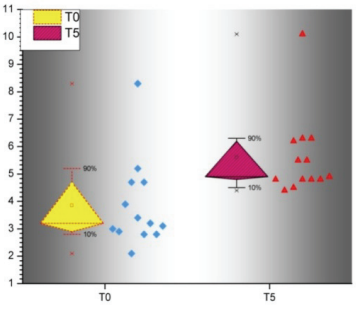

Figure 3. Hemoglobin amount evolution from day of admission and the 5th day post-transfusion

The total number of white blood cells, neutrophils and lymphocytes, has been mostly influenced by the patient's pathology.

The total number of platelets has had a rising tendency between the day of admission and the 5th day post-transfusion, their value being outside of the physiological reference range in only 4 of the patients in the 5th day after transfusion.

The majority of the 13 patients taken into the long term efficacy study of erythrocyte concentrate have reached a point in the 5th day after transfusion therapy in which they were no longer considered in danger of tissue hypoxia. Two patients remained with low red blood cell number and hematocrit level, but a rise of the hematological parameters could be seen anyway in the 5th day post-transfusion comparing to admission day.

Some of the studied values have been increased and influenced by the body's own compensatory mechanisms as well that involve an increased production and release of reticulocytes. These are precursor cells that the body produces in a much faster rate and greater amount to compensate the lack of red blood cells in case of an anemia caused of no matter what. This response of the bone-marrow can be seen in 3 to 5 days after clinical signs of anemia are visible. A greater number of reticulocytes have been observed on admission in comparison to the 5th day after transfusion therapy in 12 of the 13 patients that took part of the study. Production of these cells decreases only if the patient is no longer anemic or if the cell production capacity of the bone-marrow has been suppressed or compromised.

CONCLUSIONS

The erythrocyte concentrate can be used safely even in critically ill patients, immune-suppressed, or in case of an exaggerated immune response.

A clear dosage of this blood product has not been set yet; every administration has to be tailored to the patients needs. The equation used to calculate administered dose, failed to give an amount that would get the desired effect. However, it must be taken into account that most patients in this study have been diagnosed with immune-mediated hemolytic anemia that could have contributed to the failure of reaching the calculated hematocrit level.

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HISTOSTRUCTURAL APPRECIATION OF THE FORESTOMACH FIRST COMPARTMENT MUCOSA IN SHEEP

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Abstract

The mucosa of the ruminal wall was analyzed and measured in the different areas. First involved in this study was the ventral sac mucosa, and after were the pillars' region and the intermediary area between the reticulum and the rumen. Sheep from the white variety of the indigenous ovine breed Țurcană (Ovis aries) were used, the pieces of interest being collected and processed using conventional histological techniques, obtaining numerous seriated slides. After they were photographed and analyzed, we have been able to identify in the structure of the mucosa a cornified stratified squamous epithelium, lamina propria, and a densification of connective fibers. All three components of the mucosa form the ruminal papillae which reach the maximum height in the ventral sac area. We tried to classify them in organized groups, according to their average shape, length and width, by the thickness of the epithelium that lines each papilla, and the proportion occupied by the connective axis. In the area of the pillars, where the ruminal papillae are missing, the mucosa has the tendency to form extremely reduced folds, based on the thickening of the epithelium, that will subsequently attract the lamina propria. In the rumino-reticular junctional area, the papillae are reduced to the average length of 496 µm. The connective densification disappears, and in the deep layer of the mucosa, muscle fibers that detach from the superficial layer of the tunica muscularis and that will constitute the future papillary muscle, can be observed.

Key words: *sheep, histology, mucosa, papillae, ruminal wall.*

INTRODUCTION

The outstanding biological value, production efficiency and diversity of products made sheep very popular, as farmers have always sought to select and refine the ovine husbandry system.

In order to enhance the biological potential, selection, amelioration, as well as genetic exploitation of the structural diversity of local and imported breeds has been taken into account in configuring a precise, efficient and clear program of genetic improvement for each morpho-productive type. Thus, the lambs that resulted from crossing the Țurcană breed with other breeds specialized in meat production are well suited for fattening, and the adult sheep have the most diverse lines of operation, specialized for milk, meet, skin and wool production (Pascal, 1998 and 2002).

The economic results are directly influenced by the proper functioning of the digestive system and how the ingested forage is prepared for digestion, as the ruminal compartment has a very important role. The type of forage fed to the animals even in the first weeks of life will greatly influence the histostructure of this very important digestive compartment (Lane *et al*., 2000).

Studies concerning the volume of the ruminal digestion and degree of absorption, consistent with ruminal wall morphology focused on different races of large ruminants, in which a series of researches were carried out, in order to have morphometric (Melo *et al*., 2013) or structural (Graham and Simmons, 2005) assessments of the ruminal mucosa.

The climatic conditions in the northern European region, with great green masses and its digestion in game species have greatly influenced the morphology of the rumen papillae (Soveri and Nieminen, 2007).

Regardless of the ruminant specie, the histostructure of the rumen mucosa is characterized (Frandson *et al*., 2009) by the presence of papillae of different height, shape and diameter, with a marked tendency to cluster in certain areas of the organ (Tudor *et al*., 2005; König *et al*., 2007; Constantinescu and Schaller, 2012).

The whole rumen mucosa consists of stratified squamous keratinized epithelium (Cornilă,

2001), accompanied by *lamina propria* and a connective densification, sometimes mistaken for *muscularis mucosae* (Banks, 1993; Eurell and Frappier, 2006; Bacha and Bacha, 2012), which resulted in research showing a possible configuration of smooth muscle cells at this level (Ikemizu *et al*., 1994; Kitamura *et al*., 2003).

Therefore, this study analyzed and measured the mucosa of the rumen wall in the areas of the ventral sac, of the pillars and in the intermediary area between the reticulum and the rumen highlighting the histostructural peculiarities of the Ţurcană sheep breed.

MATERIALS AND METHODS

Sheep from the white variety of the indigenous ovine breed Ţurcană (*Ovis aries*) were used, initially represented by three seven-year-old adult females. Four more females of the same age were later added to the study, resulting in a total number of seven ovines used for this project, which is still ongoing. The sheep originated from an individual holding that owns a herd of approximately 200 of these ovines in a Subcarpathian area from Romania.

Slaughtering was done in a slaughterhouse which owns a specialized line for sheep, organized in accordance with the current European Community norms. Upon slaughter, sheep weighed between 45 - 48 kg.

The pieces of interest were collected and processed in the Histology laboratory of the Faculty of Veterinary Medicine of Bucharest using conventional histological techniques, obtaining numerous seriated slides stained with Hematoxylin and Eosin method and modified Mallory technique.

The examination of the histological slides was done using the Olympus CX42 optic microscope, and the images were captured with a Olympus E-330 photo camera and the quick CAMERA PHOTO 2.3 software, which allowed digital editing of images and a morphometric appreciation of the analyzed structures.

RESULTS AND DISCUSSIONS

In the structure of the mucosa, we could identify cornified stratified squamous epithelium, *lamina propria* and a densification of connective fibers. All three components of the mucosa form the ruminal papillae (Figure 1).

The basement membrane of the epithelium in the apical area of the ruminal papillae appears to be deformed by the compression imposed by the *lamina propria* in the shape of some thin, elongated, close and ordered folds that are permanently in contact with a capillary network (Figure 2).

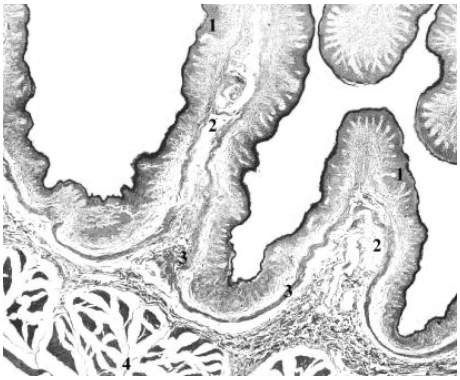

Figure 1. Photomicrograph of the ruminal wall, in the ventral sac area, showing straight papillae with wide, round apex/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification;
4. External muscle layer.

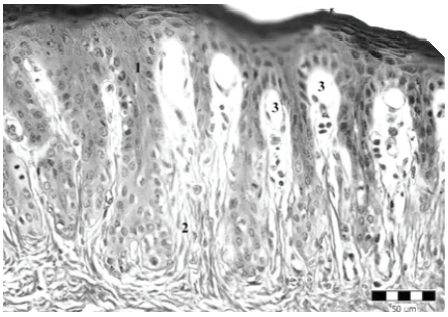

Figure 2. Epithelial detail of the apical papillary region/ Mallory stain, x400
1. Cornified stratified squamous epithelium;
2. *Lamina propria*; 3. Capillaries.

In the ventral sac area, the majority of the papillae reach the maximum height.

The studied sections reveal the presence of some papillae of different shapes and sizes that we tried to classify in organized groups, according to their average shape, length and width, by the thickness of the epithelium that covers them, and the proportion occupied by

the connective axis. The closer the papillae appear to be, the shorter and pointier they seem to be.

Tall papillae that have a relatively constant width are seen in the rumen mucosa of the ventral sac. Some are finger-like, straight and rounder towards the apex, like Constantinescu and Schaller (2012) observed generally in ruminants and not particularly in sheep. The average length is 2912 μm, the base has 695 μm diameter and the apex 641 μm. The connective axis is 422.4 μm wide, and the average thickness of the epithelium is 175 μm, varying between 58 and 294 μm.

Other papillae are extremely reduced, their length reaching only 331 μm, and they have a remotely conical aspect. The width of the base is 642 μm, of the tip 159 μm, and the average is 298.3 μm. The thickness of the epithelium is 74.5 μm, varying between 60 and 82 μm, and the connective axis is 452.8 μm, occupying mainly the area of the base of the papillae. In the case of these papillae, the connective densification does not extend into the papillae, but remains confined to the base. Also, curved papillae with oval shape, and an average height of 3039.5 μm have been observed in the ruminal wall. The connective axis and the epithetlium measure 1141 μm at the base, 1047.4 μm at mid-length, and 658 μm at the tip of the papillae. The papillary apex appears rounded. The interpapillary distance is 220 μm. These papillae have an intensely vascularized connective axis and the tendency to become curved and branched. Their apical epithelium forms regulated, thin, even, dense folds (Figure 3).

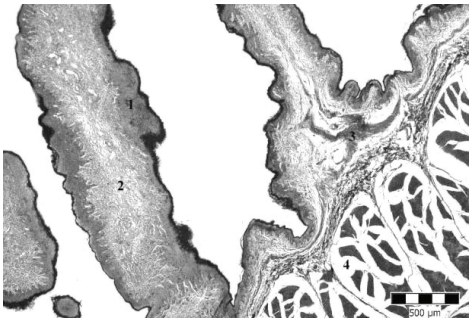

Figure 3. Reduced papillae, and finger-like papillae, straight (left) and thin (right), in the ventral sac area/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification; 4. External muscle layer.

A series of rumen papillae have a narrowed basal region, of only 379 μm, giving an aspect of shrink structure. These papillae have an average length of 2215 μm and predominate in the ventral sac. The average width is 770.8 μm, with a widened tip measuring 910 μm and a base of 497.5 μm. The connective axis stretches interepithelially 484.3 μm, and the epithelium is 136.4 μm thick, varying between 66 and 213 μm (Figure 4).

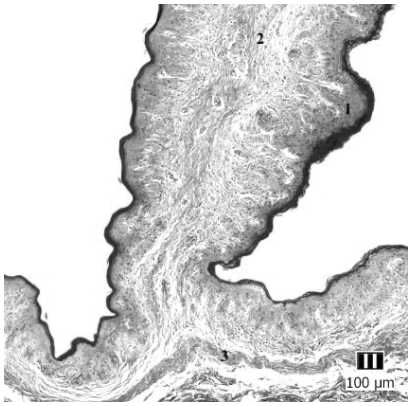

Figure 4. Photomicrograph of the ruminal wall, in the ventral sac area, showing papillae in the narrowed area/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification;
4. Internal muscle layer.

The rarest papillae in the ventral sac were the branched ones. These have an average length of 3278 μm. The average width is 1312 μm, 619.5 μm towards the apex and 992 μm at the base. The connective axis has an interepithelial stretch of 827 μm, and the epithelium has an average thickness of 152 μm, varying between 67 and 238 μm.

In the structure of the rumen mucosa, the *muscularis mucosae* layer is absent (Ikemizu *et al*., 1994; Kitamura *et al*., 2003); instead there is a bundle of dense connective fibers (Figure 5).

In the area of the pillars, the rumen papillae are missing. The mucosa has the tendency to form extremely reduces pleats, based on the thickening of the epithelium, that will subsequently attract the *lamina propria.* The rumen mucosa has an average thickness of 321.5 μm, of which the epithelium makes up for 175.3 μm, and *lamina propria* for 104.5 μm. *Stratum corneum* is very thin, of 9 μm.

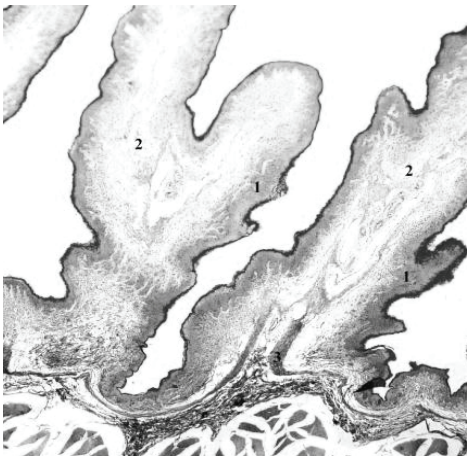

Figure 5. Photomicrograph of the ruminal wall showing branched papillae, in the ventral sac area/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification;
4. Internal muscle layer.

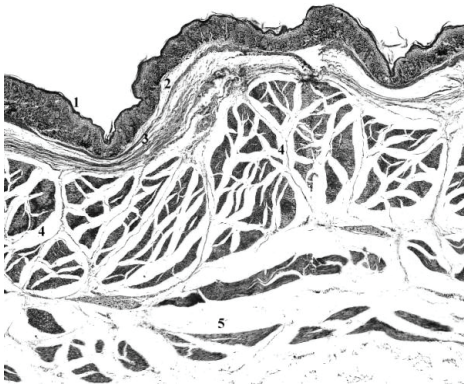

Figure 6. Histostructure of the ruminal wall in the pillars' area/ Haematoxylin-Eosin stain, x40
1. Cornified stratified squamous epithelium; 2. *Lamina propria*; 3. Connective densification;
4. Internal muscle layer; 5. External muscle layer.

It is made up of soft stratum corneum, showing nuclei of the superficial pavement cells in its structure. The connective densification is very thin, up to 41.7 μm and is very close to the epithelial basement membrane (Figures 6 and 7).

In the rumino-reticular junctional area, the papillae are reduced to the average length of 496 μm, only slightly taller than the inter-papillary mucosa, that reaches an average thickness of 447 μm, of which the epithelium is 218 μm, and *lamina propria* 229 μm. The stratum corneum reaches the average thickness of 27.7 μm.

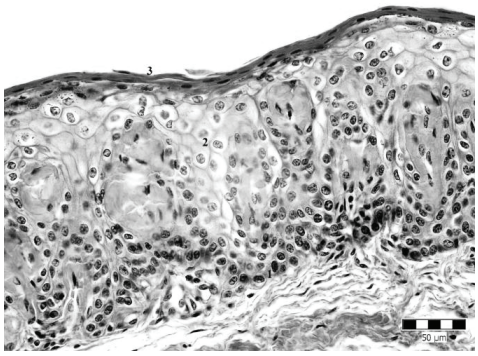

Figure 7. Histostructural detail of the ruminal mucosa in the pillar area/ Mallory stain, x400
1. Basal layer; 2. *Stratum spinosum*;
3. *Stratum corneum*.

The connective densification disappears, and in the deep layer of the mucosa, muscle fibers that detach from the superficial layer of the *tunica muscularis* and that will constitute the future papillary muscle, can be observed (Figure 8).

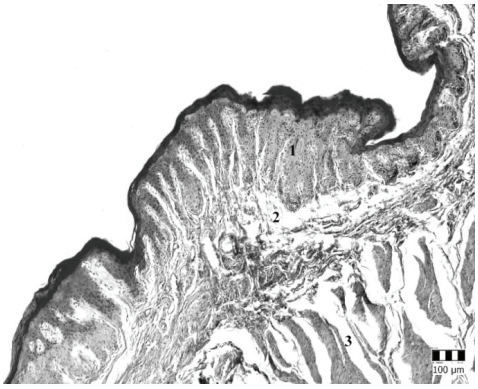

Figure 8. Histostructure of papillae in the ruminal wall, near the ruminal-reticular junction/
Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. *Lamina propria*; 3. External muscle layer.

The morphometric analysis of the different structures in different macroscopic areas of the rumen wall revealed a series of characteristic elements. It may be noticed that the thickest mucosa (447 μm) is seen at the rumen-reticular fold, and the lowest value at the pillars (321.5 μm).

There repartition of the mucosal components differs by area. The average thickness of the epithelium and of the *lamina propria* is maximal in the mucosa of the rumino-reticular fold, but the thinnest epithelium is the one lining the ruminal papillae in the ventral sac

area. The *lamina propria* is least represented at the pillar mucosa (Table 1).

Table 1. Average morphometric values in different areas of the ruminal mucosa

Area of the ruminal wall	Mucosal thickness (µm)	Epithelial thickness (µm)	*Lamina propria* thickness (µm)	Connective densification thickness (µm)	Papillar length (µm)
Ventral sac area	335.8	126.3	142	67.5	2900
Pillars	321.5	175.3	104	41.7	-
Rumino-reticular fold	447	218	229	-	496

The whole rumen mucosa is sustained by a fibrous connective dense structure on its length (Ikemizu *et al*., 1994; Kitamura *et al*., 2003), except for the rumen-reticular junction, where fragmented connective bundles detach from the superficial area of the inner layer of the muscularis in order to structure on the *muscularis mucosa*, the future papillary muscle of the reticular wall (Figure 9).

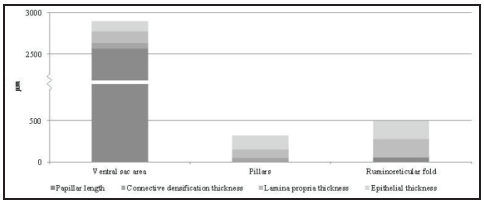

Figure 9. Proportional comparative representation of the ruminal mucosa components

The morphometric and histostructural analysis of the rumen papillae revealed significant differences in shape and size that allowed a classification of them into study groups. This sort of classification has not resulted so far out of specialized literature, neither for ruminants in general, nor for a particular breed of sheep. Neither Bacha and Bacha (2012) nor Soveri and Nieminen (2007) have not attempted to classify the structures of the ruminal mucosa, only writing a generalized view of them in sheep, and respectively in another ruminant, the forest reindeer (*Rangifer tarandus fennicus*).

The rumen papillae have a height that largely varies between 3278 µm and 331 µm, the tallest being the branched ones, and the others being the shortest papillae.

The structure that varies the least is the epithelium, which maintains its characteristics in all papillae.

The widest base ruminal papillae are both reduced, conical ones, and narrow, finger-like ones, the difference being of only 181µm.

The papillae most dilated in the apical are represented by the papillae with shrunken basis, and the narrowing ones are the reduced papillae. A correlation between the shrunken papillae and the interpapillary distance cannot be established. There are short, pointy papillae, where the papillae tend to be closer.

By analyzing the values given by the width of the connective axis and of the average width, we observed that the papillae with the widest base and the thickest connective axis have the greatest average thickness.

By individually measuring each papilla we have seen that the papillae with a length of 3000 µm predominate in the structure of the ruminal mucosa, while the short papillae of 331µm and the branched ones, of 3278 µm, are exceptions (Table 2, Figure 10). This average height is much larger than the average 1000 µm usually noticed in ruminants by Constantinescu and Schaller (2012).

Table 2. Average morphometric values for different papillae formed by the ruminal mucosa at the level of the ventral sac

Papillae	Average length (µm)	Average width middle area (µm)	Average width apex (µm)	Average width base (µm)	Epithelial thickness (µm)	Width of connective axis (µm)
Finger-like straight papillae	2912	691.8	641	695	175 (58-294)	422.4
Finger-like thin papillae	2457	498	412	1148	114.8 (79-157)	229.8
Short papillae	331	298.3	159	642	74.5 (60-82)	452.8
Conical papillae	1339.5	935.8	465.5	1329	119.5 (50-206)	886
Basally strangled papillae	2215	770.8	910	497.5	136.4 (66-213)	484.3
Branched papillae	3278	1312	619.5	992	152 (67-238)	827
Elongated oval papillae	3039.5	1047.4	658	1141	156.5 (60-250)	672.5

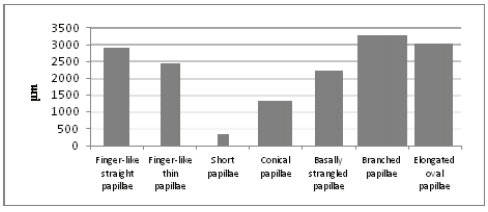

Figure 10. Morphometric appreciation of the ruminal papillae at the level of the ventral sac mucosa

CONCLUSIONS

The papillary epithelium retains its similar proportions throughout the ventral sac.

The most dilated papillae in the apical area were represented by basally narrowed ones.

The very pointed ones are actually the shortest in height.

A direct correlation between the basally narrowed papillae and the reduction of the interpapillary distance cannot be established: where the papillae tend to be closer, they appear shortened and pointier.

The thinnest epithelium is seen on the rumen papillae in the ventral sac.

The average thickness of the epithelium and of the *lamina propria* reaches the maximal value in the rumen-reticular fold area.

The fibers that will constitute into the papillary muscle of the reticular folds separate from the uppermost structure of the internal layer of the *muscularis externa* in the rumen-reticular fold area.

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INVESTIGATION OF ANTIOXIDANT COMPOUNDS IN FLUOROTIC SHEEP

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Abstract

Fluorosis, a condition which usually affects the formation of bone and teeth in human and animals, is an important health problem in Van and Agri provinces. This study was performed to determine the levels and the changes of antioxidant compounds in fluorotic sheep. 30 fluorotic sheep and 20 healthy Morkaraman sheep of 3-4 years old were used as living research materials. The diagnosis of fluorosis was confirmed by clinical examinations. The urine fluoride level was determined. Blood of all animals was taken from vena jugularis by appropriate techniques and analyzed for glutathione peroxidase (GPx), glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), sialic acid (SA) and lipid-bound sialic acid (LSA). The levels of these parameters in healthy and fluorotic group were: 1028-416.8 mU /ml, 23.23-50.16 mg/ dl, 9.25- 7.88 mU/ ml, 1.62-0.56 nmol/ ml, 51.19-46.33 mg/ dl and 9.77- 12.16 mg/dl, respectively. Urine fluoride (F) levels were 1.65 ppm in healthy and 23.84 ppm in fluorotic sheep groups. Statistical differences was found between the average values of healthy and fluorotic groups as $p \leq 0.0001$ in GPx and GSH, $p \leq 0.001$ in MDA, $p \leq 0.05$ in SA, $p \leq 0.01$ in LSA and $p \leq 0.001$ in urine F levels. No statistical differences were found in SOD levels. ($p \geq 0.05$) The results obtained in this study indicate that important changes were found in antioxidant systems of fluorotic sheeps.

Key words*:* antioxidant, blood, fluorosis, sheep, urine.

INTRODUCTION

Fluorosis is a result of excessive fluoride (F) intake for a prolonged time and occurs in two different forms as acute and chronic. Ionic F in plasma increases and F accumulates in the body. Various changes occur after chronic administration of F in blood, brain, liver and musculoskeletal system in human and animals. Excess F intake can inhibit the activity of many enzymes. Chronic fluorosis can be commonly observed in industrial areas, volcanic and tectonic areas. The Eastern part of Turkey has volcanic structure eruption of volcano enriched soil, with ground and underground water contaminated with F. The high level of F can be dangerous to all living creatures in this area. Especially Muradiye, Caldiran, Tendurek and Mt. Agri had been severely affected. The levels of F have been found elevated in plant, soil and biological samples from animal and human (Underwood, 1966; Jones 1977; Shupe et al., 1984; Ergun et al., 1987; Walton, 1988).

Antioxidants defense system inhibits the free radical production functions for the scavenger of free radicals, repair of cells, increase the capacity of antioxidant stops the secondary chain reactions. Superoxide dismutase, glutathione peroxidase, tocopherols, glutathione, retinol, ascorbic acid are taken into account for antioxidant compounds. Many studies indicate that generation of lipid peroxidation product, free radicals and altered antioxidant defense systems are closely related with fluorosis (Han et al., 2000; Freemann and Cropo, 1982).

Sialic acid is a monosaccharide with nine carbon backbone and also name for N-acetylneuraminic acid (NANA) which is widely distributed in animal tissue, especially in glycoproteins, mucoproteins, glycolipids and gangliosides. Sialic acid also contributes to creating negative charge on the cell surface. SA reduction were observed in some chronic diseases which causes to produce excess glycoprotein. Metastatic cancer cells often have sialic acid rich glycoprotein (Dnistrian et al., 1982; Traving and Schauer, 1998).

There are many researches on the F level of the biological material such as teeth, urine, bone, milk or blood, but limited study has been done on the antioxidant levels of fluorotic sheep.

The study was designed to investigate the effect of F on antioxidant systems. For this purpose the levels of F in the urine, the amounts of glutathione, SA, LSA, MDA and the activities of GPx, SOD in blood have been determined in healthy and fluorotic sheep.

MATERIALS AND METHODS

Thirty chronic fluorotic sheep raised in Van and surrounding villages of Caldiran and twenty healthy sheep from the Research Farm of Faculty of Veterinary Medicine in Van were used as living research materials.

The urine samples from all sheep were analyzed for F amount by fluorometers (Singer et al., 1969)

Blood samples were taken from v. jugularis, centrifuged at 3000 rpm for 15 minutes and plasma were separated into erythrocytes and washed three times with 0.9 % NaCl at 2000 rpm for 8 minutes. The washed erythrocytes were transferred to Eppendorf tubes. The erythrocyte package was used to determine the activity of GPx and SOD enzymes using Calbiochem enzyme kit assay (Ransod Superoxide Dismutase Enzyme Kit, Ransel Glutathione Peroxidase Enzyme Kit).

GSH levels were determined in whole blood spectrophotometrically using Ellman's Reagent at 412 nm (Beutler et al., 1963). MDA levels were measured in whole blood spectrophotometrically according to color formation with thiobarbituric acid at 412 nm. (Gutteridge, 1995; Sushil et al., 1989).

Blood samples taken into tubes without anticoagulant were centrifuged at 2500 rpm for 10 minutes and sera was separated.

The serum SA were determined spectrophotometrically using Erlich reagent comparing the reading obtained at 525 nm to a standard curve developed from a known sample of N-acetyl neuraminic acid (NANA) under the same conditions (Gerbaut et al., 1973).

LSA levels were also determined spectrophotometrically using resorcinol solution then reading at 580 nm the extracted blue color present in the organic layer, determining the amount of lipid bound sialic acid by comparing the reading obtained at 580 nm to a standard curve developed from a known sample of N-acetylneuraminic acid (NANA) under the same conditions with a special formula (Dnistrian et al., 1982; Katopodis et al., 1982).

All data were statistically evaluated using unpaired t-test.

RESULTS

All the average values of analyzed parameters were shown in Table 1. The differences between averages of two groups were analyzed and statistical importance was found as F levels ($p \leq 0.001$), activity of GPx ($p \leq 0.0001$), GSH ($p \leq 0.0001$), in MDA ($p \leq 0.001$), SA ($p \leq 0.05$) and LSA ($p \leq 0.01$).

Table 1. Biochemical parameters of urine and blood of healthy and fluorotic sheep

Parameters	n	Control X±SEM	n	Fluorotic Sheep X ± SEM	T	p
Fluoride (urine) (ppm)	10	1.65 ± 0.35	18	23.84 ± 4.74	4.672	***
Glutathione peroxidase (mU/ml)	20	1028 ± 80.92	28	416.8 ± 25.06	7.214	****
Glutathione (mg/dl)	20	23.23 ± 0.75	30	50.16 ± 4.22	6.285	****
Superoxide dismutase (U/ml)	19	9.25 ± 0.88	30	7.88 ± 0.45	1.383	NS
Malondialdehide (nmol/ml)	20	1.62 ± 0.24	30	0.56 ± 0.12	3.980	***
Sialic acid (mg/dl)	20	51.19 ± 1.81	27	46.33 ± 1.65	1.967	*
Lipid-bound sialic acid (mg/dl)	20	9.77±0.54	27	12.16±0.57	2.947	**

* p≤ 0.05, ** p≤ 0.01, ***p ≤0.001, **** p≤0.0001, NS non-significant

DISCUSSIONS

As of the geological structure, Eastern Anatolia is young and has high volcanic activity. Fluorosis has been determined in people and animals living in this region. High fluoride levels were determined in drinking waters. Consuming the plant and water with high amount F is the main causes of fluorosis. (Dobaradaran et al., 2008a; Dobaradaran et al., 2008b)

Fluoride can be excreted from body by kidney therefore, the measurement of F in urine is used to diagnose the fluorosis. Ergun et al. (1987) had reported a urine F levels of 8.11-1.49 ppm, in sheep raised in the Eastern and respectively

Eagen region. Şendil and Bayşu (1973) determined the sheep urine F levels between 3.80-30.61 and 3.80-26.61 ppm in Muradiye and Dogubeyazit town. In the present study the amount of urine F of the control and fluorotic sheep were found as 1.65-23.84 ppm, respectively ($p \leq 0{,}001$) and supported the endemic and extensive fluorosis in this area.

Chinoy and Dipti (2000) studied the effects of protein on fluorosis. They added 5-10-20 mg/kg NaF (Sodium fluoride) to the protein-deficient diets of rats and observed that the activity of liver GSH, SOD and CAT were decreased and lipid peroxidation was increased. Rats feed with added protein diets didn't show any significant changes. The authors concluded that protein addition prevented the F intoxication.

Shivarajashankara et al. (2001) feed Wistar albino rats with 100 ppm added NaF for 4 months and analyzed the parameters of antioxidants system. While the activities of GPx, erythrocyte GSH, and ascorbic acid levels were increased, SOD and uric acid levels were decreased.

Han et al. (2000) investigated the antioxidant enzymes, lipid peroxidation and free radicals levels in low Se and Cu condition with endemic fluorosis in cattle. ROS and MDA levels in fluorosis group were increased, while GPx and erytrocyte SOD levels were decreased. They concluded that high amounts of F affected the antioxidant system, and decreases the lipid peroxidation LPO and ROS levels or free radical metabolic imbalance and antioxidation disorder were the main factors causing endemic fluorosis.

In the present study the levels of GSH and GPx in fluorotic and healthy sheep were found as 50.16-23.23 mg/dl ($p \leq 0.001$), and 416.8-1028 mU/ml ($p \leq 0.001$), respectively. Both parameters were significantly changed because of the oxidative stress in fluorotic sheep.

The function of SOD enzymes is to eliminate superoxide radicals in the body. The activity of SOD enzymes in fluorotic and healthy sheep were 7.88-9.25 U/ml respectively. The differences between groups were not significantly important. Wu et al. (2015) have studied on the oxidative stress of bone tissue in rats with chronic fluorosis treated with anti-oxidant, the oxidative damage of lipid, protein and DNA. They concluded that the activity of SOD and CAT of tissues are inhibited and suppression function of hydroxy free radical is decreasing under fluorosis influence, which results in protein damage. Oxidative stress is considered to be one of the mechanisms of skeletal fluorosis.

Animal species, environmental conditions, F intake by food and water, time and amount of F intake, the antagonistic compounds with F in the medium, the age of the animals are considered significant factors in the development of symptoms of fluorosis and effects of F on animals. Some animals can have adaptive phase as reported by Shivarajashankara et al. (2001). They reported that adaptive changes could be occurred in the antioxidant system of blood, brain, liver, and protection mechanism can be formed against the oxidative stress of F intoxication.

The lipid peroxidation product, malondialdehyde, must be determined in chronic diseases. In subclinical mastitis the MDA level was increased as a result of oxidative stress (Gutteridge, 1995). Güven, and Kaya (2005) have studied the effect of fluorosis on blood malondialdehyde (MDA) and reduced glutathione (GSH) levels in yearling Tuj ewe-lamb. Lipid peroxidation and GSH levels in the fluorotic group were significantly higher than controls (MDA $p<0.01$, GSH $p<0.05$). They concluded that fluorine may cause an increase in lipid peroxidation in cases of fluorosis. In this study MDA levels in healthy and fluorotic sheep were 1.62-0.56 nmol/ml ($p \leq 0.001$). Fluorosis generally stimulates the formation of ROS and increases lipid peroxidation. In contrast to this statement, here, low MDA levels were determined in fluorotic group than the controls. MDA could be a sign for the low lipid peroxidation in our cases .

Yur et al (2013) found that in the kidney tissues, the MDA levels and SOD activities in the fluorosed sheep showed nonsignificant increases, but the GSH level and GPx activities significantly decreased. They concluded that different degrees in the pro-oxidant/antioxidant status of soft tissues such as kidney, liver, and muscle were affected by F intoxication.

Sialic acid is mainly located in the cell, gangliocyte membrane and in glycoproteins. The SA increases in some chronic disease such

as tuberculosis, liver cirrhosis, chronic bovine hematuria, lymphatic leukemia and some parasitic diseases were reported by (Freeman and Cropo, 1982; Traving and Schauer, 1998). The sialic acid and lipid-bound sialic acid levels of fluorotic and healthy sheep were found as 46.33-51.19 mg/dl ($p \leq 0.05$) and 12.16-9.77 mg/dl ($p \leq 0.01$), respectively. Increases of LSA could be caused by the inhibitory effect of F on aerobic glycolysis enzymes and possible destruction and degeneration of erythrocyte membrane.

CONCLUSIONS

As conclusion, fluorosis was detected in sheep raised in the Van and Agri provinces and surrounding villages. The levels of GSH, LSA were increased and SA, GPx, SOD and MDA levels were decreased in the blood of fluorotic sheep. These changes could be the result of oxidative stress of fluorosis and these changes must be considered during clinical and scientific studies.

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BIODIVERSITY OF THE CORONARY ARTERIES IN CATTLE - MACROSCOPIC STUDY

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Abstract

Lately, experimental medicine used the ruminants as experimental animals. On the sheep were achieved even heart experimental surgery. However, the literature about the vascularization of the heart is not very numerous especially regarding large ruminants. This study was conducted to provide supplementary data for the literature. The study was carried out on a total of 12 specimens in which the hearts were dissected after insertion into the arteries of the contrast dye. It has been found that right coronary artery is smaller than the left. Subsinusal branch is given by left coronary artery, being an extension of the left circumflex branch in subsinusal groove. Right ventricular wall is crossed by 5-6 main collaterals of right coronary artery, some of their terminal branches showing anastomosis with corresponding branches of the paraconal branch of the left coronary artery. Right coronary artery is much better represented than the left. Its terminals have a size nearly equal.

Key words: *cattle, heart, coronary arteries.*

INTRODUCTION

Besides the experimental surgery on pig, some authors (Shofti et al., 2004) used the sheep as experimental model.

The use of sheep as an animal model seemed to be ideal for simulating human cardiac surgical procedures due to the size of the chest cavity, which can accommodate devices and surgical instruments intended for human use. Due the human-sized and comparable anatomical distribution of the coronary arteries, similarity in diameter of the left and right internal thoracic arteries (LITA and RITA), and ease of harvesting saphenous veins of suitable length and diameter, makes the sheep a more suitable model for such purposes (Shofti et al., 2004; Hill, 2015)

Talking about heart anatomy in cattle there are works on histological structure of the coronary arteries (Bylina, 2004; Ocala, 1993) or cardiac innervation (McKibben, 1969) but data are not very numerous regarding the cardiac vasculature (Bhargava, 1970). For this reason a detailed macroscopic study of coronary arteries in cattle was conducted.

MATERIALS AND METHODS

The research was conducted on 12 hearts from slaughtered animals. The weight of animals ranged between 90 and 150 kg. After collecting, the hearts were washed with water, including cavities. It was aimed to eliminate the residual blood present in the lumen of the coronary artery by compressing them from the terminal to the origin. Subsequently, the contrast substance (AGO) was introduced in the coronary arteries at the level of the aortic bulb. Injected pieces were placed in 10% formalin solution for one week. After washing to remove formaldehyde they have been dissected by the classical method. Latter the most representative pieces were photographed.

Identification and description was achieved using Nomina Anatomica Veterinaria - 2005.

RESULTS AND DISSCUSIONS

The morphology of right coronary artery

The right coronary artery arises from the cranial part of the aortic bulb and is directed cranially, on the right side of pulmonary arterial trunk, between this and the right auricle.

After reaching the coronal sulcus, it is located at the ventral side of the right atrium, then, engages in the coronary groove on the atrial surface of heart. It does tortuous course at the base of the right atrium, up to subsinusal interventricular groove.

The first collateral issued by right coronary artery, is voluminous. It is directed at an acute angle, on the left side of this.

This collateral emits near the origin a branch detached from its right side, in the upper third of the cranial edge of the right ventricle.

Subsequently, the first collateral of the right coronary artery emits a flexion relatively smooth branch, reaching the origin of previous third of the right side of pulmonary arterial trunk.

Finally, the terminal trunk of the first collateral of the right coronary artery branch out in the upper third of the right ventricle, ventrally to the pulmonary trunk origin.

It is distributed in the arterial cone and intertwines with branches issued by the left coronary artery that supplies the same portion of ventricular mass.

The second collateral of the right coronary artery originates also from the right side of the main trunk at the front end of the coronal sulcus. This arises from a caliber similar to the first collateral. After a trajectory of 2 cm, emits a deeper branch descending in the thickness of the previous wall of the right ventricle and a superficial branch. The latter has an oblique path, descending to the left, focusing to paraconal groove that it meets horizontally at the line between the middle third and distal third of it. On his path, the collateral emits branches that can be drawn from both right and left sides on the main trunk. Through its distribution represents the main artery that supplies the auricular side (left) of the right atrium.

Third collateral is emitted by the right coronary artery close to the previous artery and has about the same caliber at origin with it. It comes down on cranial rim of the right ventricle having a rectilinear path. On the path it emits superficial branches detached from the both sides (right and left) and deep branches. Superficial branches, averaging four on each side, have obliquely and descendant path, covered with thin myocardial bundles. Two or three deep branches are detached from the deep side of the main trunk and lost in ventricular wall thickness. The last distal ramifications of the main trunk of the third collateral of the right coronary artery are exhausted at distal end of cranial rim of the right ventricle. Through its distribution this collateral ensures dominant arterial irrigation of cranial rim of the heart.

Next, right coronary artery emits 2-3 short collaterals, from the previous rim, that supply the upper third of the cranial rim of the heart. Opposite to these branches, originating on the back side of coronary artery, constantly emerge ventricular upper branches relatively short and fine that supply the previous coronary rim of the right ventricle and is exhausted before reaching the base of the right atrium (Fig. 1).

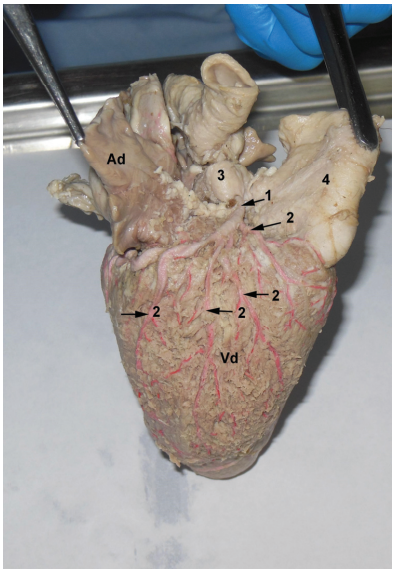

Fig. 1. The origin of the right coronary artery- dorsocranial view of the heart (original)
1-right coronary artery; 2- the main ventricular branch; 3-aortic bulb; 4- pulmonary trunk; Ad- right atrium; Vd-right ventricle

The main atrial collateral (proximal atrial artery) is emitted by the right coronary artery from its caudal edge and addresses the eccentric wall of the right atrium. This collateral emits a descending atrial branch which goes down to the base of the right atrium being exhausted into the coronary groove. Subsequent, the proximal atrial artery has a path with flexion and caudal dorsal oblique,

reaching to the level of openness of the caudal vena cava. On its way emits 2-3 ascending branches which are distributed to the right wall of the right atrium, the first of these branches, focusing cranial to the ventral edge of the right auricle (Fig. 2).

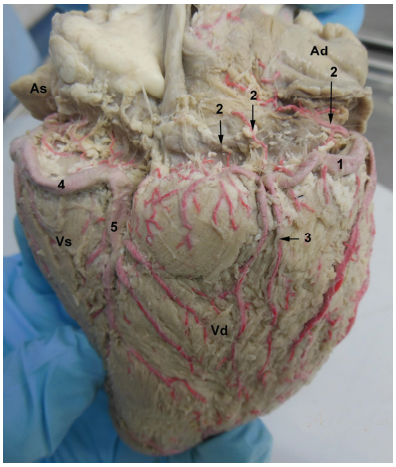

Fig. 2. The terminal branches of the right coronary artery on the right side of the heart (original)
1-the right coronary artery; 2- atrial branches; 3-ventricular branches; 4- left circumflex branch; 5-subsinuos branch; Ad- right atrium; As-left atrium; Vd-right ventricul; Vs -left ventricle

Opposite the place of detachment of proximal atrial artery from the right coronary artery emerges a strong ventricular distal branch, which obliquely descends at superficial level to the top of the right ventricle, approximately halfway between the cranial edge of the heart and subsinusal groove. This emits 2-3 fine collaterals from its right side and 4-5 branches thicker and longer from its left side.

The last of these branches descend obliquely the paraconal groove and will anastomoses with an ascending branch of interventricular paraconal artery. Through its distribution path this collateral of the right coronary artery ensures irrigation of the previous third of atrial side of right atrium (fig. 3).

After issuing this collateral, the right coronary artery emits distally 3-4 short and fine collaterals to the top edge of the right ventricle, and medial proximal emits branches to the ventral edge of the eccentric wall of right atrium.

The last of these atrial branches is better represented artery and is exhausted to atrial wall, dorsal to the opening of coronary sinus.

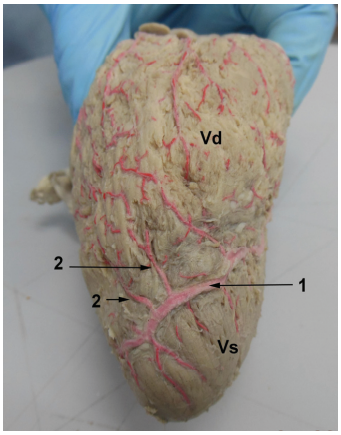

Fig. 3. The anastomosis between paraconal branch and ventricular branches of right coronary artery (original)
1- paraconal branch; 2- ascending branches (right ventricular) from the paraconal branch; Vd(Rv)-right ventricle; Vs(Lv)-left ventricle

The last two ventral collaterals of the right coronary artery often are detached as a common trunk, very short. Subsequent, they have a descendant path and slightly with flexion, oriented to the top of the heart. Through their branches, these two collaterals of the right coronary artery irrigate the middle third of the previous wall of the right ventricle.

After issuing the last two powerful ventricular collaterals, the right coronary artery leads through the coronary groove with an atrioventricular branch called right circumflex branch. This branch is distributed as a tree, emitting both ventricular branches for the coronary end of right ventricle, and atrial branches addressing ventral edge of the eccentric wall of right atrium. The finest ramifications reach the upper end of subsinusal groove.

The morphology of left coronary artery

Emerges from the left side of the aortic bulb and switches to the caudal edge of the pulmonary arterial trunk. At origin, it has almost double size of the right coronary artery. After a short path (about 2-3 cm), it reaches the paraconal groove, where it ends with a paraconal branch and a circumflex branch (Fig. 4).

At this level, from the cranial edge of the coronary artery emerges a long and relatively thin branch that engages the left side of the origin of the pulmonary trunk, reaching its cranial side.

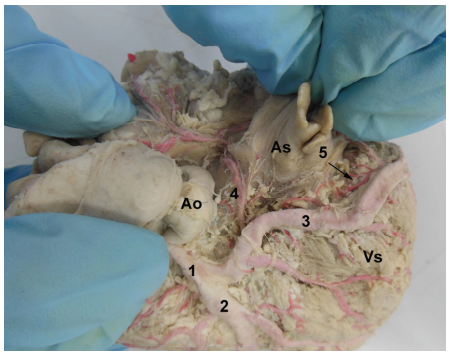

Fig. 4. The left coronary artery's terminals in coronary groove (original)
1-left coronary artery; 2-paraconal branch; 3- left circumflex branch; 4,5- the atrial branch of the left circumflex artery; Ao-aorta; As(La)- left atrium; Vs(Lv)- left ventricle.

The paraconal branch is placed in the paraconal groove, having a descendant ventro-cranial path. Near the top of the heart intersects its cranial edge, reaching the atrial side, until the distal end of the subsinusal groove. On the way, emits collateral both from the cranial edge and on the caudal edge.

Near the origin (about 0.5 cm), emits a collateral that emerges at right angle from the cranial edge. After a short path (about 1 cm) the latter will emit a dorsal branch, which will focus cranial, at a distance of approximately 0.7 cm ventral to coronal sulcus. This branch emits collaterals detached both from the upper edge and from the ventral edge (Fig. 5).

Last ramifications of this branch reach the upper third of the auricular portion of the right ventricle, ventral to the origin of pulmonary trunk. These branches are intertwined with those given by the first collateral of right coronary artery.

Through the distribution of its ramifications this artery provides irrigation of the right ventricle in the middle third of the auricular side of it. Next 3-4 cranial collaterals issued by paraconal artery are relatively thin and penetrate the ventricular mass reaching interventricular septum.

The second well represented collateral, detached from the cranial edge of paraconal artery, visible subepicardic, has a horizontal path to the cranial edge of the heart. It is highlighted on the auricular side of the right ventricle, at the boundary between the middle third and the bottom third of it.

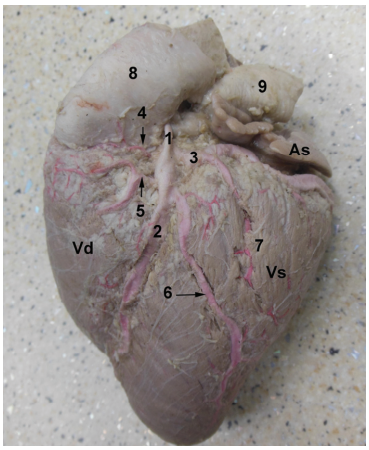

Fig. 5. The left coronary artery's terminals of the auricular face of the heart (original)
1-left coronary artery; 2-paraconal branch; 3- left circumflex branch; 4-collateral branch of left coronary artery; 5-right ventricular colaterals of the paraconal branch; 6- left ventricular colaterals of the paraconal branch; 7-the first ventricular colateral of the left circumflex branch; 8-pulmonary trunk; 9-aorta As(La)- left atrium; Vd(Rv)-right ventricle ; Vs(Lv)- left ventricle.

At the level at which the paraconal groove intersects the cranial edge of cord the paraconal branch emits a well-represented collateral with an ascendant path toward the right side of cord. This branch will anastomoses with one of ventral collaterals issued by the right coronary artery (Fig. 3).

Next, the paraconal branch passes on to cranial edge of the cord near its peak, then gets the right side being exhausted at the ventral end of subsinuos groove. During this pathway the paraconal branch emits from its cranial side 4-5 collaterals with ascendant trajectory, which is distributed in the lower middle third of cranial edge of cord and ventral end of the right ventricle, on the atrial side of it.

Paraconal branch emits the following caudal collaterals:

The first collateral is detached from the caudal edge of paraconal branch, about the same level with the first cranial collateral. By its size and scope it is the most powerful of collaterals of terminal branches of the coronary arteries. It emerges in acute angle and then focuses to the caudal edge and the top of the cord, with a descendant path and oblique, caudal ventral.

At origin this collateral emits a branch that is distributed to left ventricle on the ventral portion at the intersection of paraconal groove and coronal sulcus. Still from the main arterial trunk emerge superficial branches both from the cranial edge (3-4) and deep branches, which penetrate the thickness of the left ventricle. Finally, the main trunk of this first caudal collateral ends forked, near the caudal edge of the cord. The two terminal branches originate from the same caliber. The upper end branch has a horizontal path, crossing the caudal edge of the heart at the line between the middle third and the bottom third of it, and finally arrives on the right side of left ventricle, ending near subsinusal groove. The descendant terminal branch descends to the top of the heart, where it branches and provides irrigation to its rear half.

In conclusion, the first caudal collateral of paraconal artery is the main artery supplying auricular half ventral portion of the left ventricle, ventral half of the caudal edge of the cord and the rear portion of the peak of heart. At the top of heart, the terminal branches of paraconal artery intertwined with terminal branches of this first caudal collateral described. The next 3-4 collaterals detached from the caudal edge of the paraconal branch are relatively short (1 to 3 cm) and thin, have an oblique and descendant path caudal ventral, exhausting in the ventral third auricular portion of the left ventricle. In the terminal portion, the paraconal branch emits from the caudal margin 4-5 collaterals which descend to the top of the heart, thereby ensuring dominant irrigation of apex.

Circumflex branch presents at origin, a caliber similar to the paraconal branch. It is caudal oriented, following the coronary groove. Initially is located ventral to the left auricle, then to the ventral edge of the left atrium, until the caudal edge of it.

Subsequent, passes to the atrial side of heart, and at the proximal end of the subsinusal groove goes down through this groove as a subsinusal branch.

With a long trajectory, circumflex branch emits collaterals both from the atrial and ventricular edge, the latter being more numerous and more voluminous. The main collaterals emitted from the ventricular edge of heart, have a descendant path and are distributed in the upper half of the posterior wall of the left ventricle.

The first of these is the most developed collateral. It is oriented ventral caudal and after a trajectory of 2-3 cm, bifurcate acute angle, branches being approximately equal. Through its distribution, this collateral supplies the higher part of proximal half of auricular side of the left ventricle.

Next 3-4 collaterals emitted by circumflex branch from her lateral side are short and relatively fine branches that are lost in left ventricle mass near the coronal sulcus.

The second well represented collateral detached from the ventricular edge of circumflex branch has also an oblique path, focusing distal also to the right side to get approximately to the half caudal edge of the heart. At the caudal edge of the left ventricle from the circumflex branch, emerges a collateral which after a short trajectory of about 0.5 cm, bifurcate acute angle and is distributed in the upper third of the caudal edge of the left ventricle (Fig. 6).

Fig. 6. Ventricular collaterals of the left coronary artery - caudal view (original)
1-paraconal branch; 2- left circumflex branch; 3- ventricular branch of the paraconal artery; Vs(Lv)- left ventricle.

The following strong collateral branch (the second in volume) that emerges from the ventral edge of circumflex branch is represented by intermediate branch, which descends through the caudal groove of the heart. Finally it is finished bifurcated. It is the main artery that supplies the upper half of the parietal wall of the left ventricle.

After issuing marginal artery, circumflex branch passes to the atrial side of coronary groove. At about 0.5 cm of the origin of the interim artery emits the better represented collateral that is distributed in the upper third of the atrial side of the left ventricle.

The collaterals detached from the atrial edge of circumflex branch generally have a horizontal or ascendant trajectory and is distributed dominant to the left atrium. The first of these collaterals is best represented. It is committed to the caudal edge of the aortic bulb, ventral of left auricle in which emits 3-4 arterials, then branching giving cranial branches which are distributed in right atrium ceiling, medium branches that supply the concentric wall of the cranial vena cava at its opening in the right atrium and caudal branches that are distributed on the right side of left atrium and pulmonary veins

Next collateral detached from atrial edge of circumflex branch is located about 3 cm caudal from the previous and is oriented to the base of left atrium on its auricular side where it ends forked with a cranial and caudal branch.

At the caudal edge of the heart, the circumflex branch emits stronger collateral, from which initially emerge branches that guides cranial, to the left base of left atrium and entering the upper edge of the left ventricle from the coronal groove. Next atrial branch of this collateral goes up to the ventral side of the caudal end of the left atrium in which branches. After issuing this branch, emitted by circumflex artery collaterals path will be through the coronary trench right to the left atrium near the base of the trench subsinusal origin. On the trajectory, it emits 3-4 branches atrial addressing the ventral edge of the left atrial wall on the right, and 2-3 branches ventricular finer entering the top edge of the left ventricle from the coronal trench.

Through this collateral, the circumflex branch supplies the portion of the left atrium located caudal and ventral to opening place of the pulmonary veins. Last collateral of circumflex branch, before engaging to subsinusal groove, ends branched at coronal sulcus by fine and ventricular branches.

Subsinusal branch appears as terminal part of circumflex branch in the subsinusal groove which it pursues near to the top of the heart. On its path, emits collaterals that fall both from the cranial and caudal edge of the main trunk and of its deep side (Fig. 7).

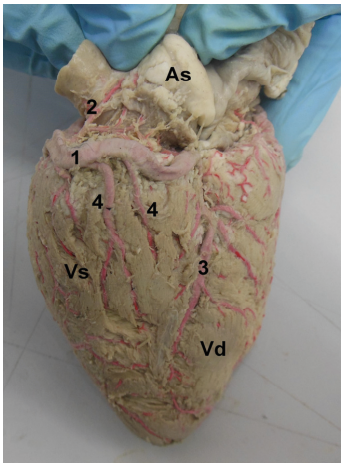

Fig. 7. Distribution of left circumflex branch- caudolateral view of right side (original)
1- left circumflex branch; 2- atrial branch; 3- subsinusal branch from 1; As – left atrium; Vs(Lv)- left ventricle; Vd- right ventricle

There are three or four cranial collaterals with an oblique trajectory cranio-ventral and have an average length of 3-4 cm. They provide irrigation of anterior wall of the right ventricle on the portion located previous to the subsinusal groove.

Caudal collaterals, numbering two or three, are shorter and finer ending each bifurcated into the right end of the left ventricle.

Collaterals detached from the deep side of subsinusal branch penetrate the thickness of the ventricular myocardium and are exhausted into the interventricular septum.

CONCLUSIONS

Right coronary artery is smaller than the left and did not reach the origin of subsinuos groove. Ventricular collaterals are bulky and numerous. They irrigate almost entirely right ventricular wall. For this reason the right ventricular collaterals of paraconal branch are reduced.

The two terminals of the left coronary artery are equal. About two thirds of three of the left ventricle is mostly irrigated by strong collateral of the paraconal branch. Upper third is supplied by the first collateral of the left circumflex branch.

After issuing four voluminous ventricular collaterals, circumflex artery continues into the subsinusal groove with the homonymous branch.

This species have been identified anastomose visible to the naked eye between the terminal branches of the arterial tree.

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DETERMINATION OF VALUES OF THE ELECTROCARDIOGRAM'S MAIN COMPONENTS REGISTERED ON CALVES AT DIFFERENT AGES

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Abstract

This study aims to determine the values of the main components of the calf electrocardiogram (ECG), because we believe that at this age, the knowledge of these values is useful in veterinary medical decisions. Duration of the ECG components (waves, segments and intervals) provides valuable information on electrical phenomena and therefore the mechanical activity of the heart during a cardiac revolution. Also, in our study we calculated the heart rate electrocardiographic (based on R-R interval), this being the main parameter for monitoring the cardiac function. To achieve the objectives we established a group of 10 healthy calves from Romanian Black Spotted breed, to whom we recorded the electrocardiograms at different ages. Electrocardiograms were then interpreted, and the results were compared with results obtained by other authors.

Key words: *age, calf, component, electrocardiogram, value.*

INTRODUCTION

Studying veterinary literature in the field, we found that there is a high incidence of heart disease in calves (myocardium dystrophies, changes in size of the compartments of the heart, pericardial effusion), and also that at this age there is a whole spectrum of pathological conditions (digestive disorders, respiratory, etc.) which are passed on cardiac function (Codreanu et al., 2012). We observed that cardiac pathology is quite present in calves especially after birth, based on the myodistrophy due to the deficiency in Vitamin E and Selenium (Codreanu et al., 2013).All these conditions (cardiac or extra-cardiac) can cause changes in electrocardiographic parameters both in terms of wave amplitude value and values of electrocardiogram's components (Brăslașu et al., 2004).This study is addressed only to the latter aspect, because we appreciate that knowing the electrocardiogram values is a very useful component for the veterinary doctor, allowing him to easily observe changes in heart rate (tachycardia, bradycardia, arrhythmias of various types).

Also, knowing the values of the ECG allows the veterinarian to obtain data on electrical events that occur in the myocardium (atrial depolarization, ventricular depolarization and ventricular repolarization), changes in levels of some components of the electrocardiogram indicating current conduction disorders of cardiac action. For the above reasons, we believe that, in calves, knowing the values of electrocardiogram has a practical significance.

MATERIALS AND METHODS

To register electrocardiogram on calves, we used a portable electrocardiograph (ECG machine), collectors type alligator and contact surface between the animal skin and catcher (rubbing alcohol or gel). For the registration of calves' electrocardiogram it is recommended that the animals are examined in a standing position (ensuring a slight contention by one person is enough) and that the hair is shaved on the area where the collectors are placed (because hair can be a barrier against bio-currents cardiac transmission from the skin to

collectors).The parameters used in our study were the recording speed of 25 millimeters per second and amplitude of 10 mm for one mV. To register electrocardiogram on calf we used limbs leads, which involve placing electrodes on the body surface as follows: red electrode behind the right olecranon, yellow electrode behind the left olecranon, green electrode at the inguinal fold region on the left side, and the black electrode at the inguinal fold region on the right side. By placing electrodes in this way, we can record 6 ECG leads: 3 bipolar leads (D I, D II and D III) and 3 unipolar leads (aVR, aVL and aVF) (Dojana et al. 2015). Electrocardiograms were recorded on a group of 10 calves at the ages of 7 days, 30 days, 60 days, 90 days, 120 days, 150 days and 180 days. The values obtained were used to calculate the length of the main components of the electrocardiogram.

RESULTS AND DISCUSSIONS

In our research, regarding the values of some components of the electrocardiogram we determined the following electrocardiographic parameters: the duration of the P-wave, P-R interval (P-Q) (which represents the atrial systole and diastole), complex ventricular (QRS interval) (representing ventricular systole and diastole), Q-T interval, T-wave, interval P-T (which represents the duration of a heart revolution), the segment T-P (which represents the duration of general diastole), R-R interval (which is the interval between two heart revolutions), the S-T segment and heart rate (calculated based on the R-R interval).

We specify that the duration of electrocardiogram's components does not depend on the lead used for recording ECG where measurements are made, because the time required for the cardiac current action to cross the entire myocardium is the same no matter of the lead system used for recording. For this reason, we analyzed and measured electrocardiograms recorded in D II and D III, these leads giving the highest amplitude ECG waves, therefore giving the electrocardiogram the best aspect regarding the calculation of the components' duration (waves, segments and intervals). Our results are presented in the following table and will be accompanied by brief comments.

Table 1. The average duration of electrocardiogram components recorded in calves of different ages (seconds)

Age	P	P-R P-Q	QRS	Q-T	T	P-T	T-P	R-R	S-T
7 days	0.04	0.12	0.04	0.24	0.06	0.28	0.10	0.4	0.12
30 days	0.04	0.12	0.04	0.24	0.06	0.36	0.12	0.50	0.10
60 days	0.04	0.12	0.04	0.24	0.08	0.36	0.14	0.52	0.12
90 days	0.04	0.12	0.04	0.24	0.06	0.36	0.20	0.54	0.10
120 days	0.04	0.12	0.04	0.24	0.08	0.34	0.20	0.56	0.14
150 days	0.04	0.12	0.06	0.26	0.08	0.40	0.24	0.64	0.12
180 days	0.06	0.14	0.04	0.28	0.08	0.42	0.28	0.72	0.18

Looking at the data shown in Table 1, it is noted that the duration of the electrocardiographic waves resent variations and the values obtained, as well as their interpretation are given below.

P-wave duration was 0.04 seconds in all the studied age groups, with one exception (in calves older than 180 days atrial depolarization lasted longer, with the value of 0.06 seconds) (Fig. 1). This change can be explained as the calf gets older, a diminution of the cardiac current's speed is located at the excitoconductory system, thanks to the increasing of beat volume.

Regarding the duration of ventricular complex, the values we obtained were 0.04 seconds at calves from all studied age groups, except for calves older than 150 days, observation which we cannot explain. We observed that our values fall between the limits encountered in veterinary literature (Brăslașu, 2000; Mendez et al., 2001). T-wave duration was between 0.06 and 0.08 seconds, with values lower than those found in veterinary literature (Brăslașu et al., 2014; Mendez et al., 2001).

Regarding the length of segments and intervals, the values obtained by us will be listed below accompanied by interpretations and comparisons with results disclosed by other authors.The average duration of the P-R interval was 0.12 seconds at all ages and 0.14 seconds for calves older than 180 days (so, atrial depolarization and repolarization takes longer at this age).

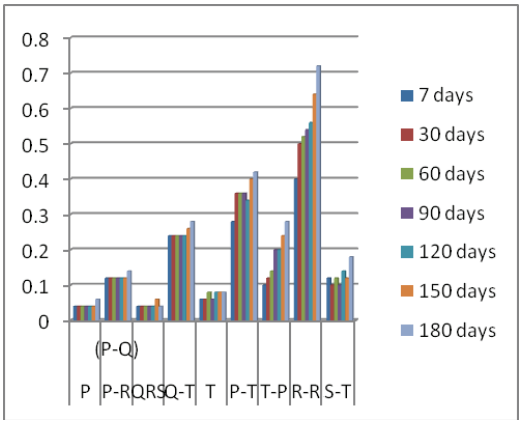

Figure 1. Mean values of electrocardiogram's components of calves at different ages

During the Q-T interval (which represents the ventricular systole and diastole) is ranged from 0.24 seconds (at calves aged between 7 and 120 days) to 0.28 seconds (calves at 180 days).

Regarding the duration of the R-R interval, which is the interval between two heart revolutions, we obtained values varied between 0.40 seconds and 0.72 seconds, which were positively correlated with age calves, and fit within the range found in the literature in the field (Brăslașu et al., 2014; Chalmeh, 2014).

During the P-T interval (which is the duration of a cardiac revolution) was ranged between 0.28 and 0.42 seconds. We observed that the correlation between this growth and calves' age is positive, signifying a prolongation of cardiac revolution along with the growth and development of the myocardium (heart enlargement and thus an increase stroke volume).

In terms of duration T-P segment (which is the duration of general diastole), the values that we obtained ranged in ascending order between 0.10 and 0.28 seconds and were positively correlated with calves' age, falling within the range found in the veterinary literature (Brăslașu et al., 2004; Mendez et al., 2001). This finding could be due to the neurovegetative balance, which is final at weaned calves, moved in compartments addressed to increase youth.

The final analyzed component was S-T segment, whose duration was between 0.10 and 0.18 seconds, with variations between studied age groups.

Values obtained for heart rate (based on R-R interval) are shown in Table 2 and their dynamic evolution can be observed in Figure 2. We mention that the heart rate was calculated at end of the recording, because at that time the animal is calm and accustomed with the sensors and with the presence of the persons who restrained it and recorded the ECG.

So, at this juncture, the heart rate values are lower compared to those from the start of the recording.

Table 2. Electrocardiographic calculated values of heart rate in calves of different ages (bpm)

Age	Mean heart rate	s
7 days	150	2.6
30 days	120	2.4
60 days	115.3	2.1
90 days	111.1	2.01
120 days	107.1	2.4
150 days	93.7	1.8
180 days	83.3	1.7

Studying the data presented, it can be observed that heart rate is negatively correlated with calves' age, the values we obtained falling between 150 bpm at 7 days old calves and 83.3 bpm at 180 old days calves. In calves aged between 30 and 90 days, heart rate changes are less obvious, falling between 120 and 111.1 bpm.

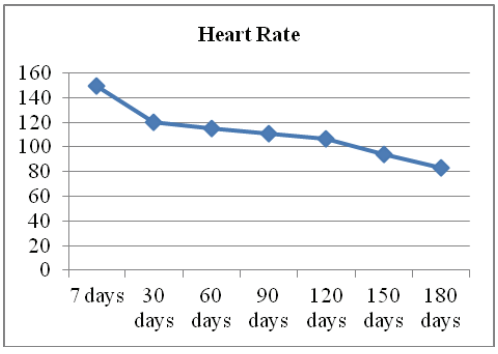

Figure 2. Changes in heart rate electrocardiographicaly calculated on the basis of R-R interval in calves at different ages

CONCLUSIONS

Some of monitored parameters (QT, PT, TP, RR, ST) increased with advancing age of the animals.

Other monitored parameters (P, PR, QRS,T) showed no changes with advancing age.

The heart rate is negatively correlated with the age of calves. In calves aged between 30 and 90 days, the heart rate changes are less obvious falling.

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MAJOR SALIVARY GLANDS TOPOGRAPHY IN RATS AND THEIR RELATION WITH THE SURROUNDING ANATOMICAL TISSUES

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Abstract

The structure of the salivary glands is different depending on the species and diet. The glandular secretion can be serous, mucous or mixed. Within the same order, for instance in rodents, there are dissimilarities between the major salivary glands, even if the diet is similar. In this study, we used five Wistar rats, originating from the University of Medicine and Pharmacy "Iuliu Haţieganu" biobase, in Cluj-Napoca. After inducing neuroleptanalgesia, the method of euthanasia was cervical dislocation and then a stratigraphic dissection was accomplished. We mention that these euthanasia methods are allowed by 2010/63/UE directive of the European Parliament and the Council from September 22nd of 2010, regarding animal protection used for scientific purposes. The external features were assessed and compared to published literature on other similar species. The macroscopic examination revealed that the major salivary glands in rats are similar to those from other species of mammals when referring to the general macroscopic aspect. The parotid gland is localised at the base of the auricular concha, without exceeding the outer ear base, extended distally in the ventral cervical region. The rats' ventral extremity of the parotid gland faces and ends with a sharp angle, toward the scapular-humeral joint, covering the jugular gutter with this layout. Aboral border of the mandibular gland is covered by ventral extremity of the parotid gland, both mandibular glands being near the external jugular veins. As a location, the mandibular glands in rats reside in the sublaryngeal and subtracheal region. Sublingual glands are located orally from the rostral pole of the mandibular glands, maintaining in a certain way the same layout as the mandibular glands.

Key words*: parotid gland, mandibular gland, sublingual gland, rat.*

INTRODUCTION

Differences can be found between the salivary glands in mammals, depending on the species and the type of food (herbivorous, carnivorous or omnivorous) (Baciu, 1970; Tache, 1994).
Amongst salivary gland functions, we include the lubrication of the first segments of the digestive tract, preparing the food for digestion, maintaining the integrity of the buccal cavity, regulating the local bacterial flora, facilitating teeth remineralization, and neutralising bacterial plaque (Yazdani Moghaddam et al., 2009; Tucker and Miletich, 2010; Yasear et al., 2012; Gal and Miclăuș, 2013).
The specialized literature we consulted on didn't contain enough information about the major salivary glands' topography, which is why we decided to undertake a macroscopic investigation of these, and keeping track of some particular aspects of the major salivary glands.

Salivary glands are extension of the digestive tract, being responsible for the secretion of saliva. These represent a group of organs which secrete a watery substance, named saliva, which fulfils several physiological functions. Thus, its role is to moisten the mouth and to dissolve food (Asari et al., 2000; Barone, 2009). Likewise, it ensures the protection of the teeth and also the soft tissues that surrounds them (Asari et al., 2000; Tucker and Miletich, 2010). Salivary glands are found in mammals, reptiles, and birds, being a feature of terrestrial species.
Their structure and development are in close relations with the diet (Barone, 2009).

MATERIALS AND METHODS

In this study, we used 5 Wistar female rats, originating from the University of Medicine and Pharmacy "Iuliu Haţieganu" biobase, in Cluj-Napoca.

The equipment used for this task was the dissection instruments and the Nikon D3000 photo camera.
In the case of our subjects, we induced neuroleptanalgesia by giving a supradose of Xylazin Bio 2% intramuscularly, and after 15 minutes a supradose of Ketamin 10, according to Ramsey's findings (2011).
After inducing neuroleptanalgesia, the euthanasia was done by the method of cervical dislocation. We mention that these methods are approved by the 2010/63/UE directive of the European Parliament and the Council from September 22nd of 2010, regarding animal protection used for scientific purposes.
After euthanizing the subjects, we performed a stratigraphic dissection of the regions containing the major salivary glands.

RESULTS AND DISCUSSIONS

In rats, the parotid gland is a paired organ, situated in the parotid region. The parotid gland is seen after making an incision ventral to the base of the external ear, then removing the skin, the parotid fascia, and the adipose tissue covering the gland.
The images obtained with the subject in latero-lateral decubitus and the latero-lateral photographic exposure show that the parotid gland is localised at the base of the auricular concha, without exceeding the outer ear base. From the ear base, the parotid gland extends distally in the ventral cervical region (Figure 1).
This aspect proves that the parotid gland can be of a cervico-cephalic type when we make a reference to the region that is being occupied by it.
Orally to the parotid gland, a disk-shaped gland can be observed. The length of this gland is only up to the proximal half compared to the length of the parotid gland. This gland is called the extraorbital lacrimal gland. The gland can be easily noticed due to having a darker colour than the parotid gland (Figure 1).
On the side of the parotid gland, glandular parenchyma can be noticed being surrounded by conjunctive septa. The medial side covers both vascular formations and formations such as muscle and nerve. As for the muscle formations, the parotid gland is in accordance with the ventral cervical muscles and the cephalic extremity of the sternocephalic and brachiocephalic muscles.
We point out that the rats' parotid gland covers the oral extremity of these muscles. In the ventral cervical region it can be observed that the parotid gland partially covers Wiborg's triangle.
This leads to the assertion that the lingual-facial vein, retro-mandibular, and the sterno-mandibular muscle tendon are partially covered.
The rats' ventral extremity of the parotid gland faces and ends with a sharp angle, toward the scapular-humeral joint, covering the jugular gutter with this layout. The nerve formations that come into contact with the parotid gland are represented by the branches of cervical spinal nerves, specifically auricular branches. The extraorbital lacrimal gland located orally by the parotid gland, touches with its medial side the branches from the facial nerve, namely the dorsal and ventral buccal nerve. The buccal nerves' origin is covered by the profound side of the parotid gland.

The mandibular gland in rats is a paired gland, located on both sides of the sagittal plane. We note that, compared to the parotid gland, the mandibular gland is darker in colour, with a reddish hue. It has an oval shape with a convex aspect of the upper ventral side, and the dorsal side contacts the underside of the parotid gland (Figure 2).

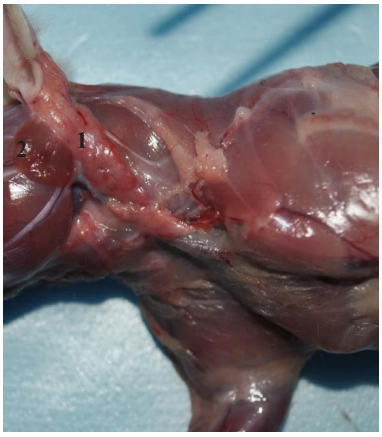

Figure 1. Parotid gland in rats (1); Extraorbital lacrimal gland (2)

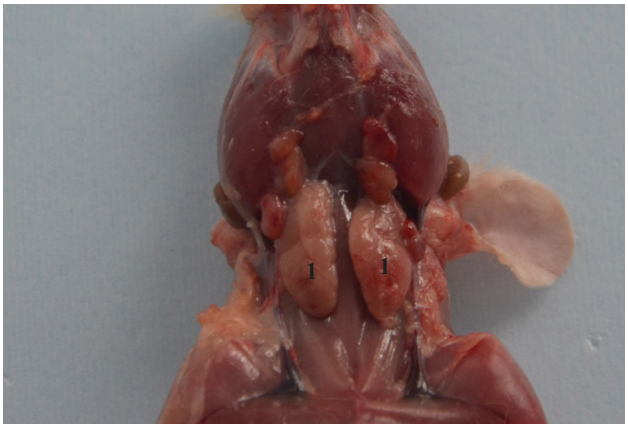

Figure 2. Mandibular glands in rats (1)

Anatomically, we mention that both mandibular glands are near the external jugular veins. As a location, the mandibular glands in rats reside in the sublaryngeal and subtracheal region. The mandibular lymph node is in direct contact with the mandibular gland, and drains the mandibular gland region (Figure 3).

Sublingual glands are located orally from the rostral pole of the mandibular glands, maintaining in a certain way the same layout as the mandibular glands, namely their location on either side of the sagittal plane. Sublingual glands are in connection with the mandibular glands. Their colour is different from the mandibular glands by having a darker shade. The above mentioned features were visible only after removing the common fascia covering both sublingual and mandibular glands (Figure 4).

The location of the sublingual glands was found at the recurved branches of the mandible, caudal from the mandibular lymph node. In terms of general appearance, the two glands appear to be situated at the medial side of the recurved branches of the mandible, in the under-hyoid region.

The vast majority of the studies were based upon the pathological, immunohistochemical or structural parts of these major salivary glands, focusing only on the visible anatomical differences in the species under study. Specialized literature shows studies about the morphology and histology of the major salivary glands (Young and Van Lennep, 1978; Pinkstaff, 1980). A considerable diversity has been observed in the structure of the salivary glands in different species of mammals by both electron microscopy and optical microscopy (Mohammadpour, 2010).

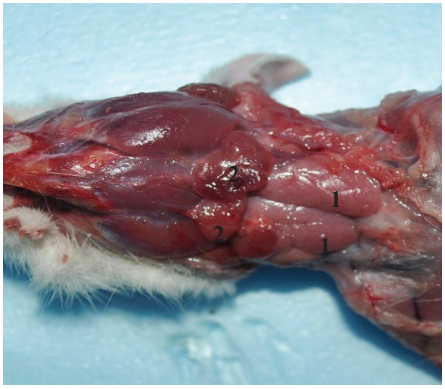

Figure 3. Mandibular glands in rats (1); Mandibular lymphnodes (2)

Some authors maintain that the rats' parotid gland consists of two distinct parts. The first portion is located above the base of the outer ear, disk-shaped, with the medial side being slightly concave, yellow-brown coloured, and the second one surrounding the first in a ventral way (Parhon et al., 1957). Later, this assumption was debunked, revealing the existence of a channel that is connected to the lacrimal groove; this portion actually being the extraorbital lacrimal gland (Jonjic, 2001; Quesenberry and Carpenter, 2004; Di Palma et al., 2006; Amano et al., 2012).

Our results are similar to those of other authors who have also found that the salivary glands in humans and rodents (mice and rats) consist of three pairs of major salivary glands: parotid, mandibular and sublingual (Saracco and Crabill, 1993; Amano, 2011). In laboratory rodents, salivary glands are described in many studies. However, the glands have certain features that prove to be an obstacle to some researchers. This border includes the small sized salivary glands, the thin diameter of the glands channels, and the short lifespan of these animals (Štembírek et al., 2012).

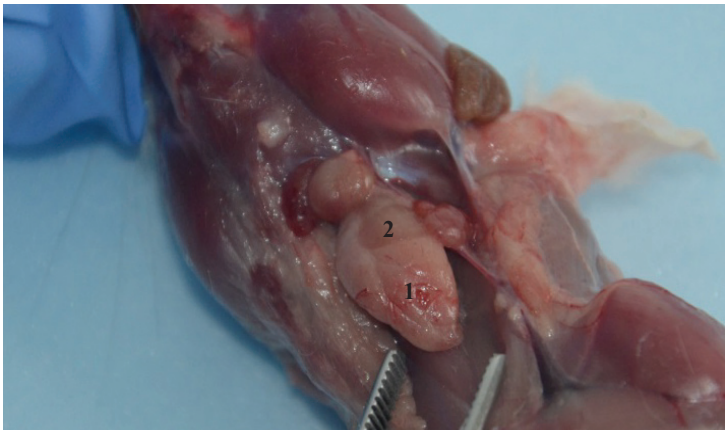

Figure 4. Mandibular gland in rats (1); Sublingual gland in rats (2)

The rats' parotid gland is located behind the ear, in the area below the ear, caudally neighbouring the mandibular gland. This gland is embedded in the subcutaneous adipose tissue. After removing the skin, the gland resembles the pancreatic structure (Jonjic, 2001).

We agree and confirm what Da Cunha Lima Marta et al. (2004) say, namely that the extraorbital lacrimal gland is found beneath the skin, on the lateral side of the cheek region, near the outer ear. As previously mentioned,

this gland has been confused with the parotid gland.

Comparable results have been reported by other researchers as well. In rodents, the sublingual glands are located together with the mandibular glands in the anterior cervical region, between the mandibular and the sternum lymph node. Both glands are enveloped by a common fascia (Amano et al., 2012). As we have seen, sublingual glands have the smallest dimensions compared with the other major salivary glands in rats (Da Cunha Lima Marta et al., 2004).

In rats, the parotid and mandibular gland are about the same size. The mandibular gland is large, elongated, and localized caudally from the mandibular angle. The sublingual gland is small, round, located on the rostral outskirts of the mandibular gland. The sublingual gland's channels run parallel to the mandibular gland, opening at the sublingual plica. Superficial lymph nodes of the head and neck are often confused with salivary glands (Suckow et al., 2006).

The parotid gland of the forest rat (*Neotoma lepigus*) is sitting on one side of the masseter muscle, a portion above the ear base and the other behind it. Cranio-ventrally, the glands give the impression that the two meet one another. In this region, they are placed partially on the mandibular glands. The mandibular glands are in contact with one another along the sagittal line of the neck (Howell, 1926).

The giant African rat (*Cricetomys gambianus*) has three pairs of major salivary glands: parotid, mandibular, and sublingual. These paired glands are well developed. The parotid is oval, flat, and lobed and it extends along the caudal margin of the mandible, towards the ventral side of the larynx and caudally facing the clavicle. The parotid channel has a trajectory towards the mouth, at the rostral edge of the masseter muscle, opening at the second upper molar (Olayemi et al., 2001). The mandibular gland is located in the ventral cervical region, flanked orally by the mandibular lymph node, and medially by its pair.

The sublingual gland is shaped like a lens, being in an intimate contact with the mandibular gland, but with a darker colour. Both channels – mandibular and sublingual – course in a rostral way, along the medial surface of the mandible, but they split up at some point and open separately.

Given the considerably higher size of the African rat compared with the Wistar rat, some authors maintain that the African rat is more suitable for medical research. These things give credit to growing the African rat as a substitute for Wistar rats regarding research (Olayemi et al., 2001).

The African rat is considered to be the future laboratory animal, as a replacement for the Wistar rat, owing to its larger size (Ikpegbu et al., 2014; Mustapha et al., 2015).

CONCLUSIONS

Unlike mice, rabbits and guinea pigs, where the end of the parotid gland slightly surrounds the back of the ear conchae, in rats, the parotid gland is located at the base of the ear, but without exceeding the base of the outer ear. In rats, the ventral extremity of the parotid gland extends ventrally, reaching the ventral cervical region. The ventral extremity of the parotid gland in rats ends with a sharp edge towards the scapulo-humeral joint, in comparison with the ventral extremity of the parotid gland in rabbits, which ends with a sharp edge in the oral region, in contact with the mandibular gland. The parotid gland contacts the mandibular gland. The joint capsule covering both mandibular and sublingual glands is seen in rats. Mandibular glands are in intimate contact with the sublingual glands. The rat has a well-developed extraorbital lacrimal gland, localized anteriorly to the parotid gland.

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MORPHOLOGICAL PARTICULARITIES OF THE TEETH CROWN IN GOLDEN JACKAL (*Canis aureus moreoticus*)

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Abstract

A thorough understanding of dental and oral anatomy is essential for a proper recognition of all members of the carnivore species and to recognize the various signs of disease. As long as the golden jackal spreading in Eastern Europe is steadily increasing, this study aims to present a detailed description of morphological features of golden jackal dental anatomy in order to be used in clinical practice and research. The anatomical crowns of the teeth from superior and inferior jaws of seven golden jackals were examined. The complete dental formula for the permanent dentition in golden jackal is I 3/3 C1/1 PM4/4 M2/3 x 2=42teeth. The inferior dental arch is anisognathic, narrower and shorter compared to the superior dental arch. The superior incisors are located slightly rostral from the inferior incisors. Their size increases from the central to the lateral incisors, each incisor crown showing a prominent cingulum and three tubercles. The canine teeth were similar in length and width, having a simple crown. The first premolar is the smallest on both dental arches, having one tubercle, while the second and third premolars have in addition a small distal tubercle. The superior forth premolar and the first inferior molar form the carnassials tooth. The superior carnassial has three distinguishing lobes: paracone, metacone and protocone. The upper molars have a short, wide and highly rough anatomical crown. The inferior carnassial is the strongest tooth with a three-lobed pattern. Inferior molars are smaller than those of the superior arch. The morphology of the crown of the golden jackal teeth is similar to that described in dogs.

Key words*: teeth crown, golden jackal, dentition.*

INTRODUCTION

The golden jackal is the most typical member of the genus Canis, having a medium size and no outstanding features. Despite to its phenotypic and genotypic features the golden jackal resembles the grey wolf and coyote rather than the black-backed jackal, side-striped jackal and Ethiopian wolf. Because of this, the most frequent comparisons were made with wolfs. Nevertheless, in scientific literature, there are few anatomical reports of various anatomical systems of the golden jackal, and a detailed morphological description of it has not been made. Compared to wolves, the golden jackals'projections of the skull is less developed. Even though the canine teeth are large and strong, they are thinner than wolfs' and the carnassials are weaker. Its relatively short facial region, weaker teeth row are related to the jackal's diet, composed of small birds, rodents, small vertebrates, insects and carrions. Denied carrion or prey, it feeds on fruits and seeds. This eating behaviour has imposed the occurrence of certain specific characteristics of the dentition. Every tooth, no matter its form and function has the same elements. The structures are crown, enamel, cementum, dentin, pulp, root and periodontal ligament. Anatomical crown is the part of the tooth that is occlusally located to the dentino-enamel junction, or the portion of the dentin of a tooth that is covered by enamel. The clinical crown is the portion of a tooth that is above the gingival margin or the exposed part of a tooth within the mouth. In the present study has been performed a detailed description of the clinical crown of the teeth in golden jackal in comparison with the domestic dog. Domestic dogs possess a heterodont, a diphyodont dentition with anelodont and brachyodont teeth (Evans and De Lahunta 2013). Compared to dogs, horses have hypsodont teeth (Konig et al. 2014). Rabbits have heterodont, diphyodont, with all teeth being elodont (aradicular hypsodont) (Quesenberry and Carpenter 2012, Stan 2014). Those three examples are the most representative among animals. The dental

formula for primitive carnivores consists of 44 teeth (three incisors, one canine, four premolars and three molars in each quadrant) but the evolved carnivore's dentition shows several adaptations to diet (Evans and de Lahunta, 2013). Domestic dog's teeth have short crown, covered only by a thick layer of enamel, obvious neck and long roots covered by cement (Barone 1997).

MATERIALS AND METHODS

Seven adult golden jackals (*Canis aureus moreoticus*) were examined, four male and three female. The subjects were hunting harvest, being part of an ongoing study of the anatomical description on various systems in golden jackal. The oral cavity and teeth were examined before and after exposure of the oral cavity. To expose the oral cavity, an incision was made on each side, starting from lips commissure, in horizontal line and parallel to the mandibular arch, followed by a vertical incision, along the recurved mandibular branch. The entire study was conducted in accordance with the Protocol on Medical Ethics and in compliance with the Directives 63/2010 of the European Parliament and of the Council on the Protection of Animals Used in Scientific Research.

RESULTS AND DISCUSSIONS

The particular anatomical configuration of the viscerocranium in jackal gives the oral cavity a long and narrow appearance (Figure 1). The wide oral slit, starting from the oral angles reaching close to the carnassials.

Figure 1. Viscerocraniumof the golden jackal with elongated appearance of the oral cavity. Wide oral slit exceeds the carnassials plan-arrow

The dental formula contained 42 teeth in all subjects:

$$I:\frac{3}{3};\ C:\frac{1}{1};\ P:\frac{4}{4};\ M:\frac{2}{3}=21\times 2=42.$$

There were no differences in shape, number and disposition between the dentition of males and females. In dogs, Lorber et al. (1979) found differences between male and female canine crown, male having longer and wider canine crowns.

Generally, in mammals the shape, position and even number of teeth can differ according to age, breed and subject (Barone 1997). Thus, canines have 32 decidual and 42 permanent teeth. In diphyodont mammals the variations of number and shape are obvious, especially regarding the decidual and permanent dentition.

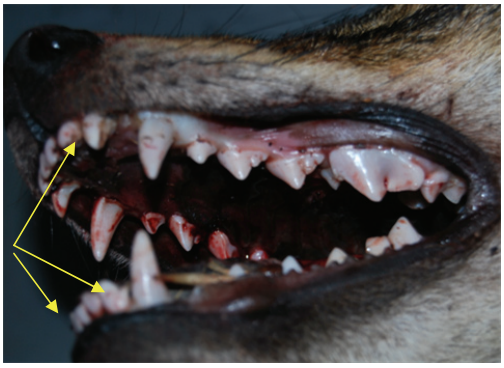

Figure 2. The short incisors crown compared with the large crowns of the majority of the teeth

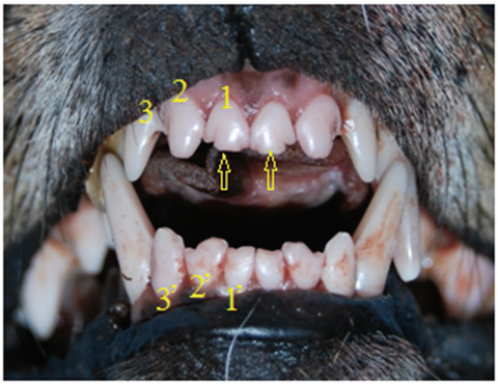

Figure 3. The short incisors crown with central prominent lobe, visible on the central incisors-arrows. Their size increased from central-1 to middle-2 and lateral-3 incisor teeth.

In canines, when the first premolar is considered deciduous, the dental formula is as follows:

$$i:\frac{3}{3};\quad c:\frac{1}{1};\quad \mathrm{m}:\frac{4}{4}=16\times 2=32,$$

In studied subjects, the incisor teeth (*Dentes incisivi*) had a short crown (*Corona dentis*) compared to the large crown of the premolar and molar teeth (Figure 2 and 3). More developed on the superior arch, their size increased from the central to lateral incisor, being rostral slightly arched (Figure 4). Their crowns were flattened and laterally compressed, heavilyfixed. The oclusal border (*Margo occlusalis*) of the crown has shown three salient cusps (lobes), the middle one being more prominent (Figure 4). The smooth vestibular surface (*Facies vestibuaris*), convex in all directions was slightly narrowed towards the neck of the teeth (Figure 4).The lingual surface (*Facies lingualis*), slightly swollen near the neck (*Cervix dentis*) showed a strong girdle (*Cingulum*) in all subjects. Its extremities from the base of the cuting edge were more obvious and formed on each side a small tubercle. The cingulum concavity delimited a small recess which subdivided the large prominent central tubercle. This tubercule was disposed along the cutting edge.The large contact surfaces (*Facies contactus*) from the incisor neck show a sharp reduction before their ending on the cingulum extremities. The oclusal border, like a delicate pointed arch (ogive), was surrounded at its base

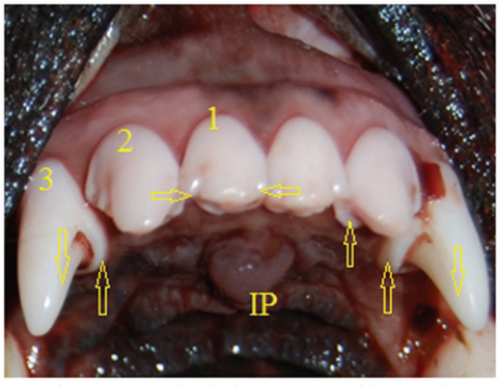

Figure 4. Superior incisors (1,2,3) with smooth, convex appearance of vestibular face. Strong cingulum on the lingual side-up arrows delineated two tubercles on its extremities-horizontal arrows, and a long, narrow central lobe of the lateral incisors-down arrows. IP-incisive papilla

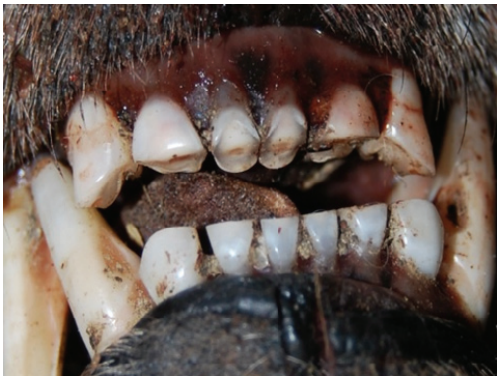

Figure 5. The tooth wear starting from the central lobe of incisors leave the occlusal surface, thick and straight. Note the obvious reduction of the crowns starting from the middle incisors and stump appearance of teeth

by the two tubercles that marked the end of the cingulum (Figure 4). These tubercles were separated from the central cusps by a small notch.Therefore, this three-lobed appearence, with a prominent central lobe like a „clover" shape or like a „lily flower" is similar with the pattern described in canines (Evans and de Lahunta 2013). This disposition announces the three tubercules pattern of the premolars and molars. The incisors neck was well marked in all subjects.

In older subjects (2 subjects) it was noticed a conspicuous wear of the teeth (Figure 5). The wearing was started primarily on the cutting edge of the central lobe (on the ogiva), which was shortened up to the two tubers on the edges (Figure 5). In this way, the occlusal edge

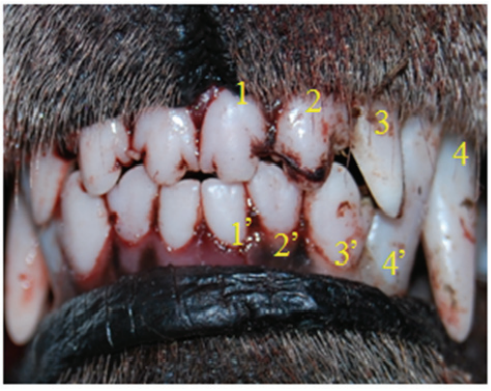

Figure 6. Superior incisors (1,2,3) are located rostral to the inferior incisors (1', 2', 3'). The superior canine tooth (4) is separated by lateral superior incisor by an interdental space, matching the inferior canine tooth (4'), in scissor like appearance

became straight and thick, the “lily flower” disappeared and the levelling appeared. The crown was strongly reduced, taking the form of a stub, the incisors distancing themselves from one another. Gums also suffered a marked process of retraction, emphasizing the appearance of stump incisors. The wear was most evident on the central incisors, progressing towards the middle and the lateral incisors (Figure 5). The wear process described here is similar to that of carnivores (Evans and de Lahunta 2013). There were few differences of size, pattern and disposition between the superior and inferior incisors. Regarding the incisors dimensions, the central were smaller than the middle, which in turn were smaller than the lateral. The obvious difference was shown on the upper jaw. Upper incisors were almost two times stronger than those of the same rank from the lower arch (Figure 4, 5 and 6). Prominent cingulum and stronger central lobe, well separated from the marginal lobes, were well defined characteristics, especially at the central incisors. The lateral incisors showed a long and sharp central lobe in absence of the distal lobe; resembling somewhat and in a small way, the canine pattern. In the occlusion of the arch the lower canine is positioned slightly distal and opposite from the superior lateral incisor.

The upper incisors exceeded rostrally to the lowers, so that, during occlusion, the sharp edges of their lingual surfaces are positioned over the vestibular surface of the lower incisors (Figure 6 and 7). Also, from the superior lateral incisor to the superior last premolar, the upper and lower teeth alternate in their disposition in the dental arch. This type of dentition is called "scissor" dentition and is described especially in dogs (Evans and de Lahunta 2013). Moreover, the central incisors only partially cover their counterparts and the adjacent parts of the inferior middle incisors. In turn, the middle superior incisors cover the occlusal edge of the two inferior lateral incisors. The superior lateral incisors were placed between the inferior lateral incisors and inferior canine teeth, a small diastema separating them from the upper canines. The dolichocephalics canine breeds retain this disposition, while brachiocephalic breeds have a marked inferior prognathism, in which the superior incisors and canines are placed more at varied distances behind their counterparts, reducing their effectiveness of cutting (Barone 1997, Verstraete and Tsugawa 2015). On each jaw, the dental arches (A*rcus dentalis superior et Arcus dentalis inferior*) described an arc, the upper one being wider and stretched compared to the lower jaw arch. The inferior dental arch showed a deeper curvature, was narrower and shorter compared to the superior arch.

Canines (*Dentes canini*), or "fangs" as they are called, were highly developed, conical shaped, having a distal (caudal) and concave tilting. Compared with the incisors, canine’s neck was less marked (Figure 8). The vestibular surface was convex and smooth. The lingual surface was crossed by a lingual groove limited by a

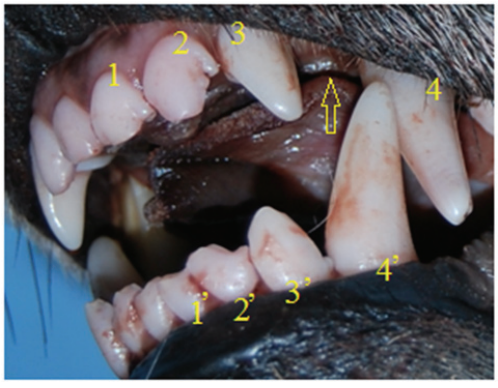

Figure 7. The crown of superior lateral incisors-3, are largest and slightly hooked caudally similar with the next canine tooth. A small *diastema*-arrow, separated the lateral superior incisors from the canine teeth

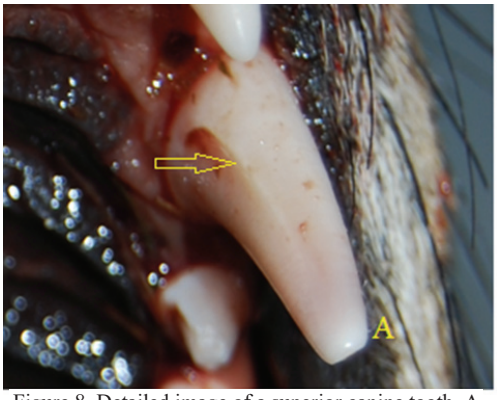

Figure 8. Detailed image of a superior canine tooth. A small ridge on the lingual side-arrow delineates a reduced groove. Note the conned shape, distally oriented and rounded apex-A, of the canine tooth

small ridge at the edge of its mesial surface (Figure 8).The superior canines appeared stronger than the lower ones, their roots being twice as long as the crown. On the distal edge, near the cingulum the canines' circumference was visibly increased. The canines were less titled on the vestibular surface, their crown being less outwards inclined. In occlusion, the lower canine is placed in front of the upper canine, which in turn, sits next to a small diastema. This diastema separates the lower canine from the first premolar (Figure 9).

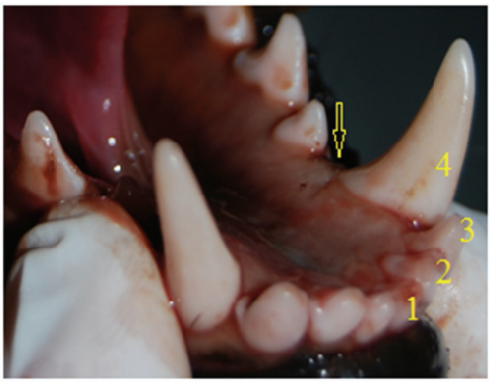

Figure 9. A small *diastema* separate the inferior canine tooth-4, from first premolar tooth-arrow

According to the anatomical rule, on each arch the premolars (*Dentes premolares*) and molars (*Dente smolares*) were classified in mesio-distal direction (rostro-caudal) in: precarnassials, carnassial or sectorius (*dentes sectorius*) and postcarnassial or tuberculosis teeth (Figure 10). Thus, the last upper premolar tooth will be described as upper carnassial and the first lower molar tooth as lower carnassial tooth. These teeth were the largest shearing teeth on both dental arches. These characteristics are similar to those of domestic dogs (Barone 1997, Evans and de Lahunta 2013). Except the last two molars, due to their blade like pattern, slicing and chapping function, on each arc all teeth have achieved a perfect secodont type of dentition. In dogs, deciduous dentition includes on the upper jaw, besides the incisors, two precarnassials, the carnassial and one postcarnassial or tuberculosis tooth. The lower jaw includes three precarnassials and one carnassial tooth (Barone 1997). In the deciduous dentition the first premolar is sometimes described as a precursor, lacking the permanent tooth (Verstraete and Tsugawa 2015), but in accordance with this paper, rather it should be considered a persistent deciduous tooth (milk) continuing in the permanent dentition. The rest of the teeth resemble the shape and disposition as in adults, but are smaller, sharper, having narrower cusps. Their occlusion is as in adults.

The permanent dentition of the golden jackals from the present study included six cheek teeth (premolars and molars) on each superior quadrant and seven cheek teeth on each inferior quadrant (Figure 10). The first three premolar teeth are the precarnassial teeth. The first was smallest with a simple, pointed crown, whose lingual surface shows a small cingulum and a reduced distal lobe (Figure 11). The next two premolar teeth, larger than the first, slightly

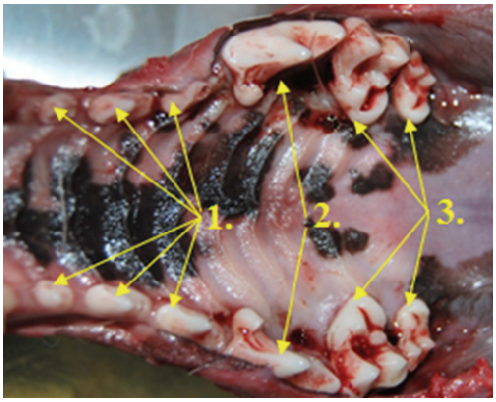

Figure 10. The upper precarnassials-1, carnassials-2 (premolars) and postcarnassials (molars)-3 teeth. Note the strong development of the carnassials and postcarnassials teeth

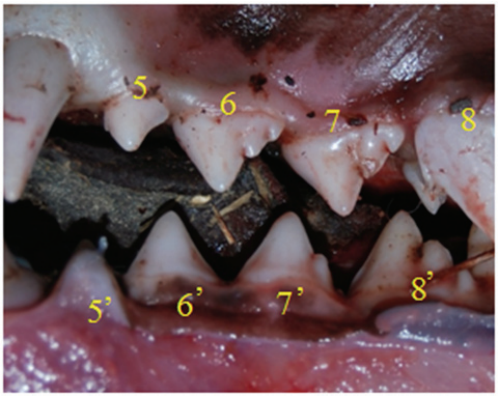

Figure 11. Upper-5,6,7 and lower 5', 6', 7', 8' premolar teeth (precarnassials). The last upper premolar-8 is the carnassial or sectorial tooth

flattened and compressed laterally show three lobes: a prominent intermediate lobe, a short and slightly detached mesial lobe and a long distal lobe (Figure 11). The last precarnassial tooth has a prominent cingulum and a well delineated distal tubercle (Figure 12).

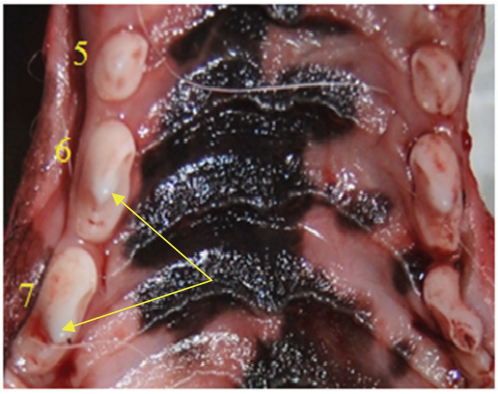

Figure 12. A small, simple crowned of the first upper precarnassial and a well developed intermediate lobe-arrows, of the last precarnassials-6,7

The superior carnassial (or the last premolar tooth) was the stongest tooth on the quadrant. (Figure 13). Three lobes were identifyed: two of them were stronger, being the tooth body, the mesial lobe, named *paracone,* being more prominent than other lobes. The mesial lobe was connected by a sharp ridge to the distal lobe, named *metacone*, which was smaller than the mesial lobe. The third lingual lobe, named *protocone,* was like a reduced, accessory lobe which was connected to the base of the main lobe (paracone) by a girdle or a small crest (Figure 13).

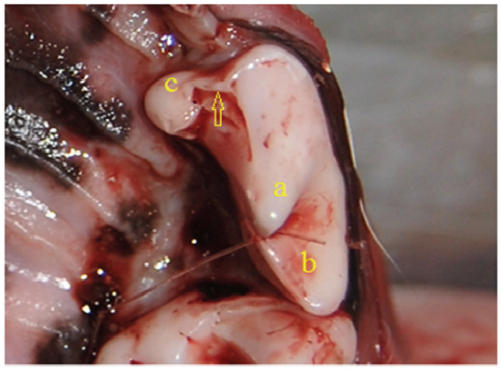

Figure 13. The upper carnassial. a-the mesial lobe (paracone); b-the distal lobe (metacone); c-the lingual lobe (protocone) connected by a small ridge to the base of paracone-arrow

The last two upper molars (or postcarnassial teeth) were well developed (Figure 14). Their crown, short and wide, very rough, was much more developed in the transverse direction than inthe mesio-distal direction. The first postcarnassial tooth (or tuberculosis tooth) was longitudinally shorter than the carnassial, but more developed transversally. Its crown was bordered by a girdle (cingulum), which was extended up to the vestibular surface at the base of two vestibular cusps (Figure 14). Of the two cusps, the mesial one, named *paracone,* was taller. On the lingual surface the cingulum inflated to form a large and short rounded lingual lobe, named *protocone.* Its occlusal surface was subdivided in small tubercles among which the heels of the lower carnassials tooth, affront.

The last postcarnassial tooth (or last molar), was smaller, the two vestibular cusps being reduced and the lingual lobe, *protocone,* being slightly larger, but less mamelonated (Figure 14).

The lower precarnassials were the four lower premolars (Figure 15). The first premolar, like its superior counterpartbut smaller than it, presented a cingulum too and a reduced distal tubercle. The following precarnassials were larger. Their crowns were three-lobed, presenting like the superior premolars, a stronger distal lobe (metaconid) extended in mesio-distal direction. From the second to the fourth premolar, the subdivision of this lobe was clearer.

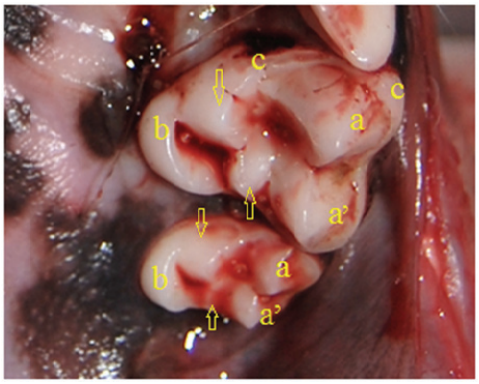

Figure 14. The upper postcarnassials. a (paracone) and a'-the two vestibular cusps; b-lingual rounded lobe (protocone) subdivided in two small tubercles-arrows. c-cingulum

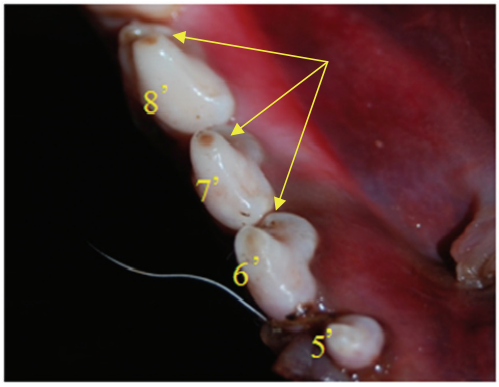

Figure 15. Lower precarnassials 5', 6', 7', 8'. The first premolar-5' is small. The distal lobe (metaconid) is prominent and subdivided, starting from the second premolar-arrows

The lower carnassial appeared stronger than the superior carnassial (Figure 16). The cingulum was relatively small, but the crown was clearly three-lobed. The intermediate lobe, sharp and strong (protoconid) was obviously flanked at its base by a small accessory tubercle distalo-lingual oriented (Figure 16). The mesial lobe (paraconid), shorter, was nevertheless visible, slightly reduced on the lingual part. The short but large caudal lobe has been subdivided into two secondary parts-vestibular (metaconid) and lingual (entoconid).

These parts were separated by a depression, adapted to receive the upper postcarnassial relief, called "heel".

The lower postcarnassials were the last two molars, much smaller than those from the upper arch. The first postcarnassial (or the second molar tooth), held a low crown, slightly wider mesio-distal than in the transverse direction. Its occlusal surface was mamelonated, the distal tubercles being the lowest. The third (or last molar), was very small, having a simple, rounded, less mamelonated crown (Figure 17).

Occlusion of the molar arch was highly efficient on the carnassials tooth due to the maximum development of these teeth. Carnassials teeth were convex on the vestibular side; their aggregate draws a kind of rostral narrow lira, especially on the upper jaw. In the inferior arch the carnassials where less divergent in caudal (aboral) direction. Thus, lower carnassials slid over the lingual surface of the upper counterparts and over the vestibular adjacent lobes of the superior postacarnassials. The sharpest and higher lobe (protoconid) of the lower carnassial, sits in the notch of the first postcarnassial tooth (between metacone and protocone), while the heel facing strong protocone of the upper postcarnassial. Therefore, this complex is permanently sharpened. Because of its positioning in the caudal part of the oral cavity this complex can apply maximum force, easily scissoring the toughest elements, (bones and tendons) without a possible separation. Other teeth have very limited role. Precarnassials are not adjacent; they are arranged alternately, inferiors being placed rostral to the superiors. Due to the reduced volume, the last postcarnassials have only a very superficial action, most often, the inferior postcarnassials are not in contact with their superior counterparts. These features are specie characteristic and are not related with the breed variations of jaws, compared to the incisors disposition, which is strongly related to the breed.

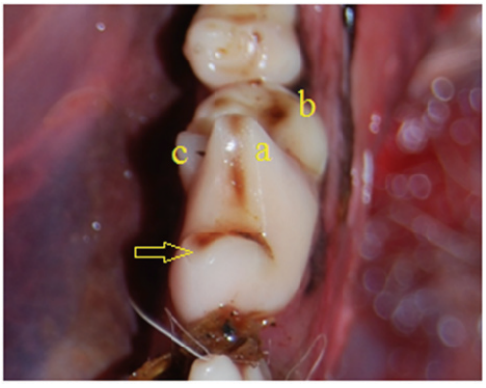

Figure 16. The lower carnassial tooth. The strong intermediate (protoconid) lobe-a. The caudal lobe was subdivided in vestibular (metaconoid)-b and lingual (entoconid)-c lobes. Small accessory tubercle at protoconid base-arrow

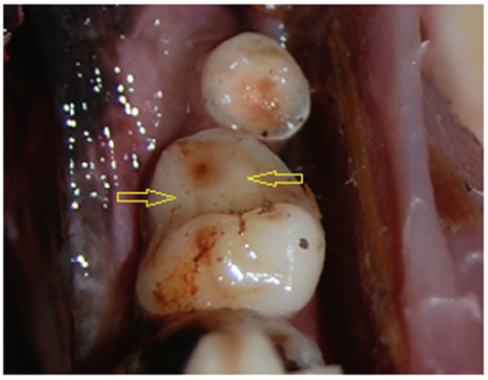

Figure 17. Small distal tubercles-arrows, of the first lower mamelonated postcarnassial tooth, and the smallest last lower precarnassial tooth

In dog the Triadan system is available to identify specific teeth. The number of a tooth is composed of three digit number each of it indicate: the first (in a system of hundreds) indicate the quadrant of the dental arch,1(00) being the upper right; 2(00) being the upper left 3(00) being the lower left and 4(00) being the lower right quadrant. The next two digits indicate the location of the tooth related with the median line, the 01 digit indicate the first central incisor, or the most mesial position of the tooth (Verstraete and Tsugawa 2015). Due to the similarities presented in this paper the Triadan system could be used for reference of the specific tooth in golden jackal.

CONCLUSIONS

The Golden Jackal dentition is similar to that of the dog being: diphyodont, heterodont, brachyodont and secodont type of dentition. In Golden Jackal, the upper dental arch is slightly longer than the lower one, the upper teeth occlusion beind made on the lingual side of the upper teeth, in a "scissor" like action.

Similar to domestic dogs, the Golden Jackal have specialized functional pair of sectorial (carnassials) teeth that consist of the last upper premolar and the first lower molar.

The Triadan system could be used to reference specific teeth in Golden Jackal.

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SERUM BIOCHEMICAL AND HISTOPATHOLOGICAL EXAMINATIONS OF SOME TISSUES OF LAMBS WITH MUSCULAR DYSTROPHY IN VAN

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Abstract

White muscle disease (WMD), is an important disease also known as 'muscular dystrophy' in the lamb and calf. White muscle disease is the result of degeneration of skeletal and cardiac muscles in lambs. Lambs mostly affected with the congenital form either born death or die a few day after birth. The disease is a manifestation of lack of selenium, vitamin E or both. Van and surrounding villiages were visited and lambs with WMD examined. The lambs with 3-10 days of age were used as research materials. Necropsy and gross examinations was performed to all lambs. The blood samples were analyzed for Vitamin E amount, Creatine kinase (CK), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) activities. The level of Vitamin E was decreased, but the other parameters significantly increased. In heart, chest and gluteal muscle lesions in lambs were found. Tissue samples were histopathologically examined. Muscular dystrophic calcification in necrotic areas as well as hyaline degeneration and Zenker necrosis were determined. In the calcified region mononuclear cell infiltration mainly macrophages, were observed. In conclusion in lamb with white muscle disease; the activities of enzymes related to muscle health were raised drastically. In gluteal, chest and especially in heart muscle the hyalin and Zenker degeneration were noted.

Key words*: Enzymes, Muscular dystrophy, lamb, tissue, Vitamin E.*

INTRODUCTION

White muscle disease (WMD) also known as "subacute enzootic muscular dystrophy" or "stiff–lamb disease" can occur in newborn lambs, but is more commonly seen in lambs up to 3 months of age. It is seen in some areas in young sheep grazing stubble or rank, dry feed or in young sheep being maintained on hay and grain rations. This condition is associated with a vitamin E deficiency.

Deficiencies of either or both selenium and vitamin E can cause weaners couring, reduced wool production, reduced ewe fertility, reduced immune response, and white muscle disease. Selenium deficiency is more common in high rainfall areas while vitamin E deficiency occurs when sheep are on dry feed for long periods. Both of them can be provided as a supplement (McDowell, 2004).

Vitamins are essential for health of all kind living organisms. Fat soluble vitamin are vitamin E, A, D and K. Vitamin E (α tocopherol) is important as a biological antioxidant for oxidant and also required for normal cell differentiation and function (Mert, 1996). Deprivation of vitamin E causes different disturbances such as disorder of reproduction, muscle function, cardiovascular system, brain and liver. But cardiac disease is really severe problems in ruminants especially in new born animals (McDowell, 2004). Although WMD was once thought to be responsive solely to selenium it is now known to also be responsive to vitamin E. Muscle dystrophy in lamb, calf and kid is generally interacted with the Se deficiency. Skeletal muscles are the most affected tissue and it is common, but the heart lession can be seen less but it is severe. It affects cardiac function, ECG pattern changes and sudden death occurs. During the Vitamin E deficiency, usually associated with a lack of green feed, myocardial changes such as hyalinization of fiber, basophilic development and Zenker necrosis could occur (Kozat et al., 2007; Deger et al., 2008). Microscopically will show severe changes in the muscle. In other cases the muscle takes on a pale 'fish-flesh' appearance (Van Metre, 2001). Treatment is accomplished by the use of both vitamin E and selenium because the condition may be caused by a

deficiency of selenium, vitamin E or both. Since the two elements compliment each other both are used in treatment. Selenium is more important in selenium deficient areas and vitamin E in selenium sufficient areas or diets (Kennedy, 2013).

MATERIALS AND METHODS

In this study, 17 lambs with 3-10 days of age from different flocks with WMD and 10 healthy lambs, raised in Van and surrounding villages, in Se deficient areas were used as research materials.

Blood samples and postmortem tissue samples of gluteal and heart muscle were taken. Blood sera were separated and analyzed for creatine kinase, aspartate aminotransferase and lactate dehydrogenase by autoanalyzer. In addition serum vitamin E levels were spectrophotometrically measured (Martinek 1964).

Tissue samples were both evaluated grossly and histopathologically. Tissues were fixed in 10% formalin solution. embedded in paraffin wax, and then stained with hematoxylin– eosin stain, examined by light microscope.

Mann Whitney-U test was used for statistical analysis and significances between groupswere calculated.

RESULTS

The serum analysis of all lambs were shown in table 1. The individual findings were also shown in table 2.

In the pathological examination 17 lambs were used. Gross Zenker necrosis was observed in heart muscle, in gluteal and chest muscle and sometimes in two different tissues (Figure 1-3) Histopathological examination of both groups were also done.

In the WMD the heart and gluteal muscles showed similar apperances such as swollen fibers, homogeneus pink and pycnotic nuclei, hyperemic vessels and hemorrhages were observed.

Furthermore hyaline degeneration and Zenker necrosis, necrotic areas with dystrophic calcification and mononuclear cell infiltration mostly with macrophages in calcified areas were clearly seen.

Table1. Some serum biochemical parameters of WMD and healthy lambs.

Parameters	n	Healthy Lambs	n	WMD Lambs	P
Vitamin E (µg/mL)	9	1.93±0.14	8	0.518±0.060	p≤0.01
Creatine Kinase (IU/L)	9	62.10±17.5	7	2804,5±67.69	p≤0.001
AST (IU/L)	9	106.14±6.36	7	675,65 ±32.53	p≤0.001
LDH (IU/L)	9	448.7±43.80	7	987.67±54.51	p≤0.001

Table2. Individual reports for gross examination of the lambs with WMD.

Number	Ages (Days)	Locations of Lesion-1	Location of Lesion-2	Severity of Lesion
1	5	Heart Muscle		Mild
2	10	Gluteal Muscle	Heart Muscle	Medium
3	3	Heart Muscle		Medium
4	3	Heart Muscle		Medium
5	3	Heart Muscle	Gluteal Muscle	Severe
6	10	Heart Muscle		Mild
7	3	Gluteal Muscle	Chest muscle	Severe
8	5	Heart Muscle		Severe
9	3	Heart Muscle		Severe
10	5	Heart Muscle		Severe
11	3	Heart Muscle		Medium
12	8	Heart Muscle	Gluteal Muscle	Severe
13	9	Gluteal Muscle	Chest muscle	Medium
14	3	Heart Muscle		Severe
15	3	Heart Muscle		Severe
16	5	Heart Muscle		Mild
17	3	Heart Muscle		Medium
18	3	Heart Muscle		Mild
19	9	Gluteal Muscle	Chest muscle	Severe
20	5	Heart Muscle		Severe
21	5	Heart Muscle	Gluteal Muscle	Severe
22	3	Heart Muscle		Severe

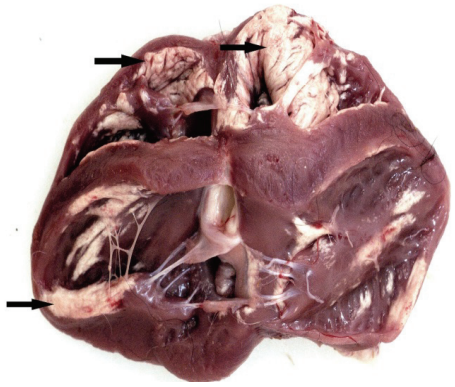

Figure 1. Zenker necrosis in heart muscle (black arrow)

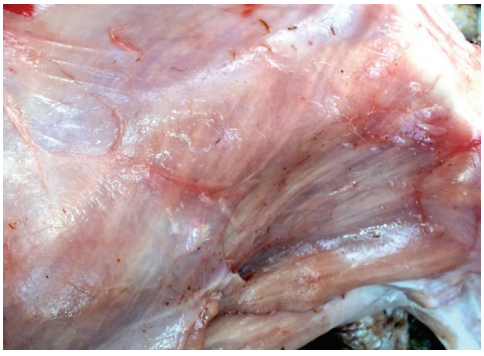

Figure 2. Zenker necrosis in gluteal muscle

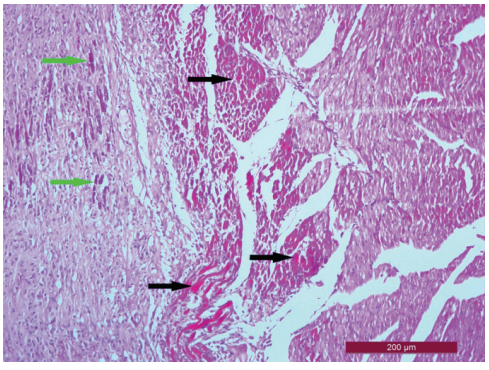

Figure 3. Dystrophic calcification (green arrow) and Zenker necrosis (black arrow) at heart muscle, and mononuclear cell infiltration. H&E Bar: 200µm.

DISCUSSIONS

Absence of Se and vitamin E cause White Muscle Disease in lambs. As known Calcium (Ca) ions is necessary for the contraction of muscle. In order to have normal muscle contraction Ca ions must go in and out of cell at necessary amounts. Excess amount of Ca are toxic for mitochondria. The peroxidation of membrane can be result of Ca infiltration to sarcoplasm and formation of mitochondrial damages in myocytes. Immidiately the energy of cells is exhausted, degeneration of myofibrils begins and intracellular Ca accumulation occurs. After these events intracellular enzymes go out to extracellular space and to blood. Troponin and AST levels increase. This events is important for the clinicopathological perspective. As result degenerative cells pass to necrosis step named Zenker necrosis. If the vitamin E levels decrease the following process is the immunodeficiency status which in secondary infection can easily occurs, immunosupression and bacterial infection can be easily seen (Hulland, 1985).

As shown in Table 1 the serum level of AST was significantly raised in lambs with WMD, $p \leq 0.001$.

Adenosin phosphate (ATP) is the universal energy currency for most of the energy requiring processes in biological systems (Lehninger, 1982). Tissues, e.g. skeletal and cardiac muscle, brain, photoreceptor cells, spermatozoa, all depend on the immediate availability of vast amounts of energy. Heart cells, which in general depend on anoxidative metabolism, energy derived from glycolysis can also contribute to the maintenance of high energy phosphate levels and contractility if oxidative phosphorylation of these cells is inhibited (Doorey and Barry, 1983).

CK activity is the greatest in striated muscle, heart tissue, and brain. The determination of CK activity is a proven tool in the investigation of skeletal muscle disease (muscular dystrophy) and is also useful in the diagnosis of myocardial infarction (MI) and cerebrovascular accidents. Increased levels of CK also can be found in viral myositis, polymyositis, and hypothyroidism (Mert, 1996). In the presented study the serum CK activity was significantly

increased ($p \leq 0.001$) there was massive degenerations in muscles.

Plasma creatine kinase (P-CK)_activities were significantly increased after physical exercise in healthy turkeys and in turkeys with genetic muscular dystrophy. Moderate exercise did not significantly affect P-CK activity in lambs. Increases in P-CK activity during expression of nutritional muscular dystrophy were readily distinguished from exercise effects; activity exceeded 160,000 mU/ml in lambs during expression of that condition. The extent of muscle damage after intramuscular administrations of some veterinary drug formulations was estimated from the total creatine kinase activity released in plasma during the 72 hours following the injection (Tripp and Schmitz 1982)

Lactate dehydrogenase (LDH) is involved in the final step of anaerobic glycolysis, catalyzes the conversion of lactate to pyruvate, consists of a system of five isoenzymes. It is not tissue-specific, being found in a variety of tissues, including liver, heart and skeletal muscle. The enzyme is tetrameric and is composed of four subunits of two molecules, M (muscle) and H (heart). Various combinations of these two molecules result in five different isoenzymes. Increased LDH5 were reported in sheep, cattle and horses, e.g. selenium and vitamin E deficient myopathy in cattle and sheep, exertional rhabdomyolysis in horses.In this research the activity of LDH was also severely incresased ($p \leq 0.001$).

Treating the heart form of white muscle disease is usually ineffective. The skeletal muscle form of the disease can be treated with supplementry selenium and/or vitamin E. Aksakal et al. (1996) tried to understand the effects of vitamin A, vitamin E, and Se in the ethiology of WMD on sheep and feed them with different diet supplemented with vitamin A+E+Se. They concluded that vitamin A like vitamin E help to maintain the normal blood paramaters in physiological levels and can be also used as prophylactic purpose. Whanger et al. (1977) reported that Vitamin E alone was more effective in the prevention of WMD than selenium alone. Ewes may be fed vitamin E prior to lambing, a therapeutic dose two to four weeks before lambing works well. In this study the vitamin E levels were low in lamb with WMD ($p \leq 0.01$).

As conclusion, muscular dystrophy is still a problem for sheep breeders. Special care and attention must give to eliminate this nutritonal disease and clinical studies must be performed to understand the changes in tissues and specimen for an applicable treatment to sick animals.

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MONITORING THE SPECIES *STAPHYLOCOCCUS AUREUS* IN DOG FAECES, IN TIMISOARA PARKS: IS THERE A ZOONOTIC RISK?

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Abstract

Stray dogs have long been regarded as a potential source of zoonotic diseases (bacterial zoonotic risk) for human. In particular, host zoonotic bacteria and parasites in the intestine of dogs were found to pose a significant risk to human health. An ensemble social change, economic and environmental, across the globe, reflects on epidemiological characteristics and pathogenesis of diseases and pathogens. And the development and supervision of bacterial zoonosis, with particular reference to multiple antibiotic resistant staphylococci isolated from dog faces, were important changes, which we refer in this study.In fecal samples from dogs were isolated Staphylococcus aureus strains pathogenic to man (MRSA), so proving dog faeces role in urban areas as a reservoir of bacteria with multiple resistance. Because the genes coding for antibiotic resistance can be transmitted between bacteria and contact between pets and their owners is tighter than in the past, our study suggests that contamination parks for children with dog feces containing such microorganisms is a problem for public health and the environment.

Key words*: staphylococci, methicillin resistance, faeces, stray dog, Timisoara, parks.*

INTRODUCTION

Staphylococci are one of the most important groups of commensal bacteria that are isolated from the skin and the mucous membranes of dogs. Moreover, they are responsible for opportunistic infections acquired in hospitals and communities, affecting mostly skin and ears, and other anatomical areas (Euzéby, 2013; Guardabassi et al., 2004; Loeffler, 2008).
An ensemble social change, economic and environmental, across the globe, reflects on epidemiological characteristics and pathogenesis of diseases and pathogens. And the development and supervision of bacterial zoonosis, with particular reference to multiple antibiotic resistant staphylococci isolated from dog faces, were important changes, which we refer in this study.
Stray dogs have long been regarded as a potential source of zoonotic diseases (bacterial zoonotic risk) for human. In particular, host zoonotic bacteria and parasites in the intestine of dogs were found to pose a significant risk to human health. People are exposed to these pathogens through direct or indirect contact with infected dogs or their feces through accidental ingestion of a zoonotic agent.
It is also important to consider that exposure to zoonotic bacteria from feces of stray dogs could present a significant health problem in the urban areas (Simoons-Smit et al., 1997; Guardabassi et al., 2004).
Parks and playgrounds frequented by children as well as stray dogs are the main areas for such illnesses declared suspicious.
The reasons for which the owners have to collect feces after their four-legged friends are based on arguments related health risks.
Dog faeces contain bacteria and parasites. If you are abandoned in public space, we get to come into contact with the faces contaminated being exposed to serious diseases. For example, if it rains, the water dissolves them, clean shoes is inevitably contaminated, sprinkle us with goo formed on clothes; if it does not

rain, dry them, grind that you inhale especially when the wind blows.
Children have the highest risk of exposure because my hands on the floor, playing with objects that touch the ground and tend to take their hands dirty in the eyes, nose and mouth.
For children's and adolescents, who play with the ball or other toys in this area, exist the risk of contracting bacteria and parasites. Flies and other insects that lay excrement and then come to us are carriers of the kitchen to the said pathogen (Tarsitano et al., 2006).

MATERIALS AND METHODS

Fecal samples

To achieve its purpose, it was collected 60 fecal samples (29 fresh and 31 old) children's play parks, located in the City.
Fecal samples were collected at random.
Also feces collected were subjected to external factors (heat, drought, rainfall, wind, etc.).
There were counted four parks primarily for children, located in different areas of the city of Timisoara.
Actual crop were used plastic containers, sterile, individually wrapped (the need for urine culture), respectively spoon and disposable gloves.
A sample was individualized and is listed on the sample box number, area of origin and date (Fig. 1).

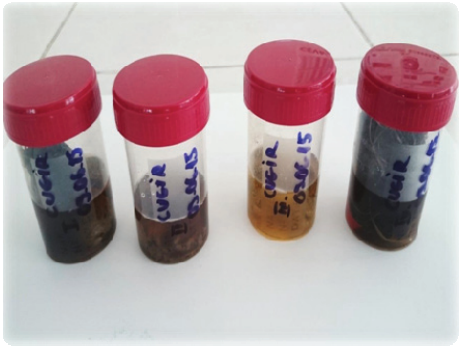

Fig. 1. Faecal samples (original).

After harvesting fecal samples were transported to the laboratory. Sample preparation was conducted in the Laboratory of Bacteriology of the Department of Infectious Diseases and Preventive Medicine in the period May-June 2015.
In the laboratory, the first step in the processsing of the stool samples was the achievement of a fecal suspension by the addition of a quantity of 5 ml of sterile physiological saline over the feces from the container used for the collection and maintenance of contact at room temperature (25-28° C) for 20-30 minutes. Subsequent the mixture was homogenized by gentle manual stirring.
For these suspensions were made insemination on a special chromogenic medium, Chromatic Detection (Mikrobiologie Labor Technik)

Chromatic Detection agar– description:

Chromogenic medium used for the enumeration and identification of microorganisms from clinical specimens and food.
Special formula allows also confirming directly the indole tests *Echerichia coli* (Table 1).

Table 1. Composition Chromatic Detection agar

Standardized formula	(g/l)
peptone	14.0
yeast extract	3.0
tryptone	6.0
sodium chloride	5.0
chromogenic mixture	13.125
agar	15.0
finale pH	7.2 +/- 0.2

Peptone, tryptone and yeast extract are a source of amino acids and vitamins. Sodium chloride maintains the osmotic balance of the environment.
Chromogenic mixture allows identification of microorganisms based on colony color and morphology.
Technique: medium surface inoculate 10 µl specimen using a sterile loop (loop bacteriological) or pharyngeal exudate rod for clinical trials.
Incubate at 37 ° C, under aerobic conditions in an incubator for 18-24 hours. Observe growth and colony color and interpretation is done according to the manufacturer, listed in the product data sheet (Table 2).

Table 2. Interpretation of results

Microorganism	Growth	Aspect of the typical colony
Escherichia coli	good	Pink
Staphylococcus aureus	good	Cream-colored
Klebsiella pneumoniae	good	Aquamarine
Proteus mirabilis	good	Brown
Pseudomonas aeruginosa	good	Yellowish
Enterococcus faecalis	good	Green Turquoise

Samples were collected using sterile cotton wool pads, attached to a plastic rod, pharyngeal exudate for harvesting.
Sowings were made by depletion of pathological material on a cotton ball on the agar surface. Next, the plates were incubated at 37 ° C in normal atmosphere for 18 -24 hours.
After interpreting the results of the Chromatic Detection agar, typical of *S. aureus* colonies were picked on nutrient broth medium with 5% sheep blood, to obtain fresh cultures necessary to carry out sensitivity testing. After 24 hour incubation, the obtained cultures were performed plating Muller Hinton medium, the specific Kirby Bauer technique (Codiţă and Buiuc 2008).

Susceptibility testing to antibiotics

Behavior towards antibiotics was tested all bacterial strains isolated Using diffusion method. A common method for determining the antimicrobial susceptibility, primarily in small laboratories and veterinary practices, is the agar diffusion test (diffusion method). This method uses paper disks impregnated with the antimicrobial substance, which are then applied to the surface of the agar medium previously impregnated with a pure culture of bacteria being tested. The diameter of the growth inhibition zone around the paper disk is inversely correlated with the minimal inhibitory concentration (MIC).
This diffusion technique is not difficult, however, it must be strictly observed and tracked area size standard for each drug separately. Any variation in the execution technique changes the relationship between the zone of inhibition and MIC, resulting in misinterpretation of results. The antibiotics tested were: methicillin - ME - 30 µg, gentamicin - CN – 10 µg, tetracycline - TE - 30 µg, ciprofloxacin - CIP - 30 µg, kanamycin - K - 30 µg, novobiocin - NV - 30 µg, doxycycline - DO - 30 µg, erythromycin - E - 15 µg, vancomycin - VA - 30 µg, ceftriaxone - CRO - 30 µg, cefoxitin - FOX - 10 µg, polymyxin B - PB - 50 IU, rifampicin - RA - 30 µg, lincomycin - L - 30 µg, cefaclor - CEC - 30 µg, pristinamycin - PT - 15 µg and ampicillin / sulbactan - SAM - 30 µg. All bio discs were manufactured by Liofilschen-Italy and interpretation of the results was performed in accordance with manufacturer's recommendations.

RESULTS AND DISCUSSIONS

After reading and interpretation of specific colonies on plates with Chromatic detection agar, seeded faecal samples from dogs were isolated microorganism with pathogenic potential for humans (Fig. 2). From 60 faecal samples, 18 samples were positive for *Staphylococcus aureus* (18/60; 30%). The results of the special chromogenic agar plating are shown in Table 3 and Fig. 2.

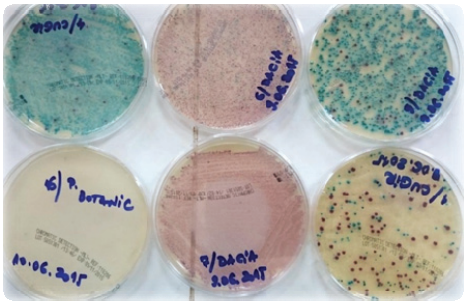

Fig. 2. Aspects of colonies in the Chromatic detection medium (original)

Enterobacteriaceae (*Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli*). Quantification of other bacteria not covered by this study.

Figure 3 presents *S. aureus* aspects of colonies in the Chromatic detection agar, according to the technical specifications given by the manufacturer. These colonies are cream colored.

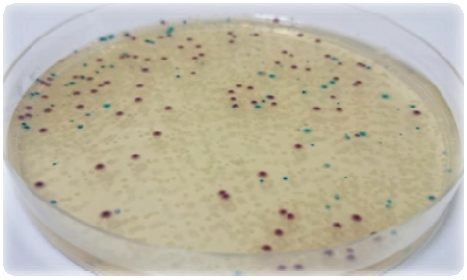

Fig. 3. Specific *S. aureus* colonies (cream) on Chromatic detection agar (original)

Table 3. Results of the inoculation in the Chromatic detection agar

Microorganism	Number of samples taken	Number of positive samples	
		No.	%
Escherichia coli	60	34	56.67
Staphylococcus aureus	**60**	**18**	**30.00**
Klebsiella pneumoniae	60	28	46.67
Proteus mirabilis	60	11	18.34
Pseudomonas aeruginosa	60	14	23.34
Enterococcus faecalis	60	43	71.67

Results of susceptibility testing to antibiotics

Most strains of staphylococci unexposed to the pressure of antibiotics are sensitive to these antimicrobials, but there have been cases where resistance was related phenomena.

Interpretation of results was done according to standards set by the Clinical and Laboratory Standards Institute (2006) Is better use for interpretation CLSI Vet 01-A4 and Vet 01-S2/2013. The results were classified into three categories: susceptible, intermediate sensitive and resistant.

The results obtained from testing the antibiotic susceptibility of strains of staphylococci isolated from the feces of dogs are given in Table 4.

Table 4. Sensitivity rate of *S. aureus* strains isolated (n = 18), compared to 17 antibiotics (Table 4 is glued to Table 3)

Antimicrobial substance name (Initials/MIC *)	interpretation sensitivity testing					
	susceptible		intermediate sensitive		Resistant	
	Nr.	%	Nr.	%	Nr.	%
Methicillin - ME - 30µg	17	94.45	-	-	1	5.56
Gentamycin – CN – 10µg	12	66.67	2	11.12	4	22.23
Tetracycline – TE – 30µg	9	50	1	5,56	8	44.45
Ciprofloxacin – CIP – 30 µg	18	100	-	-	-	-
Kanamycin – K – 30 µg	11	61.12	4	22.23	3	16.67
Novobiocin – NV – 30 µg	18	100	-	-	-	-
Doxycycline – DO – 30 µg	10	55.56	3	16.67	5	
Erythromycin – E – 15 µg	12	66.67	2	11.12	4	22.23
Vancomycin – VA – 30 µg	18	100	-	-	-	-
Ceftriaxone – CRO – 30 µg	18	100	-	-	-	-
Cefoxitin – FOX - 10µg	18	100	-	-	-	-
Polymyxin B – PB – 50UI	-	-	5	27.78	13	72.23
Rifampicin – RA – 30 µg	18	100	-	-	-	-
Lincomycin – L – 30 µg	18	100	-	-	-	-
Cefaclor – CEC – 30 µg	18	100	-	-	-	-
Pristinamycin – PT – 15 µg	18	100	-	-	-	-
Ampicillin / sulbactam – SAM – 30 µg	18	100	-	-	-	-

* MIC – The minimum inhibitory concentration

Analyzing the results of the table we can see that antibiotics sensitivity was variable depending on the group of antibiotics.

If antibiotics: novobiocin, rifampicin, pristinamycin, ciprofloxacin, vancomycin, ceftriaxone, cefoxitin, cefaclor and ampicillin / sulbactan,

considered the drug of choice for staphylococci, the number of sensitive strains were 100%, all isolates were sensitive (Table 4).
This suggests that the tested strains isolated from animals to which these antibiotics were not used. Also, it can be said that all of these antibiotics for staphylococci or kit is typically used in humans, in the treatment of staphylococcal infections in animals, respectively.
B-lactam used against (methicillin, ceftriaxone, cefoxitin, cefaclor, ampicillin with sulbactam), antibiotic sensitivity was highest, except *Staphylococcus aureus*, where they isolated one resistant strain for methicillin.
The phenomenon of antibiotic resistance, β-lactam in the case is based on genetic determinants of type plasmid and chromosomal β-lactamases governing synthesis, broad-spectrum, ensuring the resistance staph. Resistance to methicillin is transmitted by plasmids (R factor) and having a pattern common to other β-lactams (Weese 2008). For this reason, methicillin-resistant staphylococci are considered particularly with zoonotic risk, having a complex circuit or human-animal-human (Tarsitano 2006; Velescu and Tănase 2010; Bywater 2004; Weese and Van Duijkeren 2010).
Compared to aminoglycosides (gentamicin, kanamycin) and macrolides (erythromycin and vancomycin), antibiotics sensitivity was differrent, the maximum to vancomycin (Table 4). In the case of gentamicin-resistant strains were isolated four, three strains to kanamycin and 4 strains resistant to erythromycin (Table 4).
Most of the strains were resistant to polymyxin B (13 strains), through the use of topically applied preparations containing this antibiotic (Table 4).
Sensitivity to tetracycline's (tetracycline, doxycycline) was reduced 13 strains being resistant to this group of antibiotics to which resistance phenomenon is type plasmid and chromosomal (8 strains tetracycline and 5 strains to doxycycline) (Tables 4).
All strains tested were sensitive to ciprofloxacin, since the quinolone is not used in drug therapy in dogs the usual manner.
The development of resistance staphylococci to different antibiotics, it is a consequence of wasteful use in the treatment of diseases in pigs. Antibiotics used irrationally creates a selective pressure being selected and transmitted genetic determinants of type plasmid and chromosomally. Consequently, the phenomenon of multiple resistance intra- and interspecific that is transmitted. Methicillin resistance of special importance as it can be associated with resistance to β-lactams and other groups of antibiotics (Weese, 2008, Guardabasi et al., 2004, Bywater, 2004).
After testing strains of staphylococci isolated from the feces of dogs, against 17 antibiotics were identified methicillin-resistant strain and more type of resistance, β-lactams to, tetracyclines, macrolides and polymyxin B.
In the literature there is very little information available about the microbial flora present in fecal pets, especially dogs, despite the presence of Gram-positive cocci in feces dog has already been observed decades ago (Devriese and Pot, 1995; Murray, 1990, Tannock, 1995, Loeffler et. al., 2010).
In a study by Cinquepalmi et al. (2013) in Bari region - southern Italy, on a sample of 418 dog feces samples collected from the streets, have identified strains of MRSA (methicillin-resistant *S. aureus*) at a rate of 0.7%. In similar studies, Abbott et al. (2010) and Abdel-Moein et al. (2012) identified these bacteria in a proportion of 0.4% and 3%.
MRSA strains isolated from companion animals (dogs and cats) are also similar to disseminated hospital strains (Abbott et al., 2010). Dogs, for this reason, can pose major public health modules for dissemination outside hospitals MRSA strains (Abdel-Moein et al., 2012; Ferriera et.al., 2011; Morris et. al., 2012, Rich and Robert 2004).
Dog feces in urban areas can be an important source of pathogenic microorganisms with potential for both dog owners and for the community in that area, especially for children.

CONCLUSIONS

In fecal samples from dogs were isolated *S. aureus* strains pathogenic to man (MRSA), so Proving dog faeces role in urban areas as a reservoir of bacteria with multiple resistance.
Because the genes coding for antibiotic resistance can be transmitted between bacteria

and contact between pets and their owners is tighter than in the past, our study suggests that contamination parks for children with dog feces containing such microorganisms is a problem for public health and the environment.

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SARCOMA OF THE NASAL CAVITIES IN A DOG: CASE REPORT

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Abstract:

Sarcomas comprise approximately one-third of canine intranasal tumors; however few veterinary studies have described survival times of dogs with histologic subtypes of sarcomas separately from other intranasal tumors. The particularity of the case is due to nature of nasal tumor – sarcoma - something unusual on nasal tumors and then something extraordinary at the age of occurrence of this tumor – dog of 5 years age. The surgical technique consisted of nasal cavities oral approach and cutting a bone fragment length located in bony floor of the nasal cavity left, which allowed wide access to the cavity.

Key words: *dog, cronic rinithis, sarcoma.*

INTRODUCTION

This clinical case, a dog, female, 5 years old, Belgian Malinois breed was directed for consult to the Department of Pathology and Surgical Clinic of the École Nationale Vétérinaire Nantes by a veterinarian for a suspected disease to the nasal cavities or sinus.

The originality of this case derives from the long evolution of the disease (over 18 months) during which there were many additional tests done which tried to establish a diagnosis of certainty which proved to be unsuccessful, but also by the specific surgical act that managed through surgical technique to approach a certain diagnosis, to stop animal suffering and improve patient comfort due to favorable postoperative clinical outcome even if long-term prognosis is very reserved.

The particularity of the case is also due to nature of nasal tumor – sarcoma - something unusual in terms of nasal tumors and extraordinary in terms of age of tumour occurrence-dogs 5 years of age.

The surgical technique consisted of nasal cavities oral approach and cutting a bone fragment located in the bony floor of the left nasal cavity, which allowed wide access to the cavity.

CASE DESCRIPTION

ANAMNESIS

The dog presented a supurative bloody secretion to the left nostril.

MEDICAL HISTORY

Following this clinical presentation, the dog owner consulted a veterinarian and treated with 250 Ronaxan® for a infectious rhinitis, 1.5 cp / day / for 7 days. Treatment allowed for the regression of symptoms but recurrences are recorded in the coming months. Antibiotic therapy improves the patient's condition but does not cure the patient therefore an intranasal foreign body is suspected and a nasal lavage, is performed under general anesthesia followed by treatment with Antirobe® 150 (2 capsules / day/8 days). A serological examination is conducted towards aspergillosis but this is considered indecisive as far as only one positive arc was quantified.

A new recurrence is indicated with sero-suppurative secretion and treatment is initiated based on Augmentin® and treatment for a suspected aspergillosis with Enilconazole as intranasal baths (Caulkett et al., 1997) and (Mathews et al., 1998). A rhinoscopy to achieve a biopsy is performed but did not bring

additional information. A new treatment is tested based on Megasolone® (1 cp / day / 20 days) and Augmentin® (1 cp / evening - morning and 20 days) (Barret et al., 1977).
The patient was presented in the emergency room and is operated by a splenic tortion. Histological analysis did not reveal anything abnormal. On this occasion a fistulous abscess was detected in the left upper canine tooth and removed. Suppurative discharge recurrences occurred upon stopping treatment. Last treatment before being consulted in the surgery clinic was established and was the administration of Megasolone 20® (1cp / day) and Bactrium® 160 (1cp / morning and evening).
The reason for consultation was the unilateral suppurative discharge relapse with signs of obstruction of the left nostril, which causes shortness of breath.

CLINICAL EXAMINATION
Good general condition (good maintenance condition, appetite preserved) mucous pink color, TRC <2 sec, left submandibular lymph node is hypertrophied. The general examination revealed nothing abnormal except mucopurulent secretion and signs of respiratory obstruction of the left nostril with dyspnea.

ADDITIONAL TESTS: Blood count (table 1), the examination of hemostasis (table 2) have been performed before referral for surgical consultation.
Haematological examination revealed a minimum anemia.
The conclusion of examination of hemostasis: Outside anticoagulation treatment TCK ratio P/T below 1.20 is considered normal.

Table 1. The blood count of the dog

Erythrocytes	6.100.000/mm3	
Hemoglobin	14.3 g/100ml	
Hematocrit	41.3 %	
V.G.M.	68 µ3	
T.C.M.H.	23 gama	
C.C.M.H.	35 %	
Leucocytes	9.600/mm3	
Neutrophils	71%	Or 6816/mm3
Eosinophils	5%	480/mm3
Basophils	0/%	0/mm
Lymphocytes	19%	1.824/mm3
Monocytes	5%	480/mm3

Table 2. The examination of hemostasis

Quick time		***Cefaline activate time***	
TQ Control	8.0 sec	TCA Control	33 sec
TQ	8.0 sec	TCA Patient	22 sec
The ratio TCK P/T = **0,67**			

MICROBIOLOGICAL EXAMINATION:
Cyto-bacteriological examination of a sample collected from the nasal cavities and cultivated on a specific medium revealed numerous colonies of *Pasteurella multocida*. For *Aspergillus*, the result was negative. Serological examination for Aspergillosis was weakly positive in an arc, which is considered insignificant.

HISTHOPATOLOGYCAL EXAMINATION concluded that it is a chronic rhinitis presenting moderate inflammatory lesions due to a less specific pathogen in the nasal mucosa revealed as representative in the sample collected**.**

RHINOSCOPY: A rhinoscopy through posterior approach (pharynx) was carried out but could not reveal any lesions. Instead using an anterior approach rynoscopy (nasal) a signficant-sized formation located in the anterior part of the left nasal cavity. This formation could not be mobilized by our attempts to pressure lavage.

RADIOGRAPHIC EXAMINATION of the skull in dorso-ventral incidence allowed the viewing of an area of increased density in the left nostril (Gibbs et al., 1979) (Figure 1).

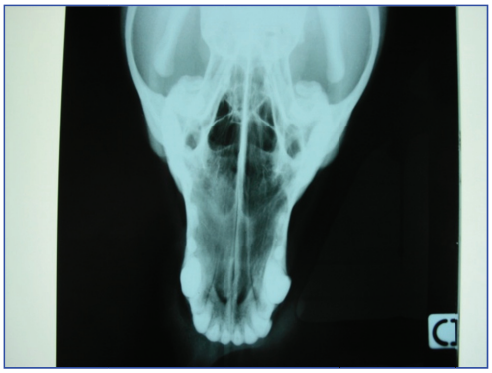

Figure. 1. Increased radiodensity in the left nasal cavity compared to the right side

SUMMARY OF CLINICAL SIGNS:

- mucous suppurative secretion located just in the left nostril
- stenosis of left nostril accompanied by noise
- submandibular left lymph node hypertrophy
- left eye epiphora

CLINICAL DIAGNOSIS

Recidivating chronic rhinitis, accompanied by a mucous-suppurative secretion, unilaterally and signs of left nostril stenosis.

ETIOLOGICAL HYPOTESES (Hamilos and Lund, 2004):

1. Etiology of inflammatory type
   - rhinitis given by a foreign body
   - rhinitis of traumatic origin
   - rhinitis associated with dental disease
2. Etiology of infectious (bacterial, fungal) and parasitic nature
   - tumors of the nasal cavities
   - sinus disease.

SURGICAL TECHNIQUES:

Rhynotomy with oral performed: Following clinical, radiological and rhynoscopical examinations the decision to proceed to the surgical act was made immediately for: diagnosis (biopsy, excision), prognosis (nature of tumor, extension, degree of differentiation) and treatment (in some cases of benign or inflammatory etiology).

The principle of the technique is based on cutting a bone fragment located in bony roof of the mouth, near the left nasal cavity and allowed wide access to the nasal cavity.

General anesthesia, the type of narco-neuroleptic-analgesia, was use, as tge the dog is intubated endotracheal, so as to avoid possible aspiration of blood resulted from the surgical technique.

Maintenance of anesthesia for a sufficient period of a particularly laborious surgical intervention was performed with isoflurane.

The dog was lying on its back and using restraining techniques of the nasal cavities floor was put in a comfortable position parallel to the operating table during the surgery.

The incision of the oral mucosa covers a longitudinal line of the left nasal cavity and then it was carefully removed.

The periosteum is lifted off with a periosteum in order to achieve a significant bone piece in terms of size. Bone resection is carried out using an orthopedic cutters, operated by an electric engine similar to that used in dentistry.

Exploring the nasal cavity is almost impossible because of the hemorrhage resulted from bone resection and injury of nasal concha cavity hiding the content.

The bleeding was controlled by conventional means including intermittent pressure compresses of the cavity by means of simple or soaked absorbable with trombase.

A surgical aspirator was used to enhance visibility (Fig. 2, 3).

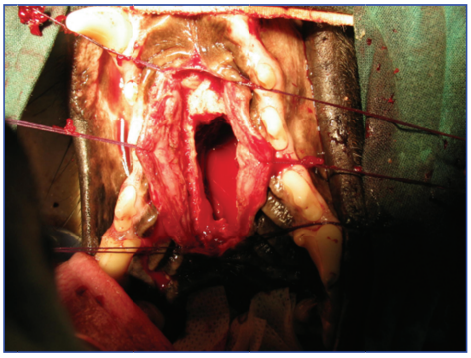

Figure 2. Cropping bone fragment on the roof of the mouth and nasal cavity to create free access using wire tractors which remove the buccal mucosa

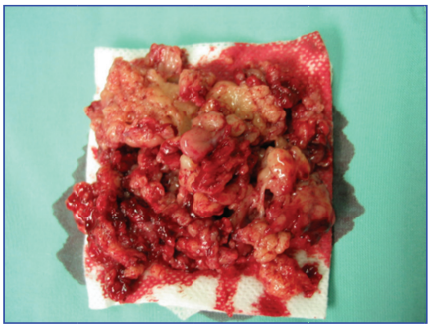

Figure 3. Fragments of the nasal cavity tumor, after extirpation

Figure 4. The patient was under medication and observation after surgery

After the bleeding was stopped, nasal tumor mass excision was carried out. The last step consists in suturing the nasal mucosa, the periosteum and buccal mucosa. It was opted for total removal of the bone fragment because the oral mucosa has a hard consistency which can substitute the necessary hardness of the oral cavity ceiling. Of course the next 3 weeks the feed was of pasty consistency.

Postoperatory, an antibiotic therapy was established based on the administration of an intravenous route of Rilexine®, Vitamin K therapy and antihemorrhagics.

The analgesic was not neglected, namely morphine administered subcutaneosuly.

A possible postoperative inflammatory edema was combated with a solution of Solu-Medrole®. The dog was under medication and observation after surgery (fig. 4).

RESULTS AND DISCUSSIONS

The conclusion of histopathological examination is that of less differentiated sarcoma. The tumor is developed in the epithelium of the nasal mucosa and upon contact with the deep bone tissue. Prognosis is reserved because there is an increased risk of recurrence. It is imperative that regular checks on the patient are performed

Their harmful action resulting from local invasion and bone destruction rather than in their metastasis. At the same time, a study conducted on a total of 504 cases of malignant intranasal tumors showed the appearance of metastases in 51 cases with frequent localizations: lymphatic system, brain and lungs. Intranasal tumors occur most likely in older dogs. The mean age for dogs affected by such tumors is about 9 years (Tabel 3, Norris, 1985).

Table 3. The frequency of cases of tumors of the nasal cavity in dogs according to age (Norris, 1985)

Age (years)	FREQUENCY
5	***0***
5-6	6
7-8	8
9-10	8
11-12	8
13-14	3
15	1
Males	16
Females	18

Regarding the histological nature of nasal cavities tumours, we distinguish epithelial tumors and tumors of mesenchymal origin (Norris and Laing, 1985).

Tumors of epithelial origin are called squamous cell or carcinomas are the most common and with a percentage of 60-75%. Adenocarcinomas are by far my most frequently encountered epithelial tumors. They come from sinus epithelium, nasal, olfactory mucosa or mucous glands of the nasal cavities.

Tumors of mesenchymal origin are more frequently fibrosarcomas followed by condrosarcomas, osteosarcomas and melanomas (Tabel 4, after Norris and Laing, 1985).

Table 4. Classification of nasal tumors in 34 dogs from the Ontario Veterinary College (after Norris, 1985)

	Norris (1985)	Mac Ewen (1980)	Morgan (1982)	Madewell (1979)	Bradley (1978)	TOTAL
EPITHELIUMA ORIGIN	23	30	35	27	13	133
Adenocarcinomas	9	19	13	10	12	63
Carcinomas	12	8	9	12	6	47
Carcinoamas with squamous cells	2	3	7	4	-	16
Undifferentiated carcinomas	-	-	4	1	-	5
Others	-	-	2	-	-	2
MESENCHYMAL ORIGIN	11	13	30	22	3	79
Fibrosarcomas	-	6	14	6	-	26
Condrosarcomas	5	3	6	8	-	22
Osteosarcomas	4	-	4	2	-	10
SARCOM	**-**	**2**	**2**	**1**	**3**	**8**
Reticulosarcomas	-	-	4	4	-	8
Hemangiosarcomas	1	1	-	1	-	3
Lymph sarcomas	1	1	-	-	-	2

From our observations, the most common clinical signs in dogs with nasal tumors are epistaxis, sneezing, muco-purulent discharge, cough, dyspnea, respiratory noises stenosis, chemozis, epiphora, maxillofacial deformity of the skull, tonsillitis. Regarding treatment undertaken in the Department of Pathology and Surgical Clinic of École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique, it aims to improve the prognosis, to prolong the patient's life by reducing clinical signs and improve comfort. When a tumor is suspected localized to the nasal cavities, suspicion reinforced by radiological examination and examination rhinoscopy, we propose a rhynotomy (exploratory and curative) be undertaken. Only surgical curettage alone is not fully effective.

Surgical curettage and complementary treatment consisting of radiotherapy and chemotherapy must be associated to prevent local recurrences.

Surgery is possible to obtain a diagnosis of certainty by excision of the tumor mass stenosis and improve patient quality of life. After bibliographical studies, radiation therapy consists in destruction of histologocally modified tissue using ionizing radiation which causes chromosome breaks, disturbing the normal mechanisms of cell division. The total dose of radiation is 3000 to 4000 rad distributed in 3-4 fractions. The period between two treatment sessions is 10-14 days. This treatment interval allows for the repair of tissues. Among the substances used as anticancer chemotherapeutic drugs one can distinguish: Vincristine, Vinblastine, Bleomycin, Adriblastina (Norris and Laing, 1985).

CONCLUSIONS

Rhinotomy with an oral approach proved to be a very good technique in addressing the nasal tumor, facilitating wide access by removing a piece of bone from the roof of the mouth and tumor curettage.

We must recall that tumors located in the nasal cavities are mostly malignant. Their location makes complete surgical treatment, impossible. It is therefore necessary to undertake other therapeutic methods such as radiotherapy and chemotherapy.

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THE INCIDENCE OF EPITHELIAL NEOPLASMS IN PETS (DOG AND CATS)

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Abstract

The authors present a retrospective study on cases of malignant epithelial neoplasms in dogs and cats, over a period of time of two years (2013-2015), diagnosed in the Medical Clinic of the Faculty of Veterinary Medicine of Bucharest, within the cytology and hematology laboratory, with a quick and easy technique as the cytomorphological exam. In this paper work, based on the studied cases (39 dogs and 11 cats), we intend to bring new information related to the incidence of these cancer forms in the two species, analising several aspects: solid tumors with various anatomical localizations (mammary gland, perianal region, testicle etc.), celullar proliferation of cavitary fluids and internal viscera solid tumors. Our study shows how of a great importance is to corroborate the clinical investigations with laboratory investigations. Our study demonstrated the possibility of double cancers or metastases, which are very important data for the therapy.

Key words*: epithelial cancer, canine, feline, cytomorphology.*

INTRODUCTION

Epithelial neoplasms are represented by the malignizations developed from the embrionary ectodermic foils, known as carcinomas (in the old nomenclature were known as epitheliomas) (Baba et al., 2007; Balint et al., 2005).
However, epithelial malignancies have also some other specific names depending on location.
For knowledge, these neoplasms have been described and classified in the major chapters of malignant neoplasms in dogs and cats (Manolescu et al., 2009; Dinescu et al., 2013).
The literature in this field abounds in information about malignant epithelial neoplasms in dogs and cats, which shows very clearly that these are the most common forms of cancer found in these species (Baba et al., 2007; Balint et al., 2005; Cowell et al., 2008; Waldron, 2001; Withrow et al., 2007). Fact that we ascertained from all the cancer forms that we investigated in twenty years, for which we analised this casuistry on global aspect, relying mainly on cytomorphological examination (Baker et al., 2000; Balint et al., 2013; Manolescu et al., 2009).

MATERIALS AND METHODS

Our study was conducted over a period of two years (November 2013 - November 2015) on a total of 50 cases (39 dogs and 11 cats) in the Medical Clinics of the Faculty of Veterinary Medicine of Bucharest.
The animals were included in our study after a clinical investigation, where tumor formations were put into evidence in various areas (mammary gland, perianal region, testicle etc.). As a result of laboratory tests and imaging (ultrasound, radiography), were detected cavitary fluids or tumors in the internal organs.
The external solid tumors were diagnosed by biopsy puncture, from which smears and prints on microscope slides were performed, stained May-Grünwald Giemsa and microscopically examined using Olympus BX 51 optical microscope.
The intra-cavitary fluids were collected, and after centrifugation, from the obtained sediment, smears were performed, stained and examined in the same way.
A third category of casuistry was the one that presented tumor formations on the internal organs, that were visualizes by ultrasound. In

these situations, after the necropsy, the tumoral formations were diagnosed by cytomorphological exams.
Another investigating method, for an important number of cases, was the nasal and gastric endoscopy.
With this method the presence and the aspects of the tumors were highlighted. Smears were realized from the harvested tissue, stained May-Grünwald Giemsa and examined at the microscope.

RESULTS AND DISCUSSIONS

In tables 1 and 2 we present details on the cases included in the study.

Table 1. The incidence of malignant epithelial neoplasms in cats

No.	Sex	Age	Breed	Location	Citmorphological diagnosis
1.	Female	14 years old	European	Mammary gland	Anaplastic adenocarcinoma with vegetated areas
2.	Female	14 years old	European	Mammary gland	Vegetant adenocarcinoma
3.	Female	14 years old	Birman	Mammary gland	Solid adenocarcinoma
4.	Female	13 years old	European	Mammary gland	Papillary vegetant carcinoma
				Inguinal lymph node	Vegetant carcinoma metastasis
5.	Female	13 years old	Persian	Peritoneal cavity (fluid)	Cholangiocellular carcinoma metastasis
6.	Female	9 years old	European	Mammary gland	Vegetant adenocarcinoma
7.	Male	13 years old	Persian	Liver	Cholangiocarcinoma
8.	Male	12 years old	Birman	Lungs	Giant cell carcinoma
				Liver	Malignant hepatoma
				Tracheobronchial lymph node	Pulmonary giant cell carcinoma metastasis
9.	Male	11 years old	Birman	Pericardic cavity (fluid)	Mucosecretant carcinoma metastasis
10.	Male	4 years old	European	Auricular tegument	Carcinosarcoma
11.	Male	3 years old	European	Kidney	Dark cell carcinoma

Table 2. The incidence of malignant epithelial neoplasms in dogs

No.	Sex	Age	Breed	Location	Citmorphological diagnosis
1.	Female	16 years old	Mixed Breed	Mammary gland	Vegetant carcinoma
2.	Female	15 years old	Caniche	Mammary gland	Vegetant carcinoma
3.	Female	15 years old	Caniche	Pericardial cavity (fluid)	Vegetant carcinoma metastasis
4.	Female	14 years old	Mixed Breed	Supraorbital tegument	Vegetant carcinoma
5.	Female	14 years old	Mixed Breed	Mammary gland	Vegetant carcinoma
6.	Female	14 years old	Mixed Breed	Urinary bladder	Vegetant carcinoma with frequent giant cell forms
7.	Female	14 years old	Caniche	Mammary gland	Vegetant carcinoma
8.	Female	13 years old	Bichon	Mammary gland	Solid adenocarcinoma with vegetated high malignant areas
9.	Female	13 years old	Cocker Spaniel	Mammary gland	Vegetant adenocarcinoma
10.	Female	12 years old	German Shepard	Mammary gland	Papillary vegetant carcinoma with giant cell reaction
11.	Female	12 years old	Mixed Breed	Mammary gland	Canalicular vegetant adenocarcinoma
12.	Female	12 years old	Spitz	Mammary gland	Vegetant carcinoma
13.	Female	12 years old	Mixed Breed	Mammary gland	Vegetant carcinoma
14.	Female	11 years old	Bichon	Mammary gland	Vegetant adenocarcinoma with xanthomatous cellular elements
15.	Female	11 years old	Bichon	Mammary gland	Vegetant carcinoma
16.	Female	11 years old	Pekingese	Mammary gland	Papillary vegetant carcinoma

17.	Female	10 years old	Teckel	Mammary gland	Cancerous mastitis
18.	Female	10 years old	Husky	Peritoneal cavity (fluid)	Infected histiocitary mesothelioma
19.	Female	9 years old	Pinscher	Mammary gland	Adenocarcinoma
20.	Female	9 years old	Pekingese	Mammary gland	Papillary vegetant carcinoma
21.	Female	8 years old	Caniche	Cervical region (tegument)	Undifferentiated vegetant carcinoma
22.	Female	8 years old	Bulldog	Peritoneal cavity (fluid)	Epithelial-like mesothelioma
23.	Female	8 years old	Caucasian Shepard	Mammary gland	Vegetant adenocarcioma
24.	Female	7 years old	Mixed Breed	Nasal cavities	Estesiocarcinoma
25.	Female	7 years old	Mixed Breed	Nasal cavities	Vegetant carcinoma
26.	Female	7 years old	Golden Retriever	Nasal cavities	Estesiocarcinoma
27.	Female	7 years old	Cocker Spaniel	Mammary gland	Small cell vegetant carcinoma
28.	Female	5 years old	Terra Nova	Pleural cavity (fluid)	Massive carcinoma
29.	Female	5 years old	Mixed Breed	Phalanx	Squamos cell carcinoma
30.	Male	16 years old	Bichon	Prostate	Vegetant carcinoma
31.	Male	12 years old	Mixed Breed	Testicle	Seminoma
32.	Male	12 years old	German Shepard	Mammary gland	Vegetant carcinoma
				Testicle	Seminoma
33.	Male	10 years old	Mixed Breed	Testicle	Seminoma
34.	Male	10 years old	German Shepard	Urinary Bladder	Primary giant cell carcinoma
				Liver	Metastatic carcinomatous masses
				Lungs	Giant cell carcinoma metastasis
				Spleen	Giant cell carcinoma – individual metastatic elements
				Pleural cavity (fluid)	Giant cell carcinoma metastasis
				Intestine	Malignant lymphoma
35.	Male	9 years old	Airdale Terrier	Nasal cavities	Papilloma with high celullar activity
36.	Male	9 years old	Shih-Tzu	Stomach	Vegetant carcinoma
37.	Male	8 years old	Mixed Breed	Perianal region (tegument)	Undifferentiated carcinoma disseminated in the perianal tissue (fig. 2)
38.	Male	7 years old	Mixed Breed	Nasal cavities	Vegetant carcinoma
39.	Male	1 year old	Akita-Inu	Stomach	Malpighian carcinoma

Our studied casuistry conducted over a period of two years, diagnosed with various epithelial neoplasms was represented by: 11 cases in cats and 39 cases in dogs. The cat's age was between 3-14 years old, and for dogs: 1-16 years old.

The frequency of the solid external tumors in cats was represented by 6 cases, in which, 4 of them were located in the mammary gland, 1 with the same location but with an inguinal lymph node metastasis, and 1 in the auricular area.

In dogs the frequency of the solid external tumors was represented by 25 cases, in which 19 of them were located in the mammary gland and the other 6 of them had different locations as: 1 in the perianal area, 1 in the supraorbital tissue, 1 in the cervical area, 1 in the phalanx, 2 in the testicle) and 1 case with double location: in the mammary gland and in the testicle.

The frequency of the solid internal tumors in cats was represented by 3 cases: 1 with hepatic location, 1 with renal location and 1 with hepatic and pulmonary location. In dogs the presence of these neoplasms was identified in 11 cases: 5 cases in the nasal cavities, 1 in the urinary bladder, 1 in the prostate, 2 in the stomach, 1 in the intestine, 1 in the urinary bladder but with multiple metastasis located in the liver, lungs and spleen.

Another location, for the epithelial tumors within the two species (dogs and cats), was in the preformed cavities (peritoneal, pericardial, pleural), which in ultrasound examination presented fluid accumulations.

The incidence of these malignant forms of cancer was increased in dogs – 78% than in cats – 22%.
The incidence of these locations in cats was represented by 2 cases (18.18%): 1 case within the peritoneal cavity and 1 in the pericardial cavity, both representing the primary carcinoma metastasis.
In dogs were identified 5 cases: 2 within the pleural cavity with serous neoplasm (1 case with histiocytic mesothelioma (fig. 4) and 1 case with epithelial-like mesothelioma (fig. 5), 2 within the pleural cavity, both cases representing the primary carcinoma metastasis and 1 case within the pericardial cavity with metastatic cells from the primary tumor (10.25%).
From our investigated casuistry in cats (11 cases) we find that from the total number of epithelial cancers, the solid epithelial tumors located in the mammary gland were represented by a 45.45% percentage and 1 case with auricular location representing 9.09%.
The cats diagnosed with epithelial neoplasms in the internal organs represented 27.27% from the total of 11 animals. Also were diagnosed 2 cases with tumors in the preformed cavities, representing 18.18%.
From our casuistry in the canine specie, from the total of 39 dogs, 25 cases (64.10%) of solid external neoplasms were diagnosed, from which – mammary gland tumors: 19 cases, and 6 cases with different locations.
The neoplasms located in some internal organs and in the nasal cavities, totalized 10 cases (25.64%).
4 dogs were diagnosed with tumors in the preformed cavities, representing 10.25%, 2 of them presented epithelial carcinoma metastasis, and the other 2 were diagnosed with serous neoplasms (mesotheliomas).
The incidence of malignant epithelial neoplasms for the solid internal tumors, from the total number of 50 cases was represented by: in dogs, of 25.64% (10 cases from 30 animals); in cats, of 27.27% (3 cases from 11 animals.
This study highlighted the existence of some rare cancers in veterinary oncology. These are represented by double cancers encountered in the following cases:
- cats with pulmonary giant cell adenocarcinoma and malignant hepatoma;
- dog with urinary bladder giant cell carcinoma (fig. 1) and malignant lymphoma in the intestine;
- dog with mammary gland vegetant carcinoma and testicular seminoma. In this case we must specify that the mammary carcinoma was in a male dog, rare encountered fact in human and veterinary medicine.
Another important issue in the case of epithelial neoplasms found in the two species, is the appearance and development of metastasis. Their detection in the clinical investigation, laboratory and imaging is extremely necessary to contribute to the establishment of cancer therapy. In our casuistry we found the following variants:
-cat with vegetant carcinoma (fig.3) of the mamma-ry gland with inguinal lymphnode metastasis;
- cat with double cancer (lungs and liver) with pulmonary carcinoma and tracheobronchial lymph node metastasis;
- cat with mucosecretant carcinoma metastasis in the pericardial fluid;
- dog with double cancer (urinary bladder, intestine): presented numerous giant cell carcinomatous metastatic masses in the liver, lungs and spleen;
- dog with vegetant carcinoma metastasis in the pericardial fluid;
- dog with massive carcinoma metastasis in the pleural fluid.
The incidence of epithelial neoplasms in dogs and cats is represented by a large variety of locations and cellular forms.

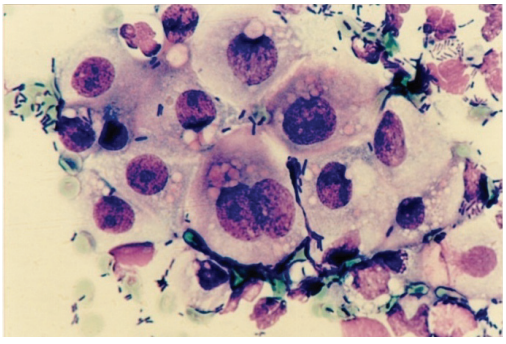

Fig. 1 – Vegetant adenocarcinoma (urinary bladder from a dog) – MGG x 1000

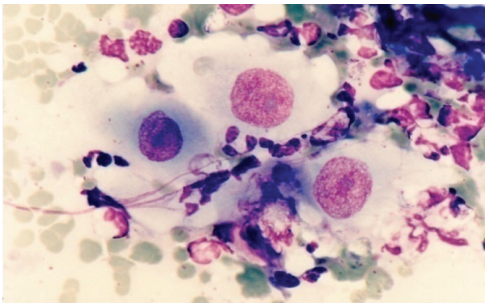

Fig. 2 – Undifferentiated carcinoma (neoplasm of the perianal tissue from a dog) – MGG x 1000

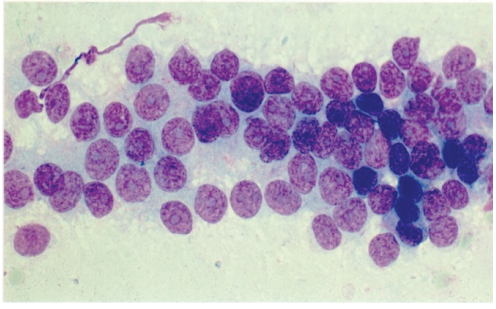

Fig. 3 – Vegetant carcinoma (mammary neoplasm from a cat) - MGGx1000

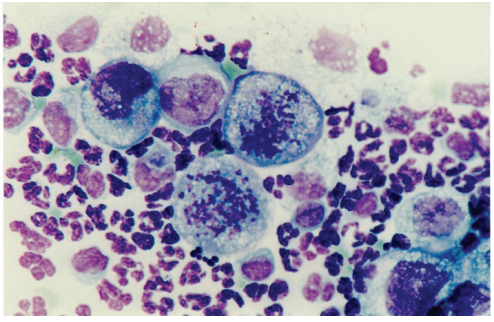

Fig. 4 – Histiocytic-like mesothelioma from a dog - MGGx1000

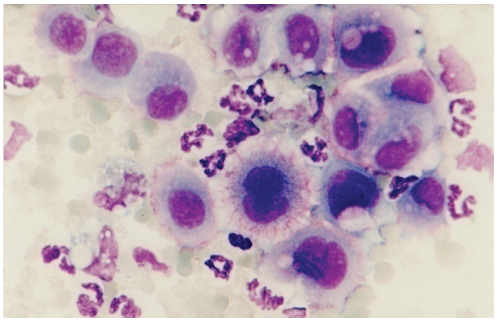

Fig. 5 – Epitelial-like mesothelioma from a dog - MGGx1000

CONCLUSIONS

1. The value of diagnose based on the cytomorphological exam is given by the speed with which is done and the high-fidelity obtained images.
2. The incidence of epithelial malignant neoplasms in the canine specie is much greater than the cases found in cats.
3. Mammary gland carcinomas are the most common, found in both species, compared to other tumor locations.
4. Corroboration of clinical investigations with laboratory and imaging is important to detect some double cancers and the metastasis; these data are helping the veterinary practitioner to conduct a better therapy.

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THE COMPARATIVE THERAPEUTIC EFFICACY OF ANTIMICROBIALS IN PIGS INFECTED WITH *MYCOPLASMA HYOPNEUMONIAE*

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Abstract

Respiratory diseases are current health problem for pig. Very often they have polietiological base which triggers defined Porcine Respiratory Disease Complex (PRDC). One of the main and permanent etiologic agents in PRDC is Mycoplasma hyopneumoniae, the causative agent of enzootic pneumonia in pigs. The disease is widespread in Bulgaria, inflicting major economic damage, resulting in high morbidity, poor feed conversion, reduced average daily gains, cost of therapy and immunization. These indicators determine treatment as necessary and inevitable in control of mycoplasma infection. The purpose of this study was to compare the therapeutic potential of enrofloxacin and florfenicol in industrial pig farms in Bulgaria. The study was conducted in pig farm breeding and fattening, with laboratory proven acute form of enzootic pneumonia. It was conducted on 260 growing pigs divided into two experimental groups. The first group was treated with enrofloxacin injective at a dose of 1 ml/10 kg., for three days, and the second with florfenicol, at a dose of 1 ml/20 kg., intramuscularly twice in 48 hours. Received clinical and epidemiological data give reason to assume that the tested schemes are effective in the control of enzootic pneumonia. As a result of the treatment to stabilize by the clinical condition of the pigs, normalization of indicators of blood and limiting morbidity and mortality. The resulting high therapeutic effect in patients treated with enrofloxacin pigs - 89.6 % and respectively florfenicol - 75.6 %, presented both as equivalent antibiotic in the treatment of enzootic pneumonia.

Key words: *pigs, M. hyopneumoniae, enrofloxacin, florfenicol, therapy.*

INTRODUCTION

Porcine respiratory disease complex (PRDC) may be of various etiology in different production systems (Halbur, 1999; Motovski, 2003). It is commonly caused by a combination of one or two viruses, *Mycoplasma hyopneumoniae* (*M. hyo*) and other bacteriological agents, which results in severe respiratory diseases and, consequently, in considerable economic losses (Ganovski, D. & I. Dinev, 1996; Motovski, 2003). One of the major PRDC etiological agents isthe causative agent of enzootic pneumonia, *M. hyo*. It damages the epithelial cells in the respiratory tract and inhibits the functions of the lymphatic system (Stipkovits et al., 2001; Opriessnig et al., 2004; Thacker, 2006).

To combat PRDC, it is generally accepted that vaccination, stress reduction and the implementation of modern breeding technologies prove useful and are thus considered necessary. However, even when utmost management care is taken, pigs can still become sensitive to bacterial infections. That is why Bosch (2004) considers the use of antibacterial agents as an integral part of the complex control measures against infectious diseases. Antibacterial agents offer several advantages in the control on enzootic pneumonia; these include flexibility of use, simple introduction via feedstock and drinking water, ease of use, possibility to optimize immunoprophylaxis programs and control of bacterial infections (Maes et al., 2011).

The antibacterials potentially active against *M. hyo* include tetracyclines, macrolides, lincosamides, pleuromutilins, fluoroquinolones, amphenicols and aminoglycosides (Hannan et al., 1989; Vicca, 2005). Of these, it is tetracyclines and macrolides that are most

commonly used for treatment of respiratory infections in pigs (Timmerman et al., 2006).
The aim of the present study was to examine the therapeutic efficacy of two injectable antibiotics in growing pigs in an industrial pig farm with acute enzootic pneumonia.

MATERIALS AND METHODS

Animals included in the study

The study included animals from a pig fattening and breeding farm with enzootic pneumonia which was clinically and laboratory confirmed after a winter respiratory outbreak. Comparative evaluation of the therapeutic effect of florfenicol and enrofloxacin was performed in 260 weaners, 2 to 3 months of age, with clinical evidence of respiratory disease. Two groups of pigs were set up:
Group 1: 140 weaned pigs were treated with the florfenicol-containing drug FLORKEM®, which contains 300 mg/ml of florfenicol. The drug was administered by intramuscular injection in a dose of 1 ml/20 kg live weight, twice at a 48-hour interval.
Group 2: 120 weaned pigs were administered the drug HIPRALONA®ENRO-I, which contains 50 mg/ml of enrofloxacin. The drug was administered three times at 24-hour intervals in a dose of 1 ml/10 kg live weight. The animals in the two groups were fed and reared under the same conditions.

Paraclinical examinations

Five blood samples were collected from each group prior to therapeutic treatment and 5 days after the beginning of treatment. The blood samples were collected from *sinus ophthalmicus*, using the closed system Venoject II, with a Butterfly needle 16G and EDTA evacuated tubes for blood counts and gel evacuated tubes for biochemical tests. Whole blood counts (RBC, HGB, PCV, WBC, LYM, PLT) were performed using an automated HemaScreen analyzer (Hospitex Diagnostics, Germany). Biochemical parameters in blood sera (total protein, albumin, glucose, urea, total bilirubin) were determined by commercially available kits (Human, Germany), using a Screen Master semi-automated chemistry analyzer (Hospitex Diagnostics, Germany).

Serological tests

Sterile blood samples (serum) were collected from 30 animals (i.e. 15 samples from each of the two groups) prior to therapeutic treatment. The samples were analyzed for the presence of specific antibodies against *M. hyopneumoniae* by *bloking* ELISA, using an INGEZIM M. HYO COMPAC diagnostic kit (Ingenaza, Spain), according to the manufacturer's instructions. The results were considered positive when OD_{450} = or<cut-off of the positive control; negative, when OD_{450} = or>cut-off of the negative control; or ambiguous, when the values fell between the positive control cut-off and the negative control cut-off.

Statistical analysis

Statistical analysis was performed using the StatMost software (StatMost 3.6, Dataxiom Software, 2003). Data represent mean values with standard error of the means (Mean±SE) determine by one-way ANOVA. Results were considered statistically significantly different when $P<0.05$.

RESULTS AND DISCUSSIONS

The clinical examination prior to treatment of the pigs from both groups showed increased body temperature (40.5°C – 41.0°C), fits of dry cough breathing difficulties and visible growth retardation. The pathoanatomical examination of four pigs that died revealed changes in the lungs characteristic of catarrhal bronchopneumonia involving the apical and cardiac lobes with well-demarcated areas of light purple discoloration. This clinical morphological analysis was indicative of respiratory disease, more specifically, of enzootic pneumonia. To confirm the diagnosis, serological tests were performed using *bloking* ELISA. The assay detected antibodies against *M. hyopneumoniae* in all tested samples. There was a sharp increase in the incidence rates, which reached 29.3 % in group 1 and 48.3 % in group 2. This required injection of the studied

antibiotics to control acute enzootic pneumonia. Prior to therapeutic treatment, five blood samples were collected from each of the two groups for hematological and biochemical tests in acute clinical form of enzootic pneumonia. The results from the hematological tests in both groups of animals are presented in Table 1. In group 1, there were changes in the red blood counts, which deviated from the reference values: a decrease in erythrocyte counts to 4.07±0.16 ($p<0.05$), in hemoglobin to 54.40±6.19 ($p<0.01$) and in hematocrit to 30.06±0.48. These values were slightly higher in group 2 animals than in group 1 but were still below the reference values: 4.45±0.18 erythrocytes; 73.20±3.28 hemoglobin ($p<0.05$) and 29.34±0.41 hematocrit. There was also a decrease in MCV in both groups: 45.38±0.42 ($p<0.05$) in group 1 and 48.20±1.53 in group 2, as compared to the reference values. The test results also showed a statistically significant decrease in MCH, which was 14.18±0.22 ($p<0.01$) in group 1 and 15.82±0.32 in group 2 ($p<0.05$). In pigs with respiratory signs, the MCHC values were found to be statistically significantly lower in both groups: 270.80±2.73 ($p<0.01$) in group 1 and 258.00±3.29 ($p<0.001$) in group 2. Deviations were also observed in the white blood counts. The leucocyte counts were reduced to 6.58±0.27 in group 1 ($p<0.05$) and to 7.88±0.27 in group 2 ($p<0.05$). The percentage of lymphocytes was lower in the two groups: 22.54±0.50 ($p<0.001$) in group 1 and 23.74±0.32 ($p<0.001$) in group 2. The mean platelet counts fell within the reference values.

In addition to the changes in the hematological profile of the pigs before treatment with the tested antibiotics, there were also changes in some of the biochemical indicators (Table 2). The results showed a decrease in the total protein content in both groups: 59.98±0.44 ($p<0.05$) in group 1 and 61.50±0.71 ($p<0.05$) in group 2. The indices that remained within the reference limits in both groups were albumin (19.46±0.28 in group 1and 18.32±0.33 in group 2) and glucose (4.72±0.29 in group 1 and 4.04±0.17 in group 2 ($p<0.05$). There were small differences in the blood urea in the two groups; the values were within the reference limits in group 1 (7.06±0.29) and slightly increased (8.60±0.33) in group 2 ($p<0.05$). The total blood bilirubin was statistically significantly increased in the diseased pigs as compared to the reference values and was 9.24±0.31 ($p<0.001$) and 7.50±0.53 ($p<0.001$), in group 1 and 2, respectively.

The results from the comparative clinico-epidemiological study of the two antibiotics are presented in Table 3. In the florfenicol treatment group (group 1), the mortality was reduced to 4.3 % and the lethality, to 14.6 %. The percentage of emergency slaughtered pigs in this group amounted to 9.7 % at the end of the experiment. In comparison, in the group treated with enrofloxacin (group 2), there was lower mortality (3.3 %) and twice as low lethality (6.9 %) as compared to group 1. A similar trend was observed in the percentage of emergency slaughtered pigs: it was reduced to 3.4 % in group 2.

Table 1. Hematological profile prior to therapeutic treatment of weaners with enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona® ENRO-I (n=5) Mean±SE	References (mean)
1. Erythrocytes – RBC	$10^{12}/1$	4.07±0.16*	4.45±0.18	5 – 8 (6.5)
2. Hemoglobin – HGB	g / l	54.40±6.19**	73.20±3.28*	100 – 160 (130)
3. Hematocrit – PCV	%	30.06±0.48	29.34±0.41	32 – 50 (42)
4. MCV	fl	45.38±0.42*	48.20±1.53	50 – 68 (60)
5. MCH	pg	14.18±0.22**	15.82±0.32*	17 – 21 (19)
6. MCHC	g / l	270.80±2.73**	258.00±3.29***	300 – 340 (320)
7. Leukocytes – WBC	$10^{9}/1$	6.58±0.27*	7.88±0.27*	11 – 22 (16)
8. Lymphocyte – LYM	%	22.54±0.50***	23.74±0.32***	39 – 62 (53)
9. Thrombocytes – PLT	$10^{9}/1$	267.20±3.71	240.20±2.22	100 – 900 (520)

*$p < 0,05$ **$p < 0,01$ ***$p < 0,001$ *(References: Nemi, C. J., 1993. Essentials of Veterinary Hematology, p. 23)*

Table. 2. Biochemical profile prior to therapeutic treatment of weaned pigs suffering from enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona®ENRO-I (n=5) Mean±SE	References (mean)
1. Total Protein – TP	g / l	59.98±0.44*	61.50±0.71*	65 – 85 (75)
2. Albumin	g / l	19.46±0.28	18.32±0.33	19 – 24 (21.5)
3. Glucose	mmol / l	4.72±0.29	4.04±0.17*	4.44 – 6.38 (5.41)
4. Blood Urea	mmol / l	7.06±0.29	8.60±0.33*	2.6 – 8.0 (5.3)
5. Bilirubin Total – T Bili	µmol / l	9.24±0.31***	7.50±0.53***	0.0 – 3.1 (1.55)

**p < 0,05 **p < 0,01 ***p < 0,001 (References: Angelov, G. et al., 1999. Klinichno-laboratorni izsledvaniya v veterinarnata meditsina, p. 106-146)*

Table. 3. Results from the clinico-epidemiological study of weaned growing pigs suffering from enzootic pneumonia

Parameters	Units	I group (n=140)	II group (n=120)
1. Treated pigs	Number	41	58
2. Age of pigs	Months	2.5	2.5
3. Medicament	-	Florkem®	Hipralona®ENRO-I
4. Active substance	-	Florfenicol 300 mg/ml	Enrofloxacin 50 mg/ml
5. Dose	ml/kg b.w.	1 ml / 20kg b.w.	1 ml / 10 kg b.w.
6. Method of administration	-	Intramuscular	Intramuscular
7. Course of treatment	Days	Twice in 48 hours	three times in 24 hours
8. Duration of the experiment	Days	21	21
9. Diseased by EP	Number	41	58
	%	29.3	48.3
10. Mortality by EP	Number	6	4
	%	4.3	3.3
11. Lethality	%	14.6	6.9
12. Emergency slaughtered	Number	4	2
	%	9.7	3.4
13. Clinically recovered	Number	31	52
14. Therapeutic efficiency	%	75.6	89.6

Table. 4. Hematological profile on the 5th day of therapeutic treatment of weaned pigs suffering from enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona® ENRO-I (n=5) Mean±SE	References (mean)
1. Erythrocytes – RBC	10^{12} / l	6.11±0.06	5.97±0.22	5 – 8 (6.5)
2. Hemoglobin – HGB	g / l	107.60±1.91	102.60±2.94	100 – 160 (130)
3. Hematocrit – PCV	%	33.66±0.98	36.08±0.78	32 – 50 (42)
4. MCV	fl	52.54±0.65	48.60±1.72	50 – 68 (60)
5. MCH	pg	14.34±0.29**	16.40±0.31	17 – 21 (19)
6. MCHC	g / l	283.20±3.25*	253.20±2.92***	300 – 340 (320)
7. Leukocytes – WBC	10^{9} / l	17.52±0.42	19.72±0.36	11 – 22 (16)
8. Lymphocyte – LYM	%	43.64±0.81***	48.90±1.44***	39 – 62 (53)
9. Thrombocytes – PLT	10^{9} / l	456.20±18.55	367.20±9.01	100 – 900 (520)

**p < 0,05 **p < 0,01 ***p < 0,001 (References: Nemi, C. J., 1993. Essentials of Veterinary Hematology, p. 23)*

Table. 5. Biochemical profile on the 5th day of therapeutic treatment of weaned pigs suffering from enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona® ENRO-I (n=5) Mean±SE	References (mean)
1. Total Protein – TP	g / l	78.08±0.90	74.76±0.76	65 – 85 (75)
2. Albumin	g / l	28.72±0.52***	30.54±0.51***	19 – 24 (21.5)
3. Glucose	mmol / l	4.92±0.14	4.50±0.44	4.44 – 6.38 (5.41)
4. Blood Urea	mmol / l	6.80±0.23	7.64±0.27	2.6 – 8.0 (5.3)
5. Bilirubin Total – T Bili	μmol / l	8.64±0.35***	7.30±0.32***	0.0 – 3.1 (1.55)

**p < 0,05 **p < 0,01 ***p < 0,001 (References: Angelov, G. et al., 1999. Klinichno-laboratorni izsledvaniya v veterinarnata meditsina, p. 106-146)*

For paraclinical examination, a new set of blood samples were collected from the two groups of animals on the 5th day of treatment. The results (table 4) showed a trend for the red blood counts to return to normal in both groups: erythrocytes (6.11±0.06 in group 1 and 5.97±0.22 in group 2); hemoglobin (107.60±1.91 in group 1 and 102.60±2.94 in group 2); hematocrit (33.66±0.98 in group 1 and 36.08±0.78 in group 2). The MCV values were back to normal in group 1 (52.54±0.65), whereas in group 2 they were slightly below the reference values (48.60±1.72). MCH remained slightly below the reference values: 14.34±0.29 in group 1 (*p<0.01*) and 16.40±0.31 in group 2. A similar trend was observed in MCHC, which was statistically significantly reduced in both groups: 283.20±3.25 in group 1 (*p<0.05*) and 253.20±2.92 in group 2 (*p<0.001*). Moreover, both antibiotics led to a normalization in the leucocyte counts (17.52±0.42 in group 1 and 19.72±0.36 in group 2), as well as in the percentage of lymphocytes (43.64±0.81 (*p<0.001*) in group 1 and 48.90±1.44 (*p<0.001*) in group 2). The comparison of the platelet counts prior to and after treatment showed an upward trend in both groups (456.20±18.55 in group 1 and 367.20±9.01 in group 2) but without exceeding the reference values.

The results from the biochemical tests carried out after the therapeutic treatment (Table 5) showed that the total protein content in the blood samples collected from both groups was within the reference range: 78.08±0.90 in group 1 and 74.76±0.76 in group 2. The albumin content was slightly above the norm in both groups: 28.72±0.52 (*p<0.001*) in group 1 and 30.54±0.51 (*p<0.001*) in group 2. Regarding the blood glucose, there were no significant differences between the samples collected prior to and following the treatment with either antibiotic; it was 4.92±0.14 in group 1 and 4.50±0.44 in group 2. It was also obtained that in both groups the blood urea was within the reference range: 6.80±0.23 in group 1 and 7.64±0.27 in group 2. The total bilirubin values were significantly increased in both groups after the antibiotic treatment, the values being 8.64±0.35 (*p<0.001*) in group 1 and 7.30±0.32 (*p<0.001*) in group 2.

At the end of the experiment (on day 21), the clinical condition of the pigs in both groups was improved and stable. The rectal temperature, albeit varying from one pig to another, was within the normal range (38.0–38.5°C). There were no clinical signs of respiratory disease (such as cough, dyspnea, anorexia or depression) in the antibiotically treated pigs. These observations were in accordance with the obtained data showing normalization of the clinical status of the treated pigs. The results from this study demonstrate the high therapeutic efficacy *in vivo* of both enrofloxacin (89.6 %) and florfenicol (75.6 %) in the control of acute enzootic pneumonia.

Our results are in agreement with the reports of Hannan et al. (1989) and Vicca (2005) about the good therapeutic efficacy of antimicrobial drugs from the amphenicol and fluoroquinolone group against *M. hyopneumoniae*. The good therapeutic effect achieved by enrofloxacin (89.6 %) against acute enzootic pneumonia suggests that, despite reports on cases of acquired antibiotic resistance in *M.*

hyopneumoniae to some fluoroquinolones (Vicca et al., 2004; Vicca, 2005), it could be considered not a serious problem in the therapy of enzootic pneumonia.

Antimicrobials, as a strategic choice of therapy against enzootic pneumonia, give good results when applied appropriately and at the appropriate moment. Parenteral therapy is preferred in the case of acute disease, whereas treatment with the forage (*per os*), as a preventive measure in metaphylaxis.

Despite the fact that antimicrobials do not protect pigs against infection and do not completely eliminate the microorganisms in the respiratory tract of diseased pigs, this type of therapeutic agents can alleviate the clinical signs of enzootic pneumonia as well as the severity of pathological lesions in the lungs. Most importantly, they can reduce the mortality from the disease and, in turn, the economic losses. Further studies are needed to determine whether administration of the tested antibiotics could prevent the transmission of *M. hyopneumoniae* from infected pigs to healthy ones.

CONCLUSIONS

Both tested schemes for therapy in acute enzootic pneumonia caused by *M. hyo* with enrofloxacin in a dose of 1 ml/10 kg b.w. administered intramuscularly three times in 24 hours and florfenicol in a dose of 1 ml/20 kg b.w. administered intramuscularly twice at 48 hours, proved to be effective in the control of disease, result of complete clinical recovery of pigs, normalization in indicators of blood and most importantly - reducing mortality.

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OESOPHAGEAL FOREIGN BODY IN A CAT: CASE REPORT

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Abstract

Oesophageal foreign body are relatively rare compared with gastrointestinal ones,but they can be encountered in clinical practice. Dogs are more likely to have oesophageal foreign bodies than are cats due to their indiscriminate eating habits. The most common oesophageal foreign bodies encountered are bones, needles, fish hooks and dental chews. Usually occurs with an object for which the size, texture or shape does not permit free passage through the oesophagus into the stomach causing the object to becomeentraped.
A fourteen years old cat was presented to our clinic with dysphagia, retching, regurgitation, ptyalism, lack of appetite and obvious signs of discomfort. From the anamnesis it resulted that the cat was feed two days before consultation with chicken that contained bones. After clinical examination ancervico-thoracic radiography was made, based on radiologic exam the diagnosis was oesophageal obstruction.
Because of the shape and dimension of the foreign body endoscopy was not possible, the only treatment left was surgery. The cat was scheduled for surgery in the same day after blood analysis.

Key words: *cat, foreign body, oesophagus.*

INTRODUCTION

Esophageal foreign body are relatively rare compared with gastrointestinal ones, but they can be encountered in clinical practice. In cats esophageal obstruction is less common than other gastrointestinal obstructions (Bebchuk, 2002). Indiscriminate eaters, dogs, are more affected than cats who are more particular eaters but cases do appear. Most common foreign bodies encountered are bones, fish hooks, needles, balls and dental chews.
Exposure usually occurs because of their hunting or playing behavior (Johnson, 1994). Ingestion of avian V-shaped bones clavicula has been described as a reason of obstruction of the pharynx and proximal oesophagus (Rendano et al., 1988).
If the foreign body remains entrapped several days, repeated peristaltic waves can produce pressure necrosis of the mucosa, submucosa and external layers of the oesophagus at the contact points. The secondary esophageal damage depends on the shape, size of the object and time that it is on contact with the mucosa (Johnson and Sherding, 2000; Gualitiere, 2001). Traction with a forceps can be successful if the foreign body is situated in the proximal part of the digestive tract (pharynx and proximal esophagus) and the shape and size of the foreign body permit this.
Endoscopy permits visualization and location of the foreign body and majority can be extracted without recourse to surgery.

MATERIALS AND METHODS

A fourteen years old cat was presented for consultation to Surgery Department of the Faculty of Veterinary Medicine in Cluj with signs of dysphagia, regurgitation, retching, restlessness, lack of appetite and ptyalism.
From the anamnesis it resulted that the cat was feed with raw chicken breast two day before consultation. Also from the clinical exam it resulted that the cat was missing the following tooth's: 101, 103, 107, 201, 202, 204, 203, 301, 302, 304, 308, 402, 403, 409(Triadan system), tartar, plaque was present and some signs of gingivitis.
Based on clinical findings and anamnesis we thought of a foreign body and the patient was send for an radiological exam. The radiological exam confirmed our suspicion of a foreign body that was located on the cervical esophagus (fig. 1).

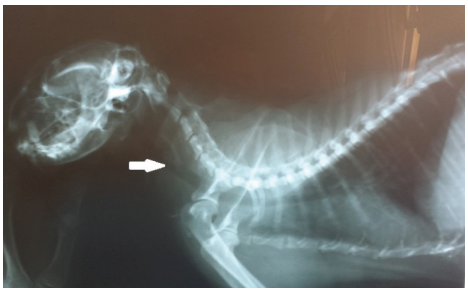

Figure 1. Foreign body located in cervical esophagus

Based on the shape (V-shape) and anamnesis we thought that it was a bird clavicula, therefore removing it with a forceps or endoscopy was not possible the only remaining therapy was surgery.Blood samples were taken for laboratory evaluation, haematology revealed a mild leukocytosis, other parameters were unremarkable. After this the cat was scheduled for surgery two hours later.

Anesthesia protocol was "magic kitty" (medetomidine 100 µg + butorphanol 1 mg + ketamin 10 mg/animal) we choose this protocol because it was a short time intervention. The surgical field was prepared aseptically and an i.v. catheter was placed for fluid therapy before and during surgery with NaCl 0.9% 20 ml/kg and glucoses 5% 10 ml/kg .

The cat was restrain in dorsal decubitus on a heated surgical table, we approached the esophagus by a ventral midline incision, separating the paired sternohyoid muscles and retracting the trachea to the right (fig. 2).

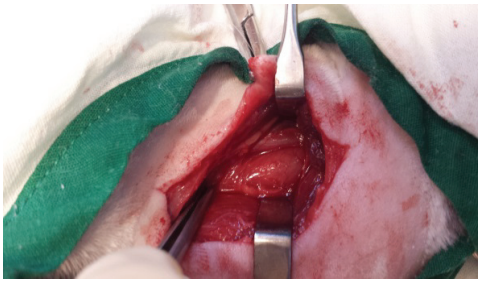

Figure 2. Ventral midline incision

Should take care not to cut the carotid artery and when retracting the trachea not damage to the recurrent laryngeal nerve. After identifying the esophagus a stab incision was made and the incision was extended as much as necessary (fig. 3).

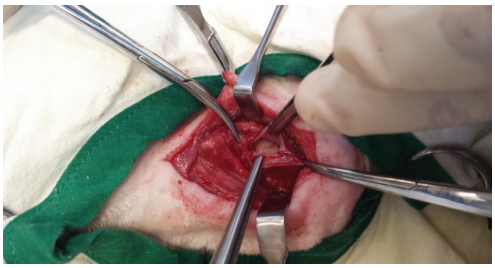

Figure 3. Esophagus incision

We proceed locking for the foreign body and extracting it from the esophagus. Because of is size and shape (clavicular bird bone) (fig. 4) the bone could not be removed until it was cut in two pieces with a scissor (fig. 5).

After removal the esophagus was checked to see if the mucosa had any injuries. Because the esophagus was unaffected, only a simple wound cleaning and a local antiseptic (methylene blue) was applied (fig. 6).

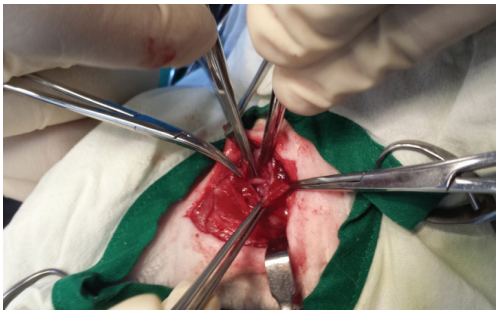

Figure 4. Entrapped bone

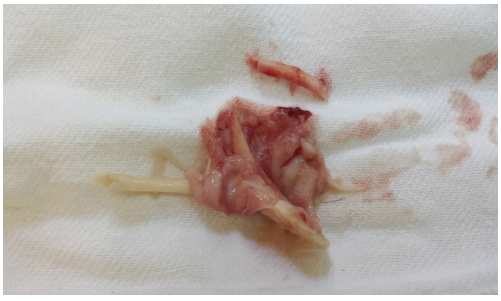

Figure 5. Removed foreign body

Next step was suturing the esophagus in two-layer pattern, first layer was mucosa and submucosa in a simple interrupted pattern with the knots inside the esophagus lumen, and the second layer apposing the muscularis and adventitia in a continues inverting pattern. The

suture material was Monocryl®3-0 (absorbable monofilament) for both layers (fig. 7).

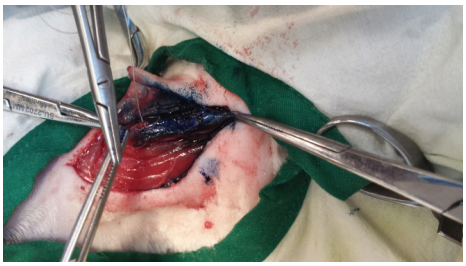

Figure 6. Local antiseptic

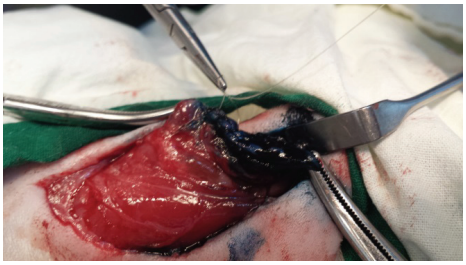

Figure 7. Suture of the esophagus

After esophagus suture the anatomical plans were apposed with a simple continues suture usingVicryl®2-0 (fig. 8) and the skin was sutured in "U" suture pattern withMersilk®2-0 (fig. 9).

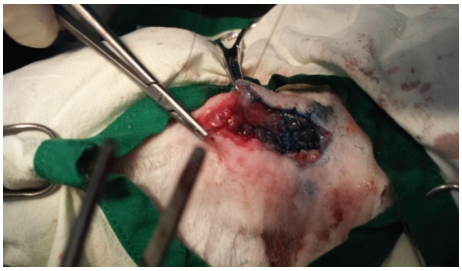

Figure 8. Apposed muscular plans

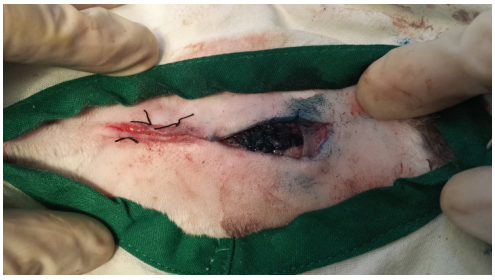

Figure 9. Skin suture

RESULTS AND DISCUSSIONS

Esophagotomy was the only treatment in this case because the shape and position of the bone, if forceps removal was tried it could result in sever damage to the oesophagus, also endoscopy would result in failure.
Postoperative care consisted of antibiotic therapy (cephalexin 20mg/kg i.m for 7 days), anti-inflammatory (meloxicam 0,2mg/kg s.c for 5 days), gastric protectors (ranitidine 2mg/kg i.v for 9 days) andoral antiseptic (methylene blue twice a day for 7 days).
First two days received only intravenous fluid therapy with NaCl 0.9% 20ml/kg, Glucoses 10ml/kg, Duphalyte®15ml/kg twice a day and vitamin C 1ml/cat/day.
After two days in the cat diet it was introduced water in small amounts andViyo Recuperation Cat®30ml/cat/day divided in three doses, in the fifth day post operatory the cat received Hill's® Prescription Diet® a/d® Canine/Feline Critical Care. Because of the animal status it was recommended a semi-solid diet for life time and meat with bones it was forbidden.
The cat was maintained under observation for three day and discharged after because she progressed well and had no signs of regurgitation after meals. The cat had a little hyperthermia (39.2^0-39.4^0C) in the first three days after it became normal. The owner came with the cat for treatment every day till the ninth day when the threads were removed.
After nine days the cat was fully recovered without any complications.

CONCLUSIONS

The number of cases of foreign body at the esophagus level in cats are rare but can appear.
In case of foreign bodies at the esophagus level it can be tried to remove with the forceps or by endoscopy but in some cases surgery is the only solution.
Surgical procedure was choose because the shape of the bone did not permit to extract it by non-surgical methods and if it was tried it could result in an esophagus rupture or perforation.
In our case the cat recovered without any complications.

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ANIMAL PRODUCTION, PUBLIC HEALTH AND FOOD QUALITY CONTROL

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INTERNATIONAL ORGANIZATIONS REGULATORY IN SAFETY FOOD FIELD

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Abstract

The paper aims to present the main international organizations with responsibilities in food safety. Not incidentally, these organizations issue legal regulations in this area. Even if they are very popular, few know their responsibilities and competencies. The main international organization that develops food safety regulations, some of them have exclusive competences in the field of food safety, as European Food Safety Authority (EFSA) or the Codex Alimentarius Commission, and others have only a component of food safety, such as World Organization for Animal Health (OIE), United Nations for Food and Agriculture (FAO), World Health Organization (WHO) and World Trade Organization (WTO).

Key words: *food safety, regulation, standards, international organization.*

INTRODUCTION

First organizations that aim to defend public health arise as a necessary objective, after the Second World War, in a world of poverty and food resources scarce, where the risks were major. Thus, under the aegis of the United Nations was founded World Health Organization, which aims from the beginning to analyze the risks for human in consumption of animal foods by functioning and by the statistics they make as an organization of epidemiological surveillance of zoonoses that are transmitted to humans through consumption and handling. Also, created under the aegis of the UN, the United Nations Food and Agriculture Organization (FAO), serves as a knowledge network. The organization, uses the expertise of its own - agronomists, foresters, fishermen, nutritionists, economists, statisticians and other professionals - to collect, analyze and disseminate data where required. FAO also published newsletters, reports, books, magazines and created numerous electronic forums. FAO uses the experience of member countries in agricultural policy, supporting planning, drafting effective legislation and creating national rural development strategies. FAO provides methods and in a few cases is a limited source of funds. In crisis situations, it is working with the World Food Program and other humanitarian agencies to protect rural areas and to help people rebuilding their lives. In 1963, after a series of meetings of representatives from FAO and WHO, has been created an organization that aims to develop and unify standards in the food industry. This organization will be called Codex Alimentarius Commission, the most important international organization in this field at the moment, and joined by most countries accepting its standards. The World Trade Organization has been created in 1994 as forum where to propose solutions and to resolve trade disputes. Later, taking into consideration the findings of these organizations, the European Union through the European Commission has initiated a series of laws, trying not to leave uncovered any area of food safety. Last step was to generate an organism to deal with food risks and to notify them to Member States in real time (Gonciarov, 2008).

MATERIALS AND METHODS

The main objective of this study is to analyze the appearance and development of the main international organizations dealing in food safety field, because in the present are many organizations that are dealing with this subject

without knowing the real importance of this. After I discovered which one they are, I have analyzed their work from the beginning until today. Very important is the legal regulations that they are elaborating, of their activities and how they are involved in every field of activity, trying to develop clear legislation and to help those involved in food safety.

RESULTS AND DISCUSSIONS

World Organization for Animal Health (OIE) has indirect responsibilities in food safety, developing regulations regarding international trade in animals and animal products and the development of standardized diagnostic methods for the diagnosis of infectious and parasitic animals. It has established collaborations with major international organizations like EU, FAO, WHO, Codex Alimentarius Commission, the WTO and other(OIE/WTO,1995). OIE has its own structure which ensures representation at governmental level, undeniable and complete independence in action. Specific structures allow access to all scientific data that serve as a basis for making decisions and implementing them very quickly in case of emergency. OIE retains today three main missions, namely:
-information on animal diseases,
-the study of methods to control these diseases,
-regulating international transport of animals and animal products (Gonciarov, 2008).

World Health Organization (WHO) is the leading international organization for human epidemiological surveillance, prepares statistical reports on health status in the world that are carried in particular by the EU and used in regulatory action. Inside WHO was created a section of "Veterinary Public Hygiene," which addressed these issues since its establishment:
- preventing and combating zoonoses,
- hygiene of food of animal origin and in particular the prevention of infections and food poisoning in humans,
- preventing and combating pollution of the environment by animal sources,
- increase knowledge of human diseases using veterinary medical sciences accumulated gains.
The organization shall establish, based on statistical data, quarterly and annual reports of health status in the world (Nastase, 2000).

United Nations for Food and Agriculture (FAO) uses the experience of the member states in elaboration agricultural policy, supporting the development of effective legislation and creating national strategies for rural development. Organized by the United Nations, FAO serves as both the developed and developing, in a joint effort to overcome hunger. FAO acts as a neutral forum where all nations negotiate agreements and subject of political debate. FAO is also a source of knowledge and information. FAO helps developing countries and countries in transition to modernize and improve their agriculture, forestry and fisheries. From its inception, FAO has focused attention on developing rural areas of the planet, given that over 70% of the world population lives in poverty. In recent years, FAO has analyzed the impact of urbanization on agriculture and nutrition, and animal health issues such as, avian flu (Gonciarov, 2008).

European Union (EU) protects the quality of food in different ways, through measures to enhance food safety and hygiene, clear labeling the rules by regulations on animal and plant health and animal welfare, by regulating pesticide residues and food additives as well as providing information on the nutritional qualities of food. The method used by the EU includes strict monitoring and control systems, while ensuring the effective functioning of the European single market (Council Directive 662/89).

Contribution of Agricultural Policy Commune-CAP-in veterinary field.

Ensuring food safety and high standards of animal health and welfare, is not just a matter of legislation. Improving food quality has always been one of the objectives of the CAP, since the introduction of quality wine label in the 80s and continuing with its further expansion in olive oil, fruit and vegetables. These efforts are currently the focus of agricultural policy and in all areas the CAP efforts are made to improve food quality (Fuerea, 2003).
Here are some examples:
• cattle identification systems and rules for labeling beef are designed to allow full traceability of meat from the store and back to the origin,

• financial incentives in rural development policy for farmers in order to improve the quality of products,
• specific measures to encourage conversion to organic farming. EU wants to ensure that all its citizens consume food with high quality standards. Food safety policy underwent a major reform as response to a series of crises such as BSE related (bovine spongiform encephalopathy) and feed contaminated with dioxin. The objective of this reform was to ensure that EU legislation on food safety is as complete as possible, and consumers have information about potential risks and measures would be taken to minimize them. Food safety begins on the farm. EU rules apply to "from farm to fork", whether the food is produced in the EU or imported from elsewhere in the world (Council Directive 425/90).
EU food safety strategy is based on four key elements:
• rules on the safety of food and animal feed,
• independent and publicly available scientific,
• enforcement of the rules and process control,
• recognize the consumer's right to choose on the basis of full information on the origin and content of the food. If you want food to be healthy, it must come from healthy animals. Keeping animals healthy through good veterinary practice and prevent the occurrence of epidemics of contagious diseases such as foot and mouth disease; swine or bird flu is a priority for the EU. But if an outbreak does occur, it is carefully monitored and steps are taken to prevent its spread. All animals and animal products must meet strict health requirements before they can be imported or marketed in the EU (Popa and Gonciarov, 2010).
Community legislation on animal welfare is based on the principle that animals should not suffer unnecessarily; this principle is reflected in clear rules on the conditions of the farming and the conditions in which farm animals can be transported and slaughtered. These rules are regularly updated in the light of new scientific data and are among the most stringent in the world. Research shows that farm animals are healthier and produce better quality food if they are properly treated (Gonciarov, 2008)
Organic farming is a production method that preserves soil structure and fertility, promotes a high standard of animal welfare and avoid using products authorized in conventional agriculture, such as synthetic pesticides, herbicides, chemical fertilizers or growth stimulators such as antibiotics, or bodies GM. Farmers resort to techniques that help maintain ecosystems and reduce pollution. The processing of organic food can only use a limited number of additives and processing aids. EU rules are guaranteeing the authenticity of organic products wherever they are produced and ensure accurate labeling. By law, use of the word 'organic' and its equivalent in any other language for food classification is reserved exclusively for organic products. The exclusive use is a guarantee for consumers about the quality and reliability of organic products they buy. EU organic logo was made available to farmers and organic food manufacturers to voluntarily use and its meaning is:
• at least 95% of the product's ingredients have been organically produced,
• the product comply with the official system of control,
• product bears the name of the manufacturer, the processor or vendor and the name or code of the inspection body (Gonciarov, 2008).
Special products.
These are natural and exceptional quality products which shows where were produced and the production methods used. Consumers and traders of food are increasingly interested in the geographical origin of food. Traditional Specialty Guaranteed logo (TSG) is used for products with distinct properties which either have traditional ingredients or are made by traditional methods. The indicators of quality provide the following advantages:
- guarantee of origin and production methods;
- send efficient commercial messages about products with high added value;
- supports companies in rural areas which receive quality products by protecting the label against fraudulent imitation (Gonciarov, 2008).

World Trade Organization (WTO) deals with the rules of trade between nations at a global level. It is a free trade organization for the commercial, a forum for governments to negotiate trade agreements, a place for them to settle trade disputes in which operates a system of trade rules. WTO began life on 1 January

1995 but the trading system is half a century old. Since 1948, the General Agreement on Tariffs and Trade (GATT) provided the rules of the system. Over the years, WTO GATT evolved through several rounds of negotiations. Last round of GATT and the largest was the Uruguay Round which lasted from 1986 to 1994 and led to the creation of the WTO. (WTO Uruguay Round Final Act, 1994).

Codex Alimentarius Commission(CCA) was born from an objective necessity. United Program FAO/WHO Food Standards, main purposes are:

- protecting consumers health and the right to food trade,
- promoting the coordinating standardization activities in the field of whole food made by governmental and non-governmental organizations.

Harmonization of food standards is generally seen as a prerequisite for consumer health protection and facilitates best international trade. For this reason, the general arrangements for application of Uruguay Sanitary and Phytosanitary Measures (SPS) and Technical Barriers to Trade (TBT) encourage international harmonization of food standards. Harmonization can only be achieved when all countries adopt the same standards. General Principles of the Codex Alimentarius specific ways in which countries can "accept" Codex standards. An increasing number of countries align their food standards or parts of them (especially safety) with the Codex Alimentarius. This generally happens for additives, contaminants and residues. Published volumes of the Codex Alimentarius are available in English, French and Spanish (Gonciarov, 2008)

European Food Safety Authority (EFSA), provides scientific advice and technical assistance to EU legislation and policies in all fields which have a direct or indirect impact on food safety and animal feed. It provides independent information on all matters within these fields and communicates risks. Authority, the Commission and the Member States shall cooperate to promote effective coherence between risk assessment, risk management and risk communication. EFSA searches, collects, analyzes and summarizes relevant scientific and technical data in its fields of competence, such as:

-food consumption and exposure of individuals to risks related to the consumption of food,
- incidence and prevalence of biological risk,
- contaminants in food and feed,
- residues (Council Regulation 178/2002).

EFSA contribute to a high level of protection of human life and health, and in this respect take account of animal health and welfare, plant health and the environment, in the context of the functioning of the internal market. The Authority shall collect and analyze data to allow characterization and monitoring of risks directly or indirectly impact on the safety of food and feed.

Competencies of EFSA also include:
- scientific advice and technical assistance on human nutrition in relation with Community legislation and, at the request of the Commission, assistance concerning notification regarding nutrition issues in the community health program,
- scientific advice on issues related to animal health and welfare and plant health,
- scientific opinions on products other than food and feed related to genetically modified organisms (Council Regulation 178/2002).

CONCLUSIONS

The main international organization that develops food safety regulations, have on one hand exclusive competences in the field of food safety, as European Food Safety Authority (EFSA) or the Codex Alimentarius Commission, and others have only a component of food safety, such as World Organization for Animal Health (OIE), United Nations for Food and Agriculture (FAO), World Health Organization (WHO) and World Trade Organization (WTO).

All recommendations, standards, general statistics developed by these organizations, are taken by the European Commission through the two DG Agriculture, DG SANCO and DG Agriculture and Food, in the elaboration of normative acts concerning food safety and others.

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BLOOD MINERAL STATUS INFLUENCE ON MINERAL NUTRITIONAL VALUE OF MILK OBTAINED FROM A DAIRY FARMING INTENSIVE SYSTEM

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Abstract

Milk from cattle species is an important part of human alimentation due to its mineral content, among other nutritional substances. Researchers have conducted studies to improve milk nutritional value, even reaching genetic manipulation in order to enrich cow milk with lysozyme, lactoferrin, and lactalbumin, components usually found in human milk. Mineral concentration in milk is an important quality parameter for human nutrition. Mineral concentrations from raw milk and blood were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) in samples taken from 15 dairy cows farmed in a dairy farming intensive system situated in the south of Romania. Blood concentrations of all analyzed minerals registered strong difference to their concentration in milk. However, strontium concentration in milk is linearly correlated to calcium concentration in milk. This finding suggests that calcium and strontium use similar transporters at cellular level and compete for the same transport system. There has not been found a positive correlation between the milk obtained from daily production and the blood level of some minerals, although it can be observed that at productions of 53.9 L/day all elements, with the exception of iron, have higher levels comparative with the lowest milk production (15 L milk), that could be explained by the differentiated feeding required by the productive capacity of animals.

Key words*: dairy cows, milk, blood, minerals.*

INTRODUCTION

Cattle research knows unexpected developments. Cow milk quality registers improved quality through genetic manipulation. In April 2011, Chinese researchers have announced the creation of a herd of 300 cows able to produce milk enriched with three components of human milk, respectively lysozyme, lactoferrin, and lactalbumin (Wang et al., 2008; Yang et al., 2011, http://www.telegraph.co.uk/news/earth/agriculture/geneticmodification/8423536/Genetically-modified-cows-produce-human-milk.html).

In June 2011, Argentinean scientists have grafted two human genes to a cow in order to produce lactoferrin and lysozyme in human breast milk specific concentrations (http://www.telegraph. co.uk/news/worldnews/southamerica/argentina/8569687/Scientists-create-cow-that-produces-human-milk.html).

Beside these genetic manipulations, research interventions by genetic selection with the aim of improving the productive performance in terms of milk and meat can be added. Considering the physiological aspects of "functioning" body including homeorhesis phenomenon it is natural in this context to ask ourselves how such interventions are beneficial for cattle homeostasis.

Trace minerals are essential for human and animal organisms. They occur in the composition of enzymes or act as a cofactor in antioxidant mechanisms. For cattle meat and milk production, performance level induces oxidative stress that can lead to health disorders (Sundrum, 2015).

Overall composition of milk (Figure 1) shows only the main categories of its constituents and the values given are average values. We notice immediately that the primary constituent of milk is water with 902 g/L, while dry weight is

only 130 g/L (http://www.ulb.ac.be/sciences/cudec/ LaitComposition.html).
Milk contains 7-8 g/L minerals as calcium, sodium, potassium, magnesium, citrates, chlorides, phosphates. Milk mineral salts are the same to blood mineral compounds, but their concentrations are different. Mineral absorption from the blood stream by the mammary gland is a selective process rather than a simple filtration (Ghergariu, 1980).

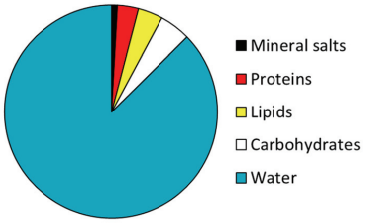

Figure 1. Global chemical composition of milk (g/L) (http://www.ulb.ac.be/sciences/cudec/LaitComposition.html)

Compared to blood mineral components, milk is richer in calcium, phosphorus and potassium, but it contains less sodium and chloride.
Average milk mineral composition is presented in Table 1.

Table 1. Average values for milk salt constituents (mg/dl) in whole milk (http://ansci.illinois.edu/static/ansc438/Milkcompsynth/milkcomp_minerals.html)

Mg	Na	Ca	K	S	P	Cl	Citrates
12	58	123	141	30	95	119	160

MATERIALS AND METHODS

Milk and blood samples were collected from a group of 15 lactating Holstein cows. The milk production of the selected group varied between 15 L/day and 53.9 L/day. The cows are representative for all lactation stages and total lactations days for the animals raised in the farm. The animals are farmed in a dairy farming intensive system from the south of Romania. Feeding is done based on physiological status and production level and water is provided *ad libitum*. A nutritionist calculated the forage rations based on productive performance, physiological status, and age using a computer software, which chose the appropriate menu for the category of animals and their productive performance. Then the mixture of ten ingredients was prepared as directed by the computer software. Samples were collected during winter period, thus winter specific feed influenced mineral concentrations in milk. Blood samples were taken from the mammary vein, collecting 10 ml from each animal, after disinfecting the puncture site with alcohol. After eliminating the first jets, milk samples were collected manually, taking 20 ml from each animal. Blood and milk samples were collected on the same day and at the same time. Plastic bottles (including lids) were used for storing the samples in order to avoid possible contamination by oligominerals contained in rubber or glass.
In order to destroy the organic matter, HNO_3 and H_2O_2 were added to milk and blood samples. Then the samples were mineralized by microwave digestion at 190^oC for 30 minutes. Suitable dilutions were made to ensure the elements fell within the calibration range.
Three standards of 0.01ppm, 0.1ppm and 50ppm were obtained from a multielement standard (MERK) containing 1000 mg/L of Ag, Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, Tl, Zn.
The instrument was calibrated using a blank and three standards for each element and, after inspection, a linear fit was applied to all elements. Samples were analyzed in a single sequence. The sample data was measured by interpolation. Instrument Configuration: Pump rate 50 rpm; Nebulizer Standard concentric; Nebulizer Argon Pressure 0.6 L/min; Spray Chamber Standard cyclonic; Centre tube 2.0 mm. RF Forward Power 1150 W; Purge Gas Argon; Coolant flow 12 L/min; Auxiliary flow 0.5 L/min; Integration times: High Wavelengths 5 seconds; Low Wavelengths 15 seconds; Analysis mode: Speedy.

RESULTS AND DISCUSSIONS

Age, lactation number and daily milk production for each cow of the study group are presented in Table 2. Cows have been assigned a number in ascending order of their daily milk production. Cow no. 2, in its second lactation registered the lowest milk iron level (0.31 mg/dl) and the highest blood sodium level (132.481 mg/dl). Cow no. 6, also in its second lactation, registered the majority of the lowest

milk mineral levels (Ca 601.5 mg/dl, K 842 mg/dl, Mg 70 mg/dl) and also the highest milk zinc level (5.35 mg/dl).

Table 2. Age, lactation number and daily milk production for each cow of the study group

Cow	Age	Lactation number	L milk/day
1	6	3	15
2	4	2	16.5
3	4	2	23.2
4	5	3	31.2
5	4	2	35.5
6	3	1	33.8
7	4	2	42.2
8	6	4	35.9
9	6	4	41.3
10	3	1	38.9
11	7	4	40.8
12	7	4	43.9
13	7	4	47.7
14	5	3	51.4
15	6	4	53.9

Cow no. 7, in its fourth lactation, registered the lowest milk zinc level (2.355 mg/dl), and also all the lowest blood mineral levels (Fe 3.254 mg/dl, Ca 1.485 mg/dl, K 5.174 mg/dl, Mg 0.318 mg/dl, Zn 0.038 mg/dl, Na 30.122 mg/dl). Cow no. 13, also in its fourth lactation, registered the lowest milk sodium level (269.65 mg/dl). Cow no.11, in its second lactation, registered the lowest milk strontium level (0.25 mg/dl).

As presented in other research (Roussel et al., 1982; Eicher, 2003), all serum values were influenced by age, and the majority of the highest milk mineral levels were registered in cows in their third lactation, as opposed to the majority of the highest blood mineral levels, which were registered in cows in their second lactation. Gadberry et al. (2003) indicated that no differences were observed in some minerals for cows and heifers.

Cow no.1, in its third lactation, registered the majority of the highest milk mineral levels (Fe 21.3 mg/dl, Ca 1471 mg/dl, Mg 174 mg/dl, Na 726 mg/dl). Cow no. 1 also registered the highest milk strontium level (4.35 mg/dl), which is thought to come from feed and water as well. The highest strontium and calcium levels were registered in the same individual, from which the conclusion drawn is that strontium concentration in milk is linearly correlated to calcium concentration in milk. This finding suggests that calcium and strontium use similar transporters at cellular level and compete for the same transport system.

Cow no. 4, also in its third lactation, registered the highest milk potassium level (1650.5 mg/dl).

Cow no.3, in its second lactation, registered the majority of the highest blood mineral levels (Fe 14.379 mg/dl, Ca 6.096 mg/dl, Mg 1.406 mg/dl), and cow no. 2, also in its second lactation, registered the highest milk sodium level (132.481 mg/dl).

Cow no. 10, in its fourth lactation, registered the highest blood potassium level (21.63 mg/dl), and cow no. 12, also I n its fourth lactation, and registered the highest blood zinc level (0.166 mg/dl).

Blood concentrations of calcium and zinc do not linearly correlate to their concentration in milk.

Milk and blood zinc levels based on daily milk productions are presented in Figure 2.

Milk and blood iron levels based on daily milk productions are presented in Figure 3.

The majority of the highest milk mineral levels are found in cow no. 1, which has the lowest daily milk production, of 15 L/day. Exceptions are the highest level of potassium (registered in cow no. 4, with a 31.2 L/day milk production) and the highest level of zinc (registered in cow no. 6, with a 35.5 L/day milk production), both milk productions being below the average productions of the analyzed cows. The highest blood mineral levels are found in cows with daily milk productions that vary between wide limits, as follows: highest sodium level is found in cow no. 2 (16.5 L/day), highest iron, calcium and magnesium levels are found in cow no. 3 (23.2 L/day), highest potassium level is found in cow no. 10 (41.3 L/day), and highest zinc level is found in cow no. 12 (43.9 L/day).

The lowest milk mineral levels are found in cows with daily milk productions that also vary between wide limits, as follows: lowest iron level is found in cow no. 2 (16.5 L/day), lowest calcium, potassium and magnesium levels are found in cow no. 6 (35.5 L/day), lowest zinc level is found in cow no. 7 (35.9 L/day), and lowest sodium level is found in cow no. 13 (47.7 L/day).

All the lowest blood mineral levels are registered in cow no. 7, with a milk production of 35.9 L/day, which is about the average daily milk production of the analyzed cows.

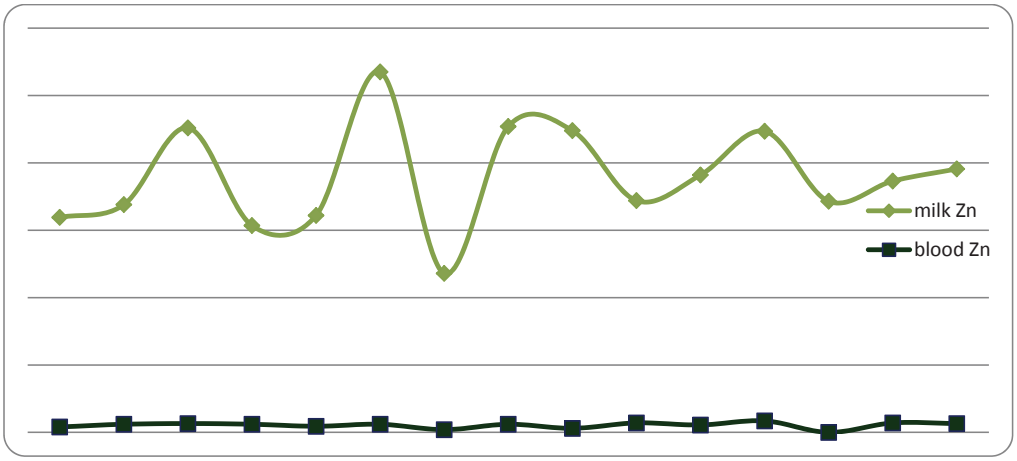

Figure 2. Milk and blood zinc levels based on daily milk productions

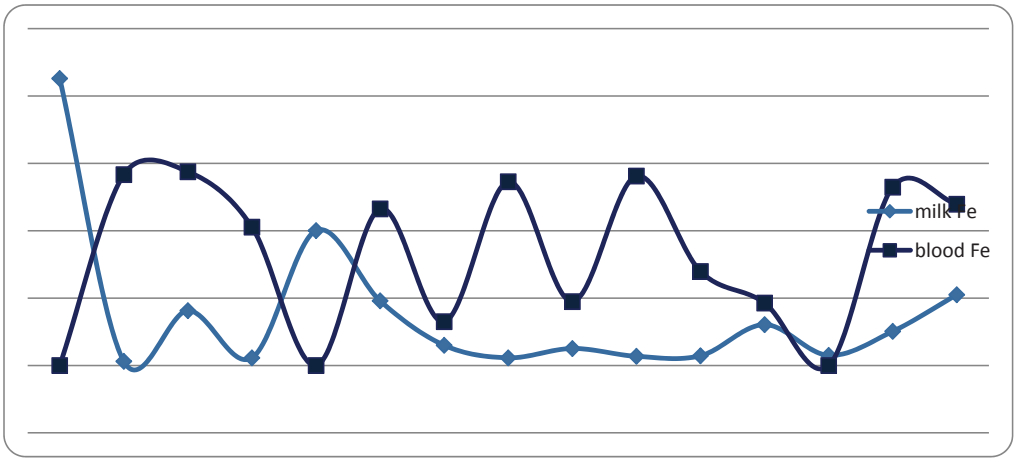

Figure 3. Milk and blood iron levels based on daily milk productions

Cow no. 1 (15 L/day milk production) had iron and sodium levels below the method detection limit. Cow no. 5 (33.8 L/day milk production) had iron levels below the method detection limit. Cow no. 12 (43.9 L/day milk production) had sodium levels below the method detection limit. Cow no. 13 (47.7 L/day milk production) had all blood mineral levels below the method detection limit.

A statistical analysis of the data by two-way ANOVA was performed in order to determine the influence of lactation number and daily milk production on the total mineral levels (Table 3 and Table 4). No significant difference was found between mineral concentrations in both blood and milk samples due to lactation number (Table 3) or due to daily milk production (Table 4).

However, in almost all determined minerals there is a strong significant difference between these two types of samples (p-value <0.0001). Fe levels in blood and milk samples registered a significant difference influenced by both analyzed variables, showed by a p-value <0.05 – lactation number (p-value = 0.0463) and daily milk production (p-value = 0.0263).

Table 3. Two-way ANOVA for total minerals concentrations (mg/dl) in blood and milk samples depending on lactation number

Element	Type of samples	Mean (mg/dl)*				Total Mean (mg/dl)	SD Mean	Std Err Mean	*p*-value
		1st Lactation	2nd Lactation	3rd Lactation	4th Lactation				
Ca	Blood	4.797[a]	5.343[a]	4.087[a]	3.079[a]	4.113	1.75	0.45	<0.0001
	Milk	1031.25[a]	934[a]	1140.67[a]	988.58[a]	1010.13	204.62	52.83	
Fe	Blood	6.822[a]	11.791[a]	7.837[a]	6.444[a]	8.199	5.64	1.46	0.0463
	Milk	5.29[a]	2.4738[a]	8.1317[a]	2.075[a]	3.821	5.5	1.42	
K	Blood	14.779[a]	16.053[a]	14.059[a]	9.182[a]	12.736	6.47	1.67	<0.0001
	Milk	1554.5[a]	1314.25[a]	1503.5[a]	1516.0[a]	1464.83	215.98	55.76	
Mg	Blood	1.118[a]	1.108[a]	0.954[a]	0.655[a]	0.897	0.45	0.12	<0.0001
	Milk	142.225[a]	122.025[a]	146.483[a]	132.425[a]	133.77	25.54	6.59	
Na	Blood	93.233[a]	113.250[a]	75.453[a]	58.589[a]	81.157	52.2	13.48	<0.0001
	Milk	393.825[a]	430.613[a]	560.733[a]	469.367[a]	467.233	137.22	35.43	
Sr**	Milk	0.355	0.359	1.677	0.41	0.642	1.029	0.266	0.3057
Zn	Blood	0.1065[a]	0.12[a]	0.1117[a]	0.0883[a]	0.104	0.04	0.01	<0.0001
	Milk	3.8775[a]	4.265[a]	3.33[a]	3.6783[a]	3.792	0.76	0.2	

* Levels not connected by the same letter are significantly different. No comparisons can be made between different elements concentration.
** One-way ANOVA was performed for strontium concentrations (mg/dl) in milk samples depending on lactation number.

Table 4. Two-way ANOVA for total minerals concentrations (mg/dl) in blood and milk samples depending on daily milk production

Element	Type of samples	Mean (mg/dl)*				Total Mean (mg/dl)	SD Mean	Std Err Mean	*p*-value
		<30 L/day	30-40 L/day	40-50 L/day	>50 L/day				
Ca	Blood	4.657[a]	4.188[a]	4.174[a]	5.044[a]	4.407	1.38	0.37	<0.0001
	Milk	1220[a]	976.8[a]	970.1[a]	878.75[a]	1010.13	204.62	52.83	
Fe	Blood	9.516[a]	7.761[a]	7.605[a]	12.605[a]	8.784	5.36	1.43	0.0263
	Milk	8.560[a]	3.487[a]	1.287[a]	3.885[a]	3.821	5.5	1.42	
K	Blood	14.750[a]	13.229[a]	9.184[a]	17.361[a]	12.736	6.47	1.67	<0.0001
	Milk	1399.83[a]	1429.10[a]	1508.10[a]	1543.50[a]	1464.83	215.98	55.76	
Mg	Blood	1.061[a]	0.9[a]	0.806[a]	1.276[a]	0.961	0.38	0.1	<0.0001
	Milk	154.533[a]	123.9[a]	135.91[a]	121.95[a]	133.77	25.54	6.59	
Na	Blood	87.776[a]	90.472[a]	88.885[a]	117.51[a]	93.643	43.73	12.13	<0.0001
	Milk	542.083[a]	392.27[a]	488.96[a]	488.05[a]	467.233	137.22	35.43	
Sr**	Milk	1.738	0.379	0.367	0.345	0.642	1.0289	0.2657	0.2454
Zn	Blood	0.110[a]	0.098[a]	0.094[a]	0.133[a]	0.104	0.04	0.01	<0.0001
	Milk	3.695[a]	3.706[a]	3.924[a]	3.82[a]	3.792	0.76	0.2	

* Levels not connected by the same letter are significantly different. No comparisons can be made between different elements concentration.
** One-way ANOVA was performed for strontium concentrations (mg/dl) in milk samples depending on milk quantity per day

Although there is no positive correlation between the milk obtained from daily production and the blood level of some minerals, it can be observed that, at productions of 53.9 L milk, all elements, with the exception of iron, register higher levels comparative with the lowest milk production (15 L milk). The explanation can be found in the differentiated feeding required by the productive capacity of animals that is done based on physiological status and production (Rotaru et al., 2012).

Because strontium was found only in milk samples, one-way ANOVA was performed for strontium concentrations (mg/dl) in milk samples depending on number of lactations and daily milk production. In both analyzed variables, no significant difference was registered, showed by a p-value > 0.05 (p-value = 0.3057 in the case of lactation number and 0.2454 for daily milk production).

CONCLUSIONS

Minerals identified in milk are identical with those found in blood, but their concentrations are different.

Blood concentrations of all analyzed minerals registered strong differences as opposed to their concentration in milk.

Strontium concentration in milk is linearly correlated to calcium concentration in milk.

In highest milk productions, all elements registered higher levels compared to the elements' values in lowest milk productions.

The lactation number or daily milk production variables did not influence the analyzed minerals levels.

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FOOD SAFETY HALAL PRODUCTS VERSUS ORDINARY PRODUCTS WITH NO RELIGIOUS PROVISIONS

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Abstract

Every person, every family, even every society has its own type of food. Between humans and his nutrition there is a close relationship, depending on many factors such as: dietary habits, age, religion, income, social status and group affiliation. The influence of religion on food consumption manifests itself differently from one nation to another, from one individual to another, depending on the type of religion and the degree of confidence of every individual who follows its religious percepts. The "halal" concept is a criterion for quality and food safety, which consists of a set of principles, standards and rules that must be applied and followed throughout the production process and supply chain, along with the HACCP system allowing monitoring all industrial transformation processes of materials and identification of the deviations that could make the food unfit for consumption. The purpose of this paper is to present an analysis of checkpoints throughout the technological process for obtaining halal meat products compared to the traditional, classical food products, to obtain the safest finished products.

Key words: *food safety, halal, meat products, religious percepts.*

INTRODUCTION

Every person, every family, even every society has its own type of food. Between humans and his nutrition there is a close relationship, depending on many factors such as: dietary habits, age, religion, income, social status and group affiliation. A major influence on food consumption is represented by the lifestyle of a person, expressed in the activities, interests and its opinions (Dindyal, 2003).

The influence of religion on food consumption manifests itself differently from one nation to another, from one individual to another, depending on the type of religion and the degree of confidence of every individual who follows its religious percepts. From this point of view there are some religions „poor" in terms of the foods restrictions, which do not specify dietary restrictions, but there are religions that forbid „the believers" to eat certain foods or food categories.

Also, there are strict specifications on some food, which although in a first phase are fit for human consumption, under certain conditions, they may become unsuitable for the population consumption (Bonne and Verbeke, 2007).

A quite high issue for public health is represented by food customs and tastes, transmitted by tradition from generation to generation and which are almost impossible to change from one generation to another.

In the context of free movement of goods and people, the population is surprised every day with new food varieties, new ways of preparing raw materials as well as new raw materials used for preparing food (Al-Qaradawi, 1993).

According to the latest studies in the field, the eating habits are changing more slowly than other more visible aspects of culture, such as language or clothing. There are also cases where eating habits are kept strictly following a feeding system imposed by religion affiliation, for example halal food for Muslims and kosher for Hebrew (Regenstein et al., 2003).

Moreover, the existence of the "halal" term on food, is an element of credibility and undeniable value by both the finished product and raw materials, ingredients and technological processes applied for obtaining it. From that flow a series of consequences, from increased consumer confidence for the product and manufacturing company, through the care it gives to the consumer by guaranteeing the

quality of the product purchased. The compliance of halal meats to religious previsions, indirectly informs the consumer that together with conventional systems for monitoring food safety and hahal meat quality is undeniable.

The "halal" concept is a criterion for quality and food safety with reference to the physico-chemical, microbiological characteristics of a food, but also of all stages of production, processing, storage, transportation and marketing of a food. It allows monitoring of all industrial transformation and identification of the non materials that could make the food unfit for consumption (Grunert, 2005).

The specific case of halal meat chain, it provided a set of principles, standards and rules to be applied and followed throughout the production process and supply chain, using HACCP as a system to ensure safety and quality for halal. The compliance with such standards is certified by applying a distinctive label for halal.

MATERIALS AND METHODS

Ensuring the quality of meat and meat products, the more that for halal, is a very important problem, due to potential contamination at any stage of a technologycal flow, in addition to normally factors incriminated and there is a risk of cross-contamination with pork or food haram. For this reason, the preventive system of potential hazards and risks analysis HACCP (Hazard Analysis and Critical Control Point), should be complemented and adapted to the requirements of halal meat (Lund, 2002).

Usually, the system guarantees the highest levels of food safety of the products obtained by applying the seven principles that form a stepwise approach in identifying potential hazards that would result in non-compliances during the flow of obtaining foods.

Lately, the food safety has become an approach much larger, from the origins and quality of raw materials, animal welfare and good practice in the food industry, including growth, feeding, transporting and slaughtering animals, until to obtain products and by- animal, processing, distribution and marketing of finished products (Verbeke, 2005). Therefore we are witnessing an expansion of the surveillance system HACCP food from the enterprise to the entire food chain from breeder animals and to the end consumer (Ali, 2010).

To produce a safe and quality meat halal, during the technological flow can be identified the control points (CP), indispensable to the HACCP plan (Riaz et al., 2004).

CP1 – Hygiene and sanitation of spaces and equipment. Hygiene, sanitation, sanitation and food security are needed in preparing halal foods. This includes various aspects of personal hygiene, clothing, workplace equipment for slaughtering animals and food processing or manufacturing. Halal food must be prepared, processed, packaged, shipped and stored in a way that they meet sanitary and hygienic requirements of the Codex Alimentarius General Principles on Food Hygiene and other relevant Codex standards. Food safety systems must prevent foreign materials contamination of food with plastic, glass or metal splinters, dust, harmful gases or fumes and unwanted chemicals (Codex Alimentarius, 2003).

CP2 – The animals which follows to be slaughtered. They must be from a species that has been accepted and grown under specific conditions halal. Are not accepted as halal, the animals that feed on dirt or own milk (Bergeaud-Blackler, 2005). These animals must not take contact with others animals or foods considered haram, which would entail non-halal animal.

CP3 – Animal welfare. The humane treatment of animals before, during and after slaughter must by assured. Boarding, during transport, from landing, until the time of slaughtering, the animals should not be stressed or suffer ill-treatment. It is also forbidden sharpening the knife in front of the animal or an animal to assist in cutting other.

CP4 – The stunning of animals. Is an technologycal operation which is not prohibited, nor is freely accepted. Some Muslim followers recommends not apply and another part are agree with stunning, applying only in certain exceptional circumstances. However, when applied, the Muslim halal inspector, who supervise the slaughtering (control and monitoring), must verify that the stunning operation is carried out in accordance with approved methods. Stunning method used must

be reversible, must not kill or cause permanent physical harm to the animal, must not cause permanent brain damage and must not penetrate or break the animal's head.
CP5 – Slaughter instruments. The knife used to slaughter must be well sharpened so that the animal does not feel pain when animal are bleeding. Slaughtering must be done once for each animal and the action slaughter is considered correct, permissible, as long as the slaughtering knife is not high on the animal during slaughter (must remain in constant contact with the animal).
CP6 – The butcher. It must be a healthy Muslim who fully understand the fundamental rules and conditions relating to the killing of animals in Islam. Animals during slaughter, butcher will not be dressed in ihram (clothing worn during the pilgrimage).
CP7 – Slaughter method. It must perform by cutting the the windpipe, esophagus, jugular vein and carotid artery, in one motion using a sharp knife, without reaching the cervical spine. As a secondary requirement, the butcher would positioned the animal to Mecca.
CP8 – The invocation. Simultaneously with cutting the great vessels, trachea and esophagus, the butcher recites the invocation to confirm that the sacrifice is done in the name of Allah, for its glorification and while respecting the religious provisions.
CP9 – The packaging. Halal food must be properly packaged using packaging materials that are not made of materials that are hard or processed or manufactured using equipment that is contaminated or considered dangerous. The packaging must be done in a clean and hygienic and in good health.
CP10 – The labeling. The materials they are made of used tags in direct contact with the product must not be harmful to health and may not be realized from raw materials which were declared prohibited. Information available on the label must contain all information necessary to identify the product and ensure the quality and wholesomeness of the food.
CP11 – Storage, sale and service of products. All halal food, which are stored, displayed, sold or served must be classified and separated at each stage in order to prevent cross-contamination of raw materials, auxiliaries or materials that are non-halal. All units refrigeration and other storage rooms must be part of a constructive plan approved by the competent authority for halal slaughter. During storage, transportation and marketing of halal meat products must be physically separate from non-halal. The equipment used in all operations should be dedicated exclusively to the production of halal.

RESULTS AND DISCUSSIONS

In recent times there has been a growing demand for halal food, which triggered the increasing of food chain stores and expanding supply specific profile. The uncertainty about the new food is manifested for those who do not know the halal food too.
To ensuring the consumer confidence and further winning their trust in these products is done primarily an correct information to the consumer on the product that it intends to acquire, but uncertainty stops him to do.
The association between different systems of food safety, which supervise the compliance of innocuousness for these products, and rules emerging from religious precepts which impose rules and more stringent, from the acceptance of animals for slaughter, may represent challenges for the younger generation who it is open to new things and after this, winning a large number of consumers for halal products.
Some risks and hazards of the HACCP plan are removed from the start when applying him for a halal product this system. For example, the biohazard represented by Trichinella spiralis in pork products, can not be considered in case of food halal, because pork is unacceptable as halal, and the slightest suspicion of contamination, causes a halal food to become non-halal.
The examination of animals before slaughter is doubled by the obligation to respect the religious requirements (Aldeeb, 2001).

CONCLUSIONS

Ensuring the safety of food supplied to the consumption population, remains one of the priorities absolutely necessary to be fulfilled, regardless of religious conception that govern the buyers.
Along with the food safety management system HACCP, the provisions specific religious

cultures, bring more support in ensuring health and consumer satisfaction.

A properly system for processing, packaging and labeling, make known to the buyer all the information it needs and could lead to uniform market food consumption, that can meet both the provisions of religious and those related to sanitation and food safety.

An sales market with safe food for population can be obtained by summing and applying all provisions, which have ultimate beneficiary food, no matter their religious or veterinary domain.

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ANALYSIS OF INTENSIVE REARING PERFORMANCES OF JUVENILE FRĂSINET CARP

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Abstract

In this study, were considered productive performance of juvenile Frăsinet carp, raised in intensive systems in a systematic ciprinicol farm. The inspection fishing activity carried out by an interval of about two weeks, at random, was body weight at different ages, and based on them, increase medium rais up. Also, main measurements were made tangible and corporal indexes have been calculated. Somatometric raw data were statistically processed and the following parameters were calculated: mean, standard error of the mean, variance, standard deviation and coefficient of variability. The summer I growth of populating, average weight of juveniles varied between 0.2-1.0 g/fish, and at the end of growth, fall, summer I juvenile has reached an average weight of 40-90 g/fish. Juveniles showed a good growth rate, according to the pond, the total average increase from populating the tanks until harvest fishing being 39.8-89.7 g/fish in 2009 (body weight increased by 199-299 times), 69.8-89.8 g/fish in 2010 (+349-449 times) and 74.0-89.0 g/fish in 2011 (+74 times). The main phenotypic features of summer carp Frăsinet, differs from the carp native populations, especially in relation to body length (total length and the standard 12.0 cm, respectively 11.5 cm,), the maximum height of the body (5.5 cm), high perimeter 11.5 cm, thickness trunk 5.4 cm, caudal of the peduncle length 3.7 cm. Except large trunk length and perimeter, the other body dimensions population analyzed is quite heterogeneous, as evidenced by the heritability coefficient values (13-28%). In first summer, the Frăsinet carp, demonstrated a correct conformation and a good body the proportionality of the optimal values of corporal indexes: 2% index of fattening, 2.1 profile index, 0.99 quality index, 47% index of thickness and 32.3% carnosity index.

Key words: *corporal indexes*, *Frăsinet carp, growth performance, juvenile.*

INTRODUCTION

Maximize the efficiency and the fisheries productions of the carp culture sector, priority objectives of routed increasing carp, requires the application of intensive production systems or growth in fish facilities systematic and strict compliance with the technologies of growing, at each stage of the evolutionary cycle. Compared to traditional systems, intensive and superintensive systems allow management and rigorous monitoring of consumption, physical and chemical factors of the aquatic environment, growth and closer supervision of the health status of the fish.

At the same time, production may well be expected, technology can be adapted to the requirements of the consumer market, and for an efficient use of the resources of food from fish ponds and obtain higher yields for each pond, polyculture can be applied. Thus, intensive and superintensive growing of culture carp in ponds (including carp Frăsinet), is an effective way to revive and relaunch the fisheries sector in Romania, sector which, due to multiple causes (drastic reduction of areas intended for fisheries in favour of crops, insufficient fodder, populating pools mainly for carp angling and less fattening and marketing lack or inadequacy of subsidies etc.) a continuous decline in the past two decades (Bura et al., 1995; Diaconescu, 2003; Bud et al., 2004). Considering the aspects previously mentioned, the purpose of this paper was to analyze the main features of juvenile Frăsinet carp production raised in intensive systems, a unit of the fisheries south of the country. This species was chosen because it is one of the local populations of carp culture preferred by fish farmers, due to the bio-productive traits

particularly favorable growth directed: manifesting high plasticity in the early years of life, great capacity of adaptation, tolerates high density growth and harsh living conditions compared to other populations and species of fish raised in Romania.
At the same time, the topic is fully in line with the current concerns of the cyprinids, because growth, both nationally and worldwide, the system intensive is used increasingly more often.

MATERIALS AND METHODS

Research was carried out in the "S.C. Trivale - Călăraşi" followed the establishment of productive performance of carp Frăsinet, during the period of intensive growth of juveniles. The increase was achieved in special pools, namely: BC 3A+3B, BC No. 1 and BP, with surface and shallow water (0.9-6.0 ha, respectively 1.0-1.5 m), which allowed easy monitoring of biological material, physical-chemical properties of communal skill water and timely intervention to remedy any shortcomings.
Working methods have been diverse and have assumed: the daily observation of behavior biological material in every watering rise in first summer; fish inspection (analytical examination) to highlight the qualities and faults of conformation and maintenance condition (body condition); gravimetria, individual weighing of respectively 100 fish on the occasion of fisheries control, about every two weeks and the calculation of the rate increase; we mean making the main body measurements (total body length, standard, ensure proper commercial, maximum and minimum height, large and small perimeter, thickness of body, head length, length of the ventral fins, dorsal, anal and caudal (Lustun, 1985; Bud and Vlădău, 2004; Turliu, 2008) using the graduated ruler, ichtiometer and the centimeter on the 50 copies, harvested in fisheries control achieved in July; calculation of corporal indexes (the index of fattening, shape, quality, thickness, carnosity (Bud and Vlădău, 2004).
Fisheries control has been carried out in different areas of the growth ponds, namely: different depths from the shore and offshore, in the areas of food and evacuation of water. Fisheries control targeted the health of the fish, and assessment of population from ponds, in order to increase or reduce the additional consumption, measures ihtio-sanitary, supplement the flow of river water to maintain the optimal physico-chemical parameters of the production environment. Raw data were processed statistically by calculating the following statistics: mean ($\bar{x}$), standard error of the mean ($S_{\bar{X}}$), variance (S^2), standard deviation (S), coefficient of variation ($CV_{\%}$) (Tacu, 1968; Sandu, 1995; Neagu, 2005).

RESULTS AND DISCUSSIONS

In all the years analyzed, in summer I, Frăsinet crap recorded a good growth rate (table 1):
- in 2009, total average growth increase from populating the ponds until harvest fishing has been 89.7 g/fish in BC No. 3A+3B and 39.8 g in the BC No. 1. Therefore, juveniles increased body weight about 299 times in the first pool and about 199 times in the basin BC no. 1.
Regardless of the pond, the intensity increased with increasing age, the maximum increase was recorded in August and September (about 20-25 g/fish).
- in 2010, the results were reversed, meaning that the total average increase of growth of the populating fishing ponds to harvest was 69.8 g/fish in BC No. 3A + 3B and 89.8 g/fish in the pond BC 1. The first type of pool, juvenile increased body weight about 349 times, and in the second pool about 449 times. This year, body mass accumulation was higher in August and September.
- in 2011, from the populating fishing ponds until harvest was recorded average total growth increase 89 g/fish in BC No. 3A + 3B (weight of juveniles increased about 89 times) and the pool named BP 74 g (weight juveniles increased about 74 times). Per calendar month, growth rate showed a similar pattern to that in 2009 and 2010.
In general, the fall of the first year of growth, culture carp must achieve an average weight of about 50 g (Grozea and Bura, 2008). Voican et al. (1981) established that at the end of the first summer, young carp reach the weight between 25 to 30 g.
Bud (1990) report the following results on the dynamics of youth weight of farmed common carp Mărtineşti: 2-3 g control fishing

conducted on 15 June, to 1 July 5-7 g, 10-12 g on 15 July, 15-20 g from 1 August to 15 August 22-25 g, 28-30 g on September 1, 32-35 g on September 15, October 1, 38-40 g, 42-50 g 15 October and 1 November 51-50 g.

The results of this study demonstrate the superiority of young Frăsinet carp to young common carp, with regard to increased growth and body weight achieved at different ages.

For example, at harvest placed on November 1, young Frăsinet carp increased in pond BC no. 3A + 3B, in 2011, had the highest average weight, about 40 grams to the young common carp analyzed by Bud (1990).

Table 1. Weight dynamics of juvenile Frăsinet carp in summer I

Fish pond	Introduction to pond	Data of control fishing and juvenile weight (g/fish)							Fishing harvest (g)
		18.06. 2009	*08.07. 2009*	*24.07. 2009*	*15.08. 2009*	*29.08. 2009*	*10.09. 2009*	-	
BC nr. 3A+3B -6 ha-	04.06.2009 0,3 g	10	15	23	35	55	70	-	90
BC nr. 1 -2,7 ha-	02.06.2009 0,2 g	8	10	15	21	30	35	-	40
		10.07. 2010	*17.07. 2010*	*24.07. 2010*	*31.07. 2010*	*14.08. 2010*	*28.08. 2010*	*12.09. 2010*	-
BC 3A+3B -6 ha-	01.06.2010 0,2 g	7,5	12,5	18,5	29	41,5	42	55	70
BC nr.1 -2,7 ha-	01.06.2010 0,2 g	12	15	20	34	41	59	80	90
BP -0,9 ha-	01.06.2010 0,2 g	10	14	19	35	46	60	75	90
		20.06. 2011	*12.07. 2011*	*29.07. 2011*	*12.08. 2011*	*30.08. 2011*	*25.09. 2011*	-	-
BC 3A+3B -6 ha-	02.06.2011 1 g	15	31	50	63	75	90	-	90
BP -0,9 ha-	02.06.2011 1 g	15	30	40	51	60	70	-	75

In a study in "Fisheries Station – Nucet", Nicolae (2004) determined the average weights between 30–50 g/crap Frăsinet values recorded during the first summer of growth, comparable to those found for the material analyzed fish in this paper.

The main phenotypic characteristics of Frăsinet carp value expressed by morphometric traits analyzed on juvenile summer I (table 2) clearly differ from those of other populations of carp of the one hand due to specific growing conditions unity "SC Trivale - Călăraşi", on the other hand, distinct morphological features of Frăsinet carp resulting intense and crosses row selection practiced during the training process of the indigenous breeds.

Character analysis of total body length, the distance measured from the tip of the snout to the imaginary line joining the tips of the caudal fin lobes, show that the 50 individual measured had an average value of 12 cm, with a coefficient of variation of 5%.

The low coefficient indicates a good homogenity of the population on this quality body due to optimal growth conditions (feeding, physical and chemical parameters of the aquatic environment) provided during the first summer juvenile.

In terms of distance measured from the tip of the snout to the end of scaly sheath (base of the caudal fin), which expresses distance standard body length, mean measurements was about 11.5 cm, with a coefficient of variation lower (7%).

Table 2. Main body dimensions values of Frăsinet carp summer I registered to control fishing conducted in July 2011

Analyzed feature	Statistical parameters analyzed				
	Media ($\bar{x}$)	Standard error ($S_{\bar{x}}$)	Variance (S^2)	Standard deviation (S)	Coefficient of variation ($CV_{\%}$)
Total length trunk (cm)	12,00	0,59	0,35	0,59	5
Standard length trunk (cm)	11,45	0,14	0,98	0,99	8
Length statutory (cm)	9,97	0,14	1,01	1,00	10
Commercial length (cm)	8,47	0,14	1,01	1,00	11
Minimum height trunk (cm)	3,90	0,14	1,02	1,00	25
Maximum height trunk (cm)	5,45	0,14	1,02	1,00	18
Large perimeter trunk (cm)	11,47	0,14	1,01	1,00	8
Small perimeter trunk (cm)	7,54	0,14	1,02	1,02	13
Thick trunk (cm)	5,37	0,14	1,02	1,00	18
Caudal peduncle length (cm)	3,70	0,14	1,02	1,00	27
Head length (cm)	3,70	0,14	1,02	1,00	27
Dorsal fin length (cm)	5,39	0,14	1,02	1,00	18
Length of ventral fin (cm)	3,57	0,14	1,02	1,00	28
Anal fin length (cm)	3,45	0,14	1,02	1,00	28
Caudal fin length (cm)	4,67	0,14	1,02	1,00	21
Body weight (g)	30	2,23	25	5	17

Percentage, standard length is about 95.4% of the total body length of juveniles, that is, in absolute terms, it is contained in about 1.05 times.

The other two lengths of particular importance for the recovery of fish consumption, both by angling and by fishery harvest had an average value of approximative 10 cm - statutory length and approximative 8.5 cm - commercial length. Expressed in relative values of total body length, length of statutory (distance measured from the middle of the eye to the tip of the caudal fin) is about 83.1%, and commercial length (distance measured from the middle of the eye to the point posterior anal fin base) is about 70.6%. The spread of values around the mean is reduced, as evidenced by the low estimated error accompanying central variables analyzed ($S_{\bar{x}}$=0.14).

Depth trunk was assessed by two measurements, ie the distance measured in the highest place of the trunk (about in the middle), which gives the maximum height dimension of the body youth and the distance measured in the region where the depth is less (caudal peduncle), distance expressing the minimum height of the body.

The values shown in Table 2, shows a ratio of 1.4 : 1.0 in favor of maximum height (5.5 cm to 3.9 cm), a result that indicates a defining characteristic of Frăsinet carp, namely, the body is muscular and stocky with convex top line, which features begin to emerge even in summer I rise.

Comparing the heights of the total body length youth, it is found that the maximum height is about 2.2 times includes long and a minimum height of about 3.1 times (on average comprise both heights are about 2.6 times). The results are comparable to the minimum provided by Bud I. (1988), which sets the values of the size of average height and body length of 1.0/3.1-1.0/4.2, adult specimen carp. Lower values determined for the population analyzed in this study are normal, because it's summer youth age category I.

For both dimensions of height (especially for minimum depth), the population of medium to large heterogeneity showed a coefficient of variation values showing 18-25%.

Analysis of body circumference, i.e. large perimeter measured at the maximum height of the body and the small perimeter determined by surrounding the body with ribbon at the caudal peduncle, suggesting good development in breadth and robustness of both the trunk and the third body segment (about 11.5 cm, respective about 7.5 cm).

As a result, small perimeter is about 65.2% of the largest perimeter, that is, in absolute terms, it comprises about 1.5 times that. In terms of variability for both traits (perimeters) population study showed a small to medium uniformity ($CV_{\%}$ = 8-13%).

Compactness body is emphasized by the high value of the character body thickness (5.4 cm), measured between the points where convexity is greatest. This attribute is less than about 2.1 times the perimeter of the large and small compared to the perimeter of about 1.4 times. Caudal peduncle showed the same average length of the head (3.7 cm), representing about 31% of total body length of juvenile (1/3.2).
Of fins, the longest presented a dorsal (5.4 cm), followed by the caudal (4.7 cm), ventral and anal (about 3.5 cm). In all cases, population heterogeneity was evident ($CV_{\%}$=18-28%).
In conclusion, for all analyzed traits except body length and large trunk perimeter, the population is quite heterogeneous, mainly due to parasitic diseases occurring throughout the period studied, the growth ponds. The occurrence of different parasites and net left its mark on both the homogeneity of most body size and the quantity of fish produced in fish harvest.
Analytical examination of the exterior which involves assessment of each region somatoscopic body in terms of shape, size, direction, catching the neighboring regions, highlighting any defects and diseases, must be completed exam synthesis. This examination aims assessment body development, body size and overall harmony. The harmony of the whole is determined by the quality of constitutive parts of the body, the quality of body regions, the way their joint and proportional development.
To assess the overall harmony, besides visual examination is necessary to study body proportions, size relationships between different regions, using for this purpose the results of measurements of body (previously presented and interpreted as absolute values) introduced formulas that are body indexes. Main body indices calculated values for Frăsinet summer I carp category, are presented in Table 3.

Table 3. The main body indexes values for Frăsinet carp summer I, 2011

Specification	Number of individuals measured	Body index value
Fattening Index	50	2.0%
Profile Index	50	2.1
Quality Index	50	0.99
Thickness Index	50	47.0%
Carnosity Index	50	32.3%

Fattening Index: {body weight/(Standard length trunk)^3} x 100 = {30/(11.45)^3} x 100 = 2.0%
Profile Index = {Standard length body / Maximum height body} = 11.45/5.45 = 2.1
Profile Index = {Standard length body / Maximum height body} = 11.45/5.45 = 2.10
Quality Index = Standard length body / Large trunk perimeter = 11.45/11.47 = 0.99
Thickness Index = (Thickness trunk / Standard length trunk) x 100= (5.37/11.45) x 100= 47.0%
Carnosity Index = (Caudal peduncle length or head / Standard length trunk) x 100 = (3.7/11.45) = 32.3%

CONCLUSIONS

The average weight of Frăsinet carp juveniles about 20 days old when transferring in growth summer I ponds (May-June) ranged from 0.2-1.0 g/fish, at the end of growth, autumn juveniles the summer I average weight reached 40-90 g/fish.
Populating density varied depending on the type of pond and the increase in monoculture or polyculture: 25000 - 80000 Frăsinet juveniles per hectare when practicing monoculture (years 2009, 2011), 20000-100000 Frăsinet juveniles per hectare option of applying polyculture (77.0% Frăsinet carp, 7.0% grass carp -*Ctenopharingodon idella* and 16.0% silver carp - *Hypophthalmichthys molitrix*, in 2010). Control fishing conducted bimonthly, showed good growth intensity of carp summer I Frăsinet according pond, the total average increase of growth of the populating fishing ponds to harvest being 39.8 - 89.7 g/fish 2009 (body weight increased by 199-299 times), 69.8 - 89.8 g/exemplary in 2010 (+ 349-449 times) and 74.0 - 89.0 g/exemplary in 2011 (+74 times).
The main phenotypic characteristics of Frăsinet carp summer I appreciated somatoscopic and somatometric in July 2011 clearly differ from those of native carp population, especially in terms of body lengths (total length 12.0 cm and length standard 11.5 cm), maximum body height (5.5 cm), high perimeter 11.5 cm, 5.4 cm thick trunk, caudal peduncle length 3.7 cm.
For all traits analyzed except large perimeter and lengths summer I Frăsinet carp is quite

heterogeneous (coefficient of heritability of 13-28%), mainly due to parasitic diseases occurring in 2011.

In the summer I had exemplary of carp Frăsinet harmonious conformation and good body proportionality, demonstrated good values of body indices.

In general, during the years examined the health status of fish material was good, except in 2011, when the control fishing conducted in July, was found the presence of ectoparasites *Lerna sp*. and *Eritrodermatita*, controlled by medication.

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PREVALENCE OF *STREPTOCOCUS SUIS* SEROTYPE 2 STRAINS ISOLATED FROM MAJOR PARTS OF FRESH PORK MEAT

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Abstract

The goal of this paper is to present the prevalence of major disease causing sertotype 2 of Streptococcus suis in meat ready for retail market shipment. Streptococcus suis is one of the most important pig pathogen causing septicemia, meningitis and other infections in affected animals. In addition this bacteria is an emerging zoonotic pathogen. Human infections are usually after close contact with pigs or their products. A total of 180 samples of raw pork meat (200 gr each) were taken at sloughterhouse. Samples were taken from different parts of pork, already prepared for market: liver, kidneys, shoulder, ham, loin, belly, and head area. Results have shown that 18 isolates were identified as Streptococcus suis serotype 2 from 180 samples examined. The rate of prevalence was 10% exactly. Serotype 2 was the most isolated serotype from fresh pork with 46,1 % of isolated S. suis serotypes followed by serotype 9, 7, 3, 1 and 4. Streptococcus suis serotype 2 had been isolated in all collected samples. Prevalence of serotype 2 in liver, kidneys, shoulder, ham, loin, belly and head was 20%, 12%, 5%, 5%, 5%, 10% and 25 % respectively. It is known that besides occupational exposure and meat processing in sloughterhouses, consuming of uncooked or partially cooked pork products is also a risk factor for infection. Therfore it should be advised that pork need to be thoroughly cooked/baked and not to be in contact with raw meat in any way.

Key words*: Streptococcus suis, serotype, pork meat, prevalence.*

INTRODUCTION

Streptococcus suis is one of the most important pig pathogen causing septicaemia, meningitis and other infections in affected animals. In addition this bacteria is an emerging zoonotic pathogen responsible for increased number of diseased humans, with illness that may be fatal. Especially during the last 15 years this increased number of human infections due this pathogen has been recorded, and while most of these cases are sporadic, in Asia two epidemics are documented.

Streptococcus suis is coccoid, facultative anaerobic, Gram positive bacterium that synthesize capsule and secrete haemolysin. Most of the strains are alpha haemolytic on sheep blood agar and seen as as single cells, in pairs or short chains on microscopic slides.

S. suis is very heterogeneous species, at the moment 33 serotypes have been recognized on the basis of the composition of capsule. During the last 25 years *S. suis* is considered to be one of the most important cause of severe economic loss in major pig breeding countries. *S. suis* is a normal inhabitant of the pigs respiratory system, mostly of the tonsils and nasal cavities, and can often be isolated from the genital and gastrointestinal systems in healthy animals. Transmission of *S. suis* among animals is considered to be mainly through the respiratory route *(*Higgins and Gottschalk, 2005*)*. Since it is a very good colonizer of the mucosal surfaces, clinically healthy pigs are the main reservoir of infection, and the most important link in the epidemiology of human infections caused by *S. suis (*Gottschalk et al., 2010*)*. *S. suis* can be also easily isolated from noses and tonsils of live pigs, as well as from pig carcasses and butchers' knives (Stanojkovic et al., 2012).

All categories of pigs can be affected by the disease caused by *S. suis*, including suckling piglets, older piglets and fatteners. Colonization of pigs with *S. suis* occurs at early stage of life,

often through vertical transmission from carrying sows. *S. suis* carriage rates may vary between herds and can range from 0% to up to 80-100% (Amass et al., 1997).

According to Silvonen et al. (1988) even if all the pigs in the herd are infected with some strains of *S. suis* clinically apparent disease varies and is usually below 5%. Meningitis is the major feature of *S. suis* infection in pigs but other organs (joints, heart, lungs, reproductive organs etc.) can also be affected.

The largest number of *S. suis* serotypes isolated from clinically ill pigs belongs to serotypes 1 to 8 (Reams et al., 1996; Higins and Gotschalk, 2001) . In the European and Asian countries, *S. suis* serotype 2 is usually the most present one (Wisselink et al., 2000), however, in some European countries with a developed pig production, such as the Netherlands, Spain and Germany, *S. suis* serotype 9 is the most common serotype causing disease in pigs.

Human infections are usually after close contact with pigs or their products. This includes farmers, farm workers, veterinarians and butchers. According to Arends and Zanen (1988) the annual risk of developing *S. suis* meningitis among abattoir workers and pig breeders has been estimated to be 3.0 cases per 100,000 population while the risk is lower for butchers, at 1.2 cases per 100,000 population in developed countries. Different from pigs infection, the main route of entry of *S. suis* in humans is thought to be through contact of cutaneous lesions, most usually on the hands and arms, with contaminated animals, carcasses or meat (Wertheim et al., 2009).

The outbreak in China in 2005 caused by *S. suis* affected more than 200 people, with almost 20% mortality rate. This epidemic has completely changed the perception of the danger which this pathogen presents to human health (Stanojkovic et al., 2014). Period of incubation ranges from just a few hours to few days (Fongcom i sar., 2001). Just like in pigs *S. suis* produces meningitis as the main feature of disease but cases of endocarditis, pneumonia, peritonitis, arthritis and other less common clinical signs can be seen as the part of generalized septicaemia (Arends and Zanen, 1988; Huang et al., 2005). Also, there have been described per acute infections related to this pathogen which were usually in the form of streptococcal toxic shock-like syndrome (STSLS) that has been associated with most of the death cases in China 2005 epidemics. In China 2005 epidemics there have been 215 cases of infection while 38 of them died mainly as a results of STSLS.

According to Hoa et al. (2011) slaughterhouse pigs are a major reservoir of *Streptococcus suis* serotype 2 capable of causing human infection. But this results were obtained from tonsil samples at the slaughterhouse. Cheung et al. (2008) examined 78 samples of raw pork lean meat from retail markets and wet markets and determined that *S. suis* can be found in every sample although in different levels (MPN/g). Authors concluded that sometimes standard culture methods can't efficiently recover *S. suis* from samples. *S*. suis was isolated from 6.1% of raw pork meat from 3 of the 6 wet markets in Hong Kong.

It can be assumed that processing and consuming of uncooked or partially cooked pork meat is also a risk factor for infection. Also, local cuisine specialities such as raw or half-cooked intestines, uterus, tonsils or fresh pig blood can be important sources of infection. In Thailand there is an increasing trend of the incidence of the disease, mainly because of consumption of half-cooked/baked meat.

Studies of *Streptococcus suis* regarding prevalence are mainly directed to clinical cases in pigs and humans and discharges, tonsils, blood, brain and spinal fluids as a specimens. There are little date of this pathogen prevalence in raw meat. In this context, the paper presents an analysis of *Streptococcus suis* presence in different parts of fresh pork meat.

MATERIALS AND METHODS

A total of 180 samples of raw pork meat (200 gr each) were taken at 4 different regional slaughterhouses in Serbia. Samples were taken randomly from different parts of pork, already prepared for market: liver, kidneys, shoulder, ham (leg), loin, belly, and head area. Mentioned parts were chosen since these are the most commonly consumed parts of pork.

All samples were homogenized, inoculated on Columbia CNA agar with 5% sheep blood and incubated aerobically for 24 h at 37 °C. Bacterial strains were selected on the basis of

colony morphology, haemolytic characteristics that they produce on blood agar (picture 1), absence of growth in 6.5% NaCl broth and their microscopic appearance. For primary identification of bacteria, classical and commercial tests API 20 Strep and Rapid ID32 STREP (bioMérieux, France) were used. In order to achieve definitive identification and determine the serotypes of the isolated strains, serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens was used.

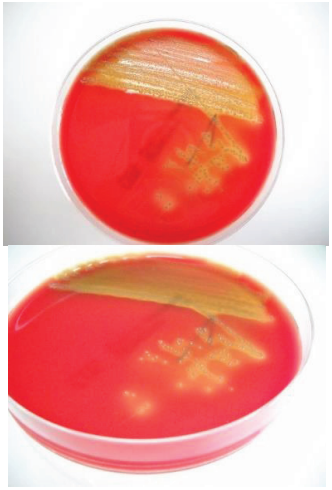

Figure 1. α Haemolysis on blood agar by *S. suis* strains

RESULTS AND DISCUSSIONS

Results have shown that 18 isolates were identified as *Streptococcus suis* serotype 2 from 180 samples examined. The rate of prevalence was 10% exactly. Except serotype 2 of *S. suis*, there have been found additional 21 strains of *S. suis* serotypes, such as serotypes 1, 3, 4, 7 and 9 with number of 3, 4, 2, 4 and 8 respectively (Figure 1). Prevalence of serotype 2 was major objective of this research as this serotype is almost always a cause of human *S. suis* infections.

Serotype 2 was the most isolated serotype from fresh pork with 46,1 % of isolated *S. suis* serotypes followed by serotype 9, 7, 3, 1 and 4. Slaughtered pigs had similar prevalence of *S. suis* strains just like those data reported for clinically ill pigs. Our data shows that serotype 2 is the most frequent serotype and in accordance to distribution of serotypes in Europe. Hoa et al (2011) found that *S. suis* serotype 2 was the most common serotype isolated from the sampled pigs, indicating that *S. suis* serotype 2 is highly prevalent in slaughterhouse pigs in southern Vietnam. In contrast to above mentioned and our research, in a study of slaughterhouse pigs in Korea, *S. suis* serotype 2 strains were absent, while serotype 9 was the most common serotype (Han et al., 2001)

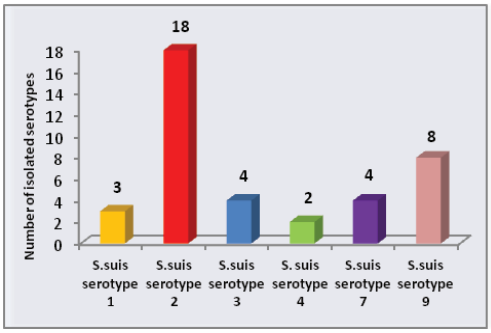

Figure 1. Number of isolated S. suis serotypes from fresh pork

Streptococcus suis serotype 2 had been isolated in all collected samples. Prevalence of serotype 2 in liver, kidneys, shoulder, ham, loin, belly and head was 20%, 12%, 5%, 5%, 5%, 10% and 25 % respectively (Table 1).

There was a significant difference in the presence of *S. suis* strains on the basis of sample collected. In this research hog head was highly contaminated with *S. suis* serotype 2 strains. This result was expected since *S. suis* is normal inhabitant of respiratory system such as tonsils, and also slaughtered pigs are held in that kind of

position that allows water to spread bacteria from hind part of the body to the head.

Noppon et al. (2014) have found overall prevalence of S. suis serotype 2 in pork of 12,8% which is similar to 10% prevalence in our research. In a research of previously mentioned authors results of prevalence of *S. suis* serotype 2 in fresh meat was 10,8% and it was not clear referring to the part of the body that fresh meat was taken from. In our study fresh meat samples from ham, loin and shoulder had lowest presence of bacteria of 5%.

Table 1. The number and percentage of isolated *Streptococcus suis* serotype 2 isolates

Meat type	Number of samples collected	Prevalence of S. suis N° (%)
Liver	25	5 (20)
Kidney	25	3 (12)
Shoulder	20	1 (5)
Ham	20	1 (5)
Loin	20	1 (5)
Belly	20	2 (10)
Head	20	5 (25)
Total	180	18 (10)

This figure is similar to results obtained by Nguyen et al. (2008) who found *S. suis* contamination of 6.1% of raw pork meat from 3 of the 6 wet markets in Hong Kong. Somehow lower isolation of *S. suis* in fresh meat from ham, loin and shoulder can be explained by fast removal of selected parts and absence of contact of these parts with other parts of pork that can be expected to be highly contaminated such as head, kidneys or sometimes skin.

Internal organs such as liver and kidneys had higher presence of *S. suis* serotype 2 strains of 20% and 12%. This is similar to 15,4% prevalence of *S. suis* serotype 2 in liver and other offal reported by Noppon et al. (2014). Although healthy at the moment of slaughter, higher kidney presence can maybe connected to findings presented by Nakayama et al. (2011) who demonstrated that *S. suis* accumulates in the kidney during *S. suis* infection.

CONCLUSIONS

Serotype 2 of *Streptococcus suis* is the most frequently isolated serotype of this bacteria in fresh pork meat. It can be readily isolated from almost every part of pork prepared to be sent to retail markets. The most contaminated parts of the pork are head, liver and kidney while ham (leg), loin and shoulder area had low prevalence of *S. suis* serotype 2 strains. It is known that besides occupational exposure and meat processing in slaughterhouses, consuming of uncooked or partially cooked pork products is also a risk factor for infection.

Therefore it should be advised that pork need to be thoroughly cooked/baked and not to be in contact with raw meat in any way.

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